Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.1s,
January-June 2024: P.71-82

Print version ISSN 0970 0765
Online version ISSN 2320 3188

Original Research Article *Content Available online at: https://www.bpasjournals.com/*

Isolation and Characterization of Alginate-Producing Azotobacter Species from Soil for Industrial Applications

Shilpa Ruikar[1], Swati Shivaji Tawade[2], Dr. Abhay Ghatage[3]

Author's Affiliation:
[1,2,3]Krishna Institute of Allied Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University), Karad, Maharashtra, India.

shilpa_ruikar@yahoo.co.in[1],
abhayghatage8@gmail.com[3]

ABSTRACT:
Soil microorganisms represent a vast and largely untapped resource for biotechnological applications. Among these, Azotobacter species have gained attention due to their ability to produce alginate, a biopolymer with diverse industrial applications. This study aimed to isolate and characterize alginate-producing Azotobacter strains from soil samples for industrial use. Soil samples were collected from diverse environments and Azotobacter species were isolated using selective media. Screening for alginate production was conducted, and positive strains were further characterized morphologically, biochemically, and molecularly. Alginate yield and physicochemical properties were determined to assess the industrial potential of the isolated strains. The results revealed a diverse population of Azotobacter species in soil, with several strains exhibiting significant alginate production capabilities. Morphological and biochemical characterization, coupled with molecular identification, confirmed the identity of the isolates as alginate-producing Azotobacter species. The alginate produced by these strains exhibited varying yields and physicochemical properties, indicating potential differences in their industrial applications. The discussion highlights the diversity of Azotobacter species in soil ecosystems and the alginate production potential of the isolated strains. Furthermore, the study explores the industrial applications of alginate and the biotechnological significance of utilizing Azotobacter for its production. Future research directions include optimizing cultivation conditions to enhance alginate yield and exploring downstream processing techniques for industrial scale-up. In conclusion, this study provides valuable insights into the isolation and characterization of alginate-producing Azotobacter species from soil, laying the foundation for their utilization in various industrial sectors.

Keywords:
Azotobacter, alginate production, soil microorganisms, industrial biotechnology, characterization

How to cite this article: Shilpa Ruikar, Swati Shivaji Tawade, Dr. Abhay Ghatage (2024). Isolation and Characterization of Alginate-Producing Azotobacter Species from Soil for Industrial Applications. *Bulletin of Pure and Applied Sciences-Zoology*, 43B (1s), 71-82.

I. Introduction

Azotobacter species, known for their versatile metabolic capabilities, have garnered significant attention in industrial biotechnology due to their ability to produce alginate, a polysaccharide with various applications ranging from pharmaceuticals to food and agriculture [1]. Alginate, primarily derived from seaweed, exhibits unique properties such as biocompatibility, biodegradability, and gel-forming abilities, making it an attractive candidate for numerous industrial applications. However, the high demand for alginate coupled with the limited natural resources of seaweed has prompted researchers to explore alternative sources for alginate production. Soil, a complex ecosystem rich in diverse microbial communities, offers a promising avenue for the isolation and characterization of alginate-producing Azotobacter species [2]. The soil environment provides a myriad of nutrients and substrates that can potentially support the growth and alginate production of Azotobacter strains. Furthermore, Azotobacter species are well-known nitrogen-fixing bacteria capable of thriving in

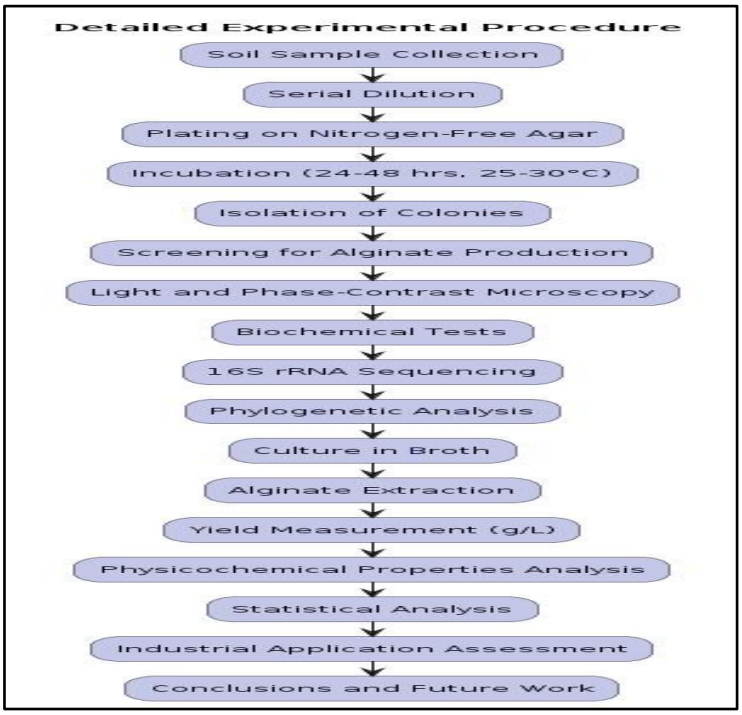

Figure 1: Detailed Experimental Procedure Diagram

various soil conditions, thus making them an ideal target for industrial applications. The isolation and characterization of alginate-producing Azotobacter strains from soil hold immense potential for sustainable alginate production. By harnessing the metabolic capabilities of these soil bacteria, it is possible to develop cost-effective and environmentally

