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Exploring marine algae for the systematic isolation of alginate lyase producers

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Abstract

Alginate, a complex polysaccharide predominantly found in seaweeds, is degraded into alginate oligosaccharides by the enzyme alginate lyase, an enzyme of high interest for its diverse applications in medicine and industry. In this study, our primary focus was to isolate microorganisms with robust alginate lyase-producing capabilities from marine algae. Through systematic screening methodologies and 16S rRNA gene sequencing, the isolate was taxonomically classified as belonging to the *Paenibacillus* genus and has been archived in gene bank with the accession number OM980630. A comprehensive optimization process was executed, testing 13 different growth media to ascertain the most conducive environment for both bacterial proliferation and enzyme activity. It was determined that Medium M-7 yielded the most promising results. Furthermore, the presence of alginate in the culture medium not only acted as a carbon source but also as an inducer for alginate lyase production. Recognizing the crucial role of alginate lyase in medical treatments, especially in managing conditions like cystic fibrosis, and its importance in industrial processes, specifically for generating bioactive oligosaccharides with applications including antitumor, antidiabetic, antihypertensive, anti-inflammatory, antimicrobial, antioxidant, anticancer, immunomodulatory, and anti-radiation activities, the identification of a new microbial source for alginate lyase production could represent a significant advancement in both healthcare and industry.

Keywords: Alginate lyase; *Paenibacillus* sp; 16S rRNA sequencing; Cystic fibrosis; Bioactive oligosaccharides

1. Introduction

Alginate, a natural polysaccharide extracted from brown algae, was first discovered by English chemist E.C.C. Stanford in 1883 [1]. While working on improving iodine dietary needs from seaweeds growing along the Scottish coast, Stanford isolated a mucilaginous substance called "algin" that displayed colloidal properties and could form a viscous solution when combined with salts such as sodium and potassium. Alginate is a widespread polysaccharide composed of two isomer residues, β - D -mannuronic acid (M) and α - L -guluronic acid (G), linked by 1→4 glycosidic bonds [2]. These bonds result in the formation of three different blocks in alginate: polyM blocks, polyG blocks, and polyMG. While some alginates may primarily consist of one type of block, others may contain all three types in a single molecule. Alginate is found in high quantities in brown algae, comprising 30-60% of the dry cell weight [2]. It is also present in some red algae and can be produced by certain bacteria, such as *Pseudomonas aeruginosa* and *Azotobacter vinelandii* [3,4]. Commercial alginates are typically extracted from marine macroalgae, including, *Macrocystis pyrifera*, *Laminaria hyperborean, Ascophyllum nodosum*, *Durvillea antarctica*, *Laminaria digitate,* and *Sargassum* spp.

Alginate extracted from brown algae, has a wide range of applications across several industries. In the food industry, it is used as a thickening, stabilizing, and gelling agent. In the pharmaceutical industry, it is used in the production of tablets, capsules, and drug delivery systems. In the biomedical field, it has found use in wound dressings, drug delivery systems, and tissue engineering due to its biocompatibility and ability to form gels. Alginate also offers solutions for

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heavy metal removal in water treatment and finds applications within the cosmetics, textile, and paper industries [5]. Alginate lyases are enzyme that facilitates alginate hydrolysis by removing the glycosidic link. Alginate lyase has been found in a wide range of organisms with varying substrate preferences, including algae, marine mollusks, marine and terrestrial bacteria, as well as some viruses and fungi [6,7]. As structural biology has advanced, numerous distinct polysaccharide lyases families' alginate lyases have been crystallographic ally described in order to understand their catalytic mechanisms.

Alginate is processed into an oligosaccharide by the enzyme alginate lyase, and this oligosaccharide has a wide range of possible uses. According to Gao *et al*, (2017) [8], traditional preparation techniques for the synthesis of AOS typically take place in very acidic and alkaline environments, causing serious environmental harm. The production of AOs using enzymes is more environmentally friendly and sustainable. Due to their unsaturated oligosaccharide architectures, AOS made using enzymatic degradation methods displayed unique bioactivities [9,10]. Marine bacterial-produced alginate lyases have been the focus of much laboratory research, but only one of these enzymes is currently available for commercial use (Sigma-Aldrich, 2021). This alginate lyase, sold by Sigma-Aldrich, is known for its high pH tolerance, high catalytic activity, and excellent heat stability, although it is quite expensive and only available in the form of a reagent.

Alginate oligosaccharides (AOs) have a variety of biological features that can help improve human health. Their bioactivities, including antitumor [11], antidiabetic [12], antihypertensive [13], anti-inflammatory [14,15], antimicrobial [16], antioxidant [17], anticancer [18], immunomodulatory [19,20] and anti-radiation [21,22] properties.

Alginate lyase also has antibiotic activity since some pathogenic bacteria have complex biofilms on their surfaces, making it difficult for conventional antibiotics to eradicate them. The ability of alginate components in *Pseudomonas aeruginosa* biofilm to hinder immune system clearance and resistance to antibiotic treatment was revealed [23,24]. Therefore, a potential therapeutic strategy for treating *Pseudomonas aeruginosa* infections is to use a purified alginate lyase-antibiotic complex [25,26]. A purified alginate lyase (AlyP1400) from a marine *Pseudoalteromonas sp.* bacterium recently showed the ability to disrupt *Pseudomonas aeruginosa* biofilm formation by decomposing alginate within the extracellular polysaccharide matrix and thereby enhancing the bactericidal activity of tobramycin, which may act as a promising approach for combination therapy [26]. Additionally, the alginate lyases are used as a potential tool for producing bioethanol, disposing of seaweed waste, and understanding the structure of alginate.