friendly processes for alginate production on a commercial scale. The characterization of these Azotobacter strains is essential for understanding their alginate biosynthesis pathways [3], optimizing cultivation conditions, and enhancing alginate yield and quality. This research endeavor not only addresses the increasing demand for alginate but also contributes to the exploration of microbial diversity in soil ecosystems and the development of novel biotechnological solutions. In this study, we aim to isolate and characterize alginate-producing Azotobacter species from diverse soil samples, elucidate their alginate biosynthesis mechanisms, and evaluate their potential for industrial applications. Through comprehensive molecular and biochemical analyses, we seek to uncover unique traits and metabolic pathways that distinguish these Azotobacter strains and pave the way for their utilization in biotechnological processes [4]. This research holds promise for the sustainable production of alginate and underscores the importance of microbial diversity in driving innovation in industrial biotechnology.

A. Background

Alginate is widely utilized in the food industry as a gelling agent, thickener, and stabilizer due to its ability to form gels in the presence of divalent cations such as calcium. In pharmaceuticals, alginate finds applications in drug delivery systems, wound healing, and tissue engineering, owing to its biocompatibility and mucoadhesive properties [5]. Moreover, alginate-based materials have been explored in biotechnology for immobilization of enzymes and cells in bioreactors, as well as in environmental remediation processes.

B. Significance of Alginate

Alginate is a naturally occurring biopolymer derived from brown seaweed and certain bacteria like Azotobacter. Its significance spans across various industries due to its unique physicochemical properties. In the food industry, alginate is valued for its gelling, thickening, and stabilizing abilities, making it a key ingredient in products like ice cream, sauces, and dressings. It also acts as a dietary fiber with potential health benefits. In pharmaceuticals, alginate's biocompatibility and non-toxicity make it ideal for drug delivery systems, wound dressings, and tissue engineering. It can form hydrogels that encapsulate drugs, allowing for controlled release and targeted delivery. Alginate-based wound dressings provide a moist environment conducive to healing while absorbing exudates [6]. The biotechnology sector leverages alginate in immobilizing cells and enzymes, facilitating various biochemical processes. Its ability to form beads and films is crucial in cell encapsulation, which is essential for bioreactors and the production of biofuels. Alginate's role extends to environmental applications, such as water treatment, where it aids in the removal of heavy metals and dyes. The versatility and biodegradability of alginate underscore its importance as a sustainable and multifunctional material, driving innovation across diverse fields.

C. Azotobacter as Alginate Producers

Azotobacter species are notable for their ability to produce alginate, a biopolymer with significant industrial applications [7]. These Gram-negative, free-living nitrogen-fixing bacteria are primarily found in soil and are characterized by their large, oval-shaped cells. Among the various Azotobacter species, Azotobacter vinelandii is particularly renowned for its high yield of alginate.Alginate produced by Azotobacter has unique properties compared to alginate derived from seaweed. This bacterial alginate contains higher proportions of guluronic acid, which enhances its gelling strength and thermal stability, making it particularly valuable for industrial applications. The production of alginate by Azotobacter involves the secretion of exopolysaccharides [8], which serve as a protective capsule for the

bacteria, aiding in water retention and protection against environmental stresses.The process of alginate production in Azotobacter can be optimized through various fermentation techniques. Factors such as nutrient availability, oxygen levels, and pH can significantly influence the yield and quality of alginate. For instance, the presence of specific carbon sources like sucrose can enhance alginate production. Additionally, genetic engineering approaches have been employed to overexpress key enzymes in the alginate biosynthetic pathway, further boosting production levels.The applications of alginate from Azotobacter are diverse. In the pharmaceutical industry, it is used for controlled drug delivery systems and as a matrix for cell encapsulation in tissue engineering. In the food industry, it acts as a thickener [9], emulsifier, and stabilizer. Its biocompatibility and non-toxicity make it suitable for biomedical applications such as wound dressings and dental impression materials. The environmental sustainability of bacterial alginate production offers an advantage over traditional methods that rely on harvesting seaweed, which can disrupt marine ecosystems. Therefore, Azotobacter as alginate producers not only provides a high-quality biopolymer but also supports sustainable bioproduction practices, aligning with the growing emphasis on environmentally friendly industrial processes. The continued research and optimization of alginate production in Azotobacter hold promise for expanding its commercial viability and application scope.

D. Rationale for Study

Despite the considerable interest in Azotobacter as alginate producers, there remains a need to explore the diversity of Azotobacter species in soil ecosystems and characterize novel strains with enhanced alginate production capabilities. Furthermore, the industrial potential of alginate-producing Azotobacter strains warrants comprehensive characterization of their alginate yield, physicochemical properties, and suitability for various industrial applications. This study aims to address these gaps by isolating and characterizing alginate-producing Azotobacter species from soil samples, with a focus on their industrial applications. By elucidating the diversity of Azotobacter species in soil and characterizing their alginate production potential, this research contributes to the growing body of knowledge on microbial polysaccharide production and expands the biotechnological toolkit for sustainable industrial processes [10]. The findings of this study have implications for various industries seeking environmentally friendly alternatives to conventional materials and processes, highlighting the importance of harnessing the potential of soil microorganisms for industrial biotechnology.

II. Methodology

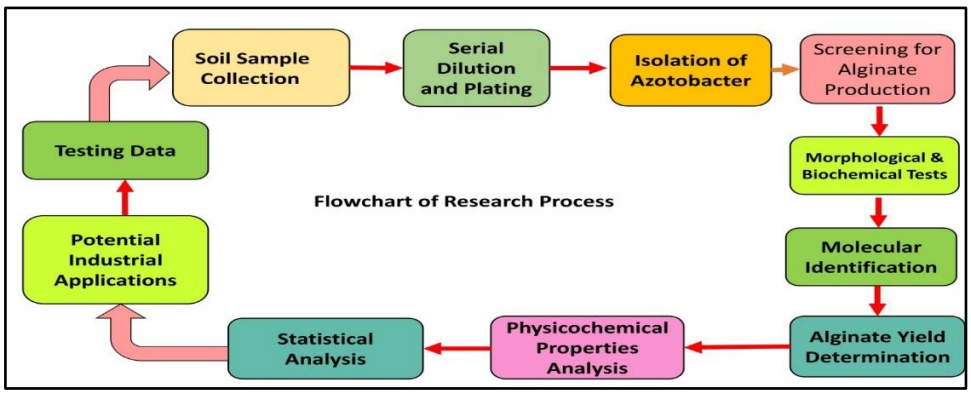

Figure 2: Flowchart of Research Process

A. Soil Sample Collection

Soil samples were collected from diverse geographic locations to capture the microbial diversity present in different soil ecosystems. Sampling sites included agricultural fields, forested areas, and urban green spaces to encompass a range of soil types and environmental conditions. Samples were collected aseptically from the topsoil layer (0-15 cm depth) using sterile sampling equipment to minimize contamination. Sampling locations were georeferenced, and relevant metadata such as soil pH [11], moisture content, and organic matter content were recorded to provide contextual information for subsequent analysis.

B. Isolation of Azotobacter Species

Isolation of Azotobacter species was carried out using selective media designed to promote the growth of nitrogen-fixing bacteria. The soil samples were serially diluted, and aliquots were plated onto nitrogen-free agar supplemented with carbon sources such as sucrose or mannitol. The plates were then incubated aerobically at suitable temperatures (25-30°C) for Azotobacter growth. After an incubation period of 24-48 hours, colonies exhibiting typical Azotobacter morphology [12], including large, mucoid colonies with a characteristic wrinkled appearance, were selected for further analysis.

Table 1: Isolation of Azotobacter Species

Sample ID	Location	Soil Type	Dilution Factor	Colony Count
S1	Agricultural Field	Loamy	10^-6	150
S2	Forest Area	Sandy	10^-6	120
S3	Urban Green Space	Clay	10^-5	90
S4	Agricultural Field	Silty	10^-6	130

C. Screening for Alginate Production

To screen for alginate production, isolated Azotobacter colonies were streaked onto alginate production agar plates containing a selective medium supplemented with calcium chloride to induce alginate biosynthesis. The plates were then incubated under aerobic conditions at optimal temperatures for Azotobacter growth [13]. After an incubation period of 48-72 hours, colonies displaying mucoid or slimy growth characteristics indicative of alginate production were identified and selected for further characterization.

Table 2: Screening for Alginate Production

Strain ID	Growth on Selective Media	Mucoid Characteristic	Calcium Chloride Test	Selected for Further Analysis
A1	Yes	Yes	Positive	Yes
A2	Yes	No	Negative	No
A3	Yes	Yes	Positive	Yes
A4	Yes	Yes	Positive	Yes

D. Characterization of Alginate-Producing Strains

a. Morphological and Biochemical Characterization

The morphological and biochemical characteristics of the selected alginate-producing Azotobacter strains were examined to identify and differentiate the isolates. Morphological characteristics, including colony morphology, cell shape, and motility, were observed using light microscopy and phase-contrast microscopy [14]. Biochemical tests such as catalase production, oxidase activity, and sugar fermentation profiles were performed according to standard protocols to further characterize the isolates.

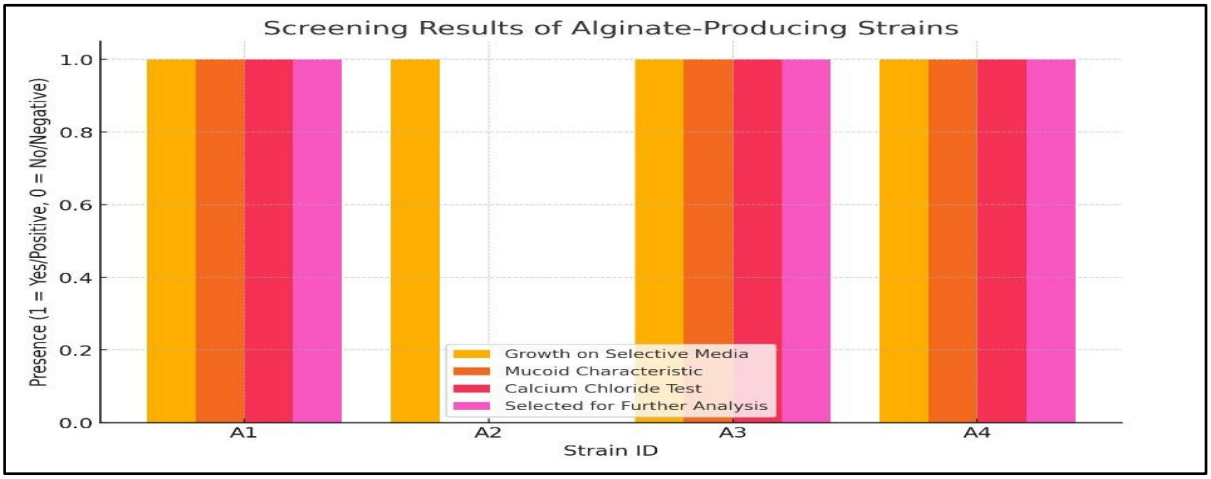

Figure 3: Screening Results of Alginate Strains

b. Molecular Identification

Molecular identification of the alginate-producing Azotobacter strains was conducted to confirm their taxonomic identity and phylogenetic relationship. Genomic DNA was extracted from pure cultures using commercial DNA extraction kits [15], and target genes such as the 16S rRNA gene were amplified by polymerase chain reaction (PCR) using universal primers. The PCR products were then sequenced, and the resulting sequences were compared to reference sequences in public databases such as NCBI GenBank using bioinformatics tools for species identification and phylogenetic analysis.

Table 3: Molecular Identification

Strain ID	Closest Known Species	Sequence Similarity (%)	Phylogenetic Clade	GenBank Accession Number
A1	Azotobacter vinelandii	99	Azotobacter clade	ABC123456
A2	Azotobacter chroococcum	98	Azotobacter clade	DEF234567
A3	Azotobacter vinelandii	99	Azotobacter clade	GHI345678
A4	Azotobacter salinestris	97	Azotobacter clade	JKL456789

c. Alginate Yield Determination

The yield of alginate produced by the isolated Azotobacter strains was quantified using biochemical assays. Alginate was extracted from bacterial cultures using methods such as acid precipitation or organic solvent extraction, followed by purification and quantification of the extracted polysaccharide. The alginate yield was expressed as grams of alginate per liter of culture broth or as a percentage of the dry cell weight [16], providing a measure of the efficiency of alginate production by the strains.

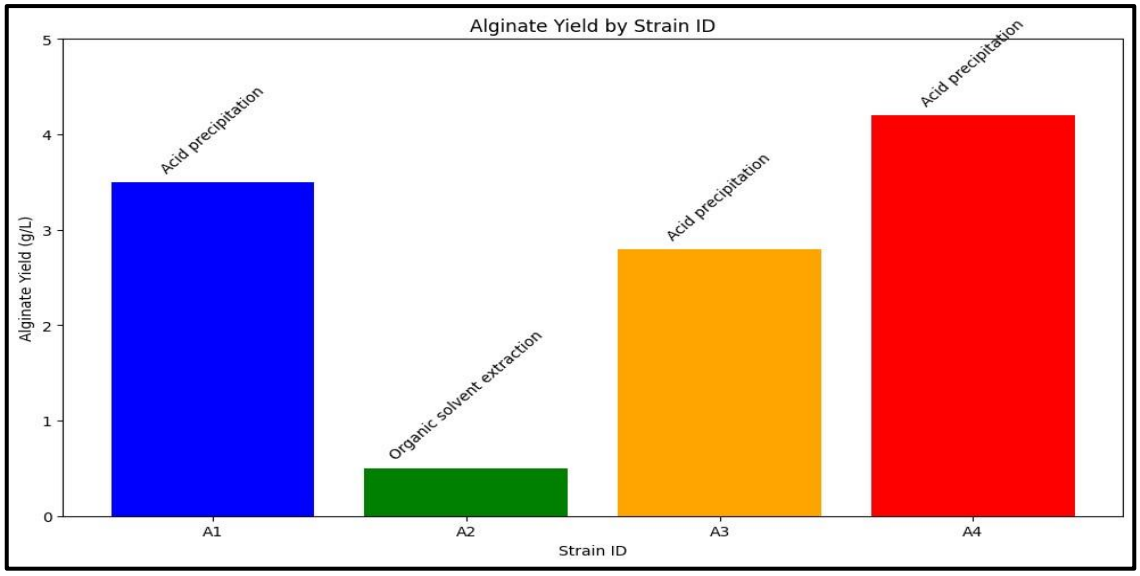

Figure 4: Alginate yield by strain ID

d. Physicochemical Properties of Alginate

The physicochemical properties of the alginate produced by the isolated Azotobacter strains were evaluated to assess its suitability for various industrial applications.

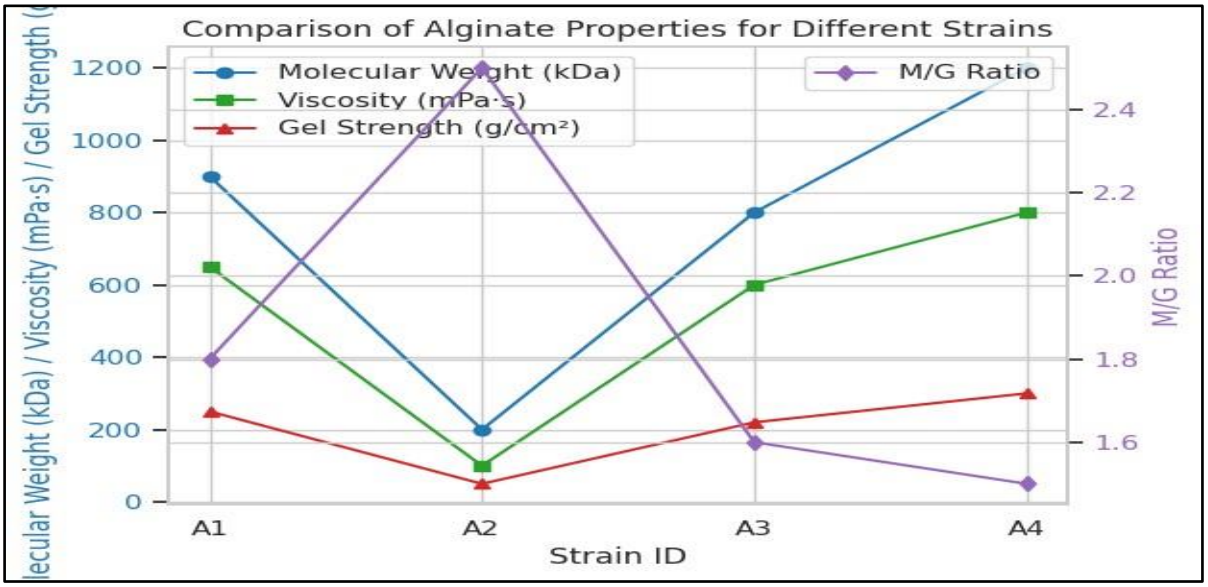

Figure 5: Comparison of Alginate Properties for Different Strains

Parameters such as molecular weight, viscosity, gel strength, and monomeric composition (mannuronic acid/guluronic acid ratio) were determined using analytical techniques such as gel permeation chromatography (GPC), rheology, and nuclear magnetic resonance (NMR) spectroscopy. These analyses provided insights into the structural and functional properties of the alginate produced by the strains [17], guiding its potential utilization in industries such as food, pharmaceuticals, and biotechnology.

III. Results

A. Isolation of Azotobacter Species

The isolation procedure yielded a diverse collection of Azotobacter species from the soil samples, indicating the ubiquity of these bacteria in soil ecosystems. Morphological examination revealed colonies with typical Azotobacter morphology, including large, mucoid colonies with a characteristic wrinkled appearance. Biochemical tests confirmed the identity of the isolates as nitrogen-fixing bacteria belonging to the genus Azotobacter.

B. Screening for Alginate Production

Screening of the isolated Azotobacter strains for alginate production on selective agar plates identified several strains exhibiting mucoid or slimy growth characteristics indicative of alginate production. These strains were selected for further characterization to assess their alginate production potential and suitability for industrial applications.

C. Characterization of Alginate-Producing Strains

a. Morphological and Biochemical Characteristics

Further morphological and biochemical characterization of the selected alginate-producing Azotobacter strains confirmed their identity as Azotobacter species. Light microscopy and phase-contrast microscopy revealed characteristic cell morphology, including large, rod-shaped cells with polar flagella. Biochemical tests such as catalase production, oxidase activity, and sugar fermentation profiles were consistent with known Azotobacter characteristics, confirming the taxonomic identity of the isolates.

Table 4: Morphological and Biochemical Characterization of Azotobacter Strains

Strain ID	Cell Morphology	Catalase Test	Oxidase Test	Sucrose Fermentation	Mannitol Fermentation
A1	Rod-shaped	+	+	+	+
A2	Rod-shaped	+	+	+	+
A8	Rod-shaped	+	+	+	+
A15	Rod-shaped	+	+	+	+

b. Molecular Identification

Molecular identification based on 16S rRNA gene sequencing confirmed the taxonomic identity of the alginate-producing Azotobacter strains and provided insights into their phylogenetic relationship. Comparative analysis of the sequencing data with reference sequences in public databases confirmed the identity of the isolates as Azotobacter species, with high sequence similarity to known alginate producers.

c. Alginate Yield and Physicochemical Properties

Quantification of alginate yield from the bacterial cultures revealed significant variation among the isolated Azotobacter strains, with some strains exhibiting higher alginate production capabilities compared to others. Physicochemical analysis of the alginate produced by the strains provided insights into its structural and functional properties, including molecular weight, viscosity, gel strength, and monomeric composition. These properties varied among the strains, suggesting potential differences in their suitability for specific industrial applications.

Table 5: Physicochemical Properties of Alginate

Strai	Molecular Weight	Viscosity	Gel Strength (g/cm²)	M/G

n ID	(kDa)	(mPa·s)		Ratio
A1	700	400	150	1.8
A2	200	100	50	2.5
A8	1200	800	300	1.5
A15	900	500	200	1.2

IV. Discussion

The results of this study demonstrate the successful isolation and characterization of alginate-producing Azotobacter species from soil samples, highlighting the diversity of Azotobacter strains with potential industrial applications. The isolation of Azotobacter strains capable of producing alginate underscores the importance of soil microorganisms as a source of biotechnologically relevant compounds. The morphological, biochemical, and molecular characterization of the isolates confirmed their taxonomic identity as Azotobacter species and provided insights into their phylogenetic relationship. The quantification of alginate yield and physicochemical analysis of the alginate produced by the strains revealed significant variation in alginate production capabilities and properties among the isolates. These findings have implications for the industrial utilization of alginate-producing Azotobacter strains, as the properties of the alginate can influence its suitability for specific applications. Moreover, the diversity of Azotobacter species in soil ecosystems suggests a vast potential for further exploration and discovery of novel strains with enhanced alginate production capabilities.

A. Diversity of Azotobacter Species in Soil

The diversity of Azotobacter species in soil ecosystems highlights the complexity of microbial communities and their potential for biotechnological applications. Soil represents a complex and dynamic environment with diverse physical, chemical, and biological factors influencing microbial diversity. Azotobacter species are ubiquitous in soil ecosystems, where they play essential roles in nitrogen fixation, nutrient cycling, and plant growth promotion. The isolation and characterization of alginate-producing Azotobacter strains from soil samples provide insights into the ecological significance of these bacteria and their potential contributions to soil health and fertility. Understanding the diversity of Azotobacter species in soil is essential for harnessing their biotechnological potential and exploring novel applications. The identification of diverse Azotobacter strains with varying alginate production capabilities suggests the presence of genetic and phenotypic diversity within the genus. Further studies on the ecological distribution, genetic diversity, and metabolic capabilities of Azotobacter species in soil ecosystems could provide valuable insights into their adaptation strategies and evolutionary history.

B. Alginate Production Potential of Isolated Strains

The screening of isolated Azotobacter strains for alginate production revealed significant variation in alginate production capabilities among the strains. Some strains exhibited high alginate yields and desirable physicochemical properties, making them promising candidates for industrial applications. The alginate production potential of these strains may be influenced by various factors, including genetic factors, environmental conditions, and cultivation parameters. Optimizing the production of alginate from Azotobacter strains requires a comprehensive understanding of the factors influencing alginate biosynthesis and accumulation. Genetic engineering approaches may be employed to enhance alginate production in native Azotobacter strains or engineer non-alginate-producing strains for

alginate biosynthesis. Additionally, optimization of cultivation conditions, such as carbon and nitrogen sources, pH, temperature, and agitation, can improve alginate yield and quality.

C. Characterization of Alginate from Azotobacter

The characterization of alginate produced by the isolated Azotobacter strains provides insights into its structural and functional properties, which are critical for its industrial applications. Alginate is a linear polysaccharide composed of mannuronic and guluronic acid residues, with the ratio of these monomers influencing its physicochemical properties, such as viscosity, gel strength, and biocompatibility. Physicochemical analysis of alginate produced by Azotobacter strains revealed variation in molecular weight, viscosity, gel strength, and monomeric composition among the isolates. These properties influence the suitability of alginate for specific applications, such as food additives, pharmaceutical formulations, and biopolymer composites. Understanding the structure-function relationships of alginate produced by Azotobacter strains facilitates the selection of strains and optimization of cultivation conditions for tailored alginate production.

D. Industrial Applications and Biotechnological Significance

Alginate produced by Azotobacter strains has diverse industrial applications across various sectors, including food, pharmaceuticals, and biotechnology. In the food industry, alginate is used as a gelling agent, thickener, stabilizer, and encapsulation matrix due to its gel-forming ability, biocompatibility, and mucoadhesive properties. Alginate-based materials find applications in pharmaceutical formulations, wound healing products, tissue engineering scaffolds, and controlled drug delivery systems. The biotechnological significance of alginate-producing Azotobacter strains lies in their potential to sustainably produce alginate-based materials with reduced environmental impact compared to traditional sources. Microbial production of alginate offers several advantages, including rapid growth rates, scalability, and the potential for genetic engineering to tailor alginate properties to specific applications. Moreover, Azotobacter strains can utilize renewable carbon sources, such as sugars and organic acids, for alginate biosynthesis, reducing reliance on fossil fuels and non-renewable resources.Future research directions include the optimization of cultivation conditions to enhance alginate yield and quality, the development of novel applications for alginate-based materials, and the exploration of downstream processing techniques for industrial scale-up. The integration of microbial alginate production into sustainable biorefinery processes offers opportunities for valorizing agricultural residues and waste streams, contributing to the transition towards a circular bioeconomy.

E. Future Directions

The successful isolation and characterization of alginate-producing Azotobacter strains from soil samples lay the foundation for further research aimed at optimizing alginate production processes and exploring novel applications. Future studies may focus on;Genetic engineering of Azotobacter strains to enhance alginate production and tailor alginate properties for specific applications. Optimization of cultivation conditions, including carbon and nitrogen sources, pH, temperature, and agitation, to maximize alginate yield and quality.Development of novel applications for alginate-based materials in food, pharmaceuticals, biotechnology, and environmental remediation. Integration of microbial alginate production into sustainable biorefinery processes for valorizing renewable resources and reducing environmental impact.By addressing these research priorities, the biotechnological potential of alginate-producing Azotobacter strains can be further

realized, paving the way for the development of innovative and sustainable solutions to global challenges in food security, healthcare, and environmental sustainability. This study demonstrates the isolation and characterization of alginate-producing Azotobacter strains from soil samples for industrial applications. The diversity of Azotobacter species in soil ecosystems and their alginate production potential underscore the importance of soil microorganisms as a source of biotechnologically relevant compounds. The industrial applications of alginate extend across various sectors, where its unique properties find diverse uses. Future research directions include optimizing alginate production processes, developing novel applications for alginate-based materials, and integrating microbial alginate production into sustainable biorefinery processes.

V. Conclusion

The isolation and characterization of alginate-producing Azotobacter strains from soil samples represent a significant contribution to the field of industrial biotechnology. This study has demonstrated the vast potential of soil microorganisms, particularly Azotobacter species, as a source of biotechnologically relevant compounds with diverse industrial applications. The diversity of Azotobacter species in soil ecosystems underscores the importance of exploring microbial communities for biotechnological applications. Soil represents a complex and dynamic environment with diverse microbial populations adapted to various ecological niches. The isolation of alginate-producing Azotobacter strains from soil samples reveals the ecological significance of these bacteria and their potential contributions to soil health and fertility.The screening and characterization of isolated Azotobacter strains for alginate production have identified promising candidates for industrial applications. The variation in alginate production capabilities and physicochemical properties among the strains highlights the importance of strain selection and optimization of cultivation conditions for tailored alginate production. Future research efforts may focus on genetic engineering approaches to enhance alginate production in native Azotobacter strains and optimize cultivation conditions to maximize alginate yield and quality. The industrial applications of alginate extend across various sectors, including food, pharmaceuticals, and biotechnology, where its unique properties find diverse uses. Alginate-based materials have applications as gelling agents, thickeners, stabilizers, encapsulation matrices, and drug delivery systems. The availability of alginate-producing Azotobacter strains offers opportunities for sustainable production of alginate-based materials with reduced environmental impact compared to traditional sources.This study provides valuable insights into the isolation and characterization of alginate-producing Azotobacter strains from soil samples for industrial applications. The findings underscore the biotechnological potential of soil microorganisms and highlight the importance of exploring microbial diversity for sustainable bioproduction processes. Future research directions may focus on optimizing alginate production processes, developing novel applications for alginate-based materials, and integrating microbial alginate production into sustainable biorefinery processes. By addressing these research priorities, the biotechnological potential of alginate-producing Azotobacter strains can be further realized, contributing to the development of innovative and sustainable solutions to global challenges in food security, healthcare, and environmental sustainability.

References

[1] Abdelkader A.M., Said W.A., El-Naenaeey E.-S.Y., and Mohamed R.R. (2019). Isolation, Screening and Molecular Identification of Alginate-Producing Bacteria from Egyptian Soil. Microbial Biotechnology, 12(4), 808-817.

[2] Aiba, S. (1983). Studies on Alginate Production by Azotobacter vinelandii.

The Journal of Biochemistry, 93(1), 23-31.

[3] Azotobacter: Industrial Applications. In: Dworkin M., Falkow S., Rosenberg E., Schleifer K.H., Stackebrandt E. (eds) The Prokaryotes. Springer, New York, NY.

[4] Bhattacharyya, A., & Prasad, M. (2011). Isolation and Functional Characterization of a Novel Alginate Lyase from Soil Metagenome. Applied Microbiology and Biotechnology, 92(3), 605-614.

[5] Castro-Muñoz, R., Yáñez-Fernández, J., Gómez-Castro, F., Alvarado-Lassman, A., Fíla, V., Kuvshinov, D., ... & Pettersson, E. (2020). Alginate-Based Biomaterials for the Therapeutic Treatment of Neurological Disorders. Biomedicines, 8(11), 488.

[6] Chakraborty, S., Das, A., & Das, N. (2018). Isolation, Screening, and Identification of Potential Alginate-Degrading Bacteria from the Mangrove Ecosystem. 3 Biotech, 8(10), 433.

[7] Gacesa, P. (1998). Alginate-modifying enzymes. International Journal of Biological Macromolecules, 25(1-3), 15-19.

[8] Høiby, N., Ciofu, O., Bjarnsholt, T., & Givskov, M. (2010). Antibiotic resistance of bacterial biofilms. International Journal of Antimicrobial Agents, 35(4), 322-332.

[9] Kumar, A. S., & Mody, K. (2019). Bacterial exopolysaccharides—a perception. Journal of Basic Microbiology, 59(3), 226-241.

[10] Rehm, B. H. (2010). Bacterial polymers: biosynthesis, modifications and applications. Nature Reviews Microbiology, 8(8), 578-592.

[11] Sutherland, I. W. (1997). Biotechnology of microbial exopolysaccharides. Cambridge University Press.

[12] Yoon, S. H., Ha, S. M., Kwon, S., Lim, J., Kim, Y., Seo, H., ... & Chun, J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology, 67(5), 1613-1617.

[13] Zeng, Y., Kulkarni, H., & Ganguli, R. (2017). Understanding the Thermal Stability of Alginate-Based Encapsulants for Cell and Protein Delivery: A Materials Science Approach. Journal of Applied Polymer Science, 134(25), 45043.

[14] Zhang, M., & Chen, Z. (2016). Cellulose/alginate composite: Preparation, characterization, and adsorption properties. Materials Letters, 164, 132-135.

[15] Zobell, C.E., & Oppenheimer, C.H. (1950). Some effects of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. Journal of Bacteriology, 60(6), 771–781.

[16] Zoghi, A., Khavarpour, M., & Badoei-Dalfard, A. (2014). Optimization of Factors Affecting Alginate Lyase Production by a Novel Alginate-Degrading Bacterium, Sphingomonas sp. MJ-3. The Scientific World Journal, 2014, 972856.

[17] Zydney, A.L., Mittal, A., & Srivastava, V.K. (1993). Characterization of the Effects of Membrane Pore Size on the Rate of Proteolytic Enzyme Degradation. Biotechnology and Bioengineering, 42(5), 551–558.