Given the complexity and breadth of issues related to alginate degradation, ranging from ecological sustainability to healthcare advancements, this study aims to isolate microbial strains proficient in alginate disassembly via enzymatic catalysis, through the action of alginate lyase. This preliminary phase of targeted microbial identification serves as the cornerstone for an extensive array of future interdisciplinary investigations, encompassing realms such as eco-friendly alginate management, breakthrough therapeutic applications, and innovative industrial processes. The successful delineation of such microorganisms offers a pivotal platform for future research, thereby paving the way for the development of sustainable, clinically relevant, and cost-efficient alginate utilization strategies.

2. Material and methods

2.1. Chemicals

The research used chemicals of an analytical grade, obtained from Merck and Hi-Media, India. All utilized media components were of a bacteriological standard.

2.2. Sample collection, isolation, screening, and selection of isolate

Marine macroalgae specimens including *Sargassum, Colpomenia, Iyengaria, Padina, Cystoseria*, and *Spatoglossum* were procured from Agar Supreme, a premier alginate production industry in TamilNadu. These algae samples were meticulously prepped and segmented into 2x2 cm squares, serving as the foundation for intricate alginate enrichment experiments. A precise mass of 10g from each sample was submerged in a sterile flask containing 100 mL of a 1% (w/v) sodium alginate solution, followed by an incubation process under an optimized temperature of 30°C for a duration of one month, ensuring alginate yield was maximized. Sterile 1% (w/v) sodium alginate solution was aseptically replenished weekly. Post-incubation, aliquots were serially diluted and streaked on sodium alginate-infused agar plates, with a precise pH of 7.5 and formulation containing 0.4% sodium alginate, 2.0% Luria broth powder, and 2.0% agar, under 150 rpm agitation. Pure cultures were differentiated and isolated based on morphology, followed by a secondary 48-hour incubation and cetylpyridinium chloride treatment to reveal hydrolysis zones indicative of positive isolates. These were further evaluated via microscopic analysis and Gram's staining. Among the different isolates screened, the

most potent isolate showing maximum hydrolysis zone was cultured in the aforementioned liquid medium, and its supernatant post-centrifugation served as the crude enzyme source. Alginate lyase activity was spectrophotometrically examined, identifying SG4 as the eminent alginate lyase producer among all isolates.

2.3. Decoding the genetic identity of isolate SG4

The selected isolated SG4 was sent to Biologia Research Pvt. Ltd., India, for 16S rRNA identification. According to the results of the 16S rRNA nucleotide sequence analysis, the bacterium was identified as *Paenibacillus* sp OM980630.

2.4. Methodology for sustaining bacterial growth and viability

Paenibacillus sp. OM980630 was cultured on pH 7.5 LB agar plates, consisting of 2.0% LB powder, 0.4% Sodium Alginate, and 2.0% Agar. Following autoclaving of medium at 121°C and 15 lb/inch2 for 15 minutes, it was dispensed into petri dishes. After verifying sterility via a 24-hour incubation at 30°C, the strain was inoculated and incubated for an additional 48 hours. Regular sub-culturing was executed every third day and a glycerol stock was formulated for preservation.

2.5. Cell mass measurement

Paenibacillus sp. OM980630's cells were extracted via centrifugation of the broth at 10,000 g for 15 min in a 4°C refrigerated centrifuge. A quantified fraction of the retrieved wet cell pellet was subjected to desiccation in an 80°C oven overnight. Subsequently, the cell slurry's absorbance was quantified at 600 nm utilizing a LABINDIA spectrophotometer. Known dried cell weights were correlated with their optical densities and plotted on a standard graph versus A_{600}.The resulting cell mass is reported as milligrams per milliliter (mg/mL) of the original culture volume, representing the dry cell weight.

2.6. Experimental procedure to quantify alginate lyase activity via DNSA methodology

To elucidate the enzymatic breakdown of alginate by alginate lyase, we employed a 3, 5-Dinitrosalicylic acid (DNSA) colorimetric assay, established methodology for quantifying reducing sugar formation. DNSA, upon reaction with reducing sugars, undergoes a colorimetric change which can be spectroscopically quantified. The assay was carried out under strictly controlled conditions in a light-insensitive environment given DNSA's photosensitive nature.

2.6.1. Reagents used:

- **DNSA Reagent:** This light-sensitive solution comprised of 1.0% (w/v) DNSA, 1.0% (w/v) NaOH, 20.0% (w/v) Potassium sodium tartrate, 0.20% (w/v) Phenol, and 0.05% (w/v) Sodium sulphite. To minimize light-induced degradation, it was stored in a brown bottle enveloped by aluminum foil at ambient temperature.
- **Alginate**: A 1% sodium alginate solution was formulated and stored at 4°C for preservation.
- **Tris-HCL Buffer:** A 0.05M solution was prepared with pH adjusted to 8.5.

2.7. Alginate lyase assay

Enzyme activity was quantified in International Units (IU), wherein one IU of alginate lyase activity is characterized as the quantity of enzyme required to liberate 1 µmol of reducing sugar (4-deoxy-L-erythro-5-hexoseulose uronic acid) per minute per milliliter at 30°C in a 0.05 M tris buffer (pH 8.5) in the presence of 1.0% (w/v) alginate solution.

2.8. Evaluation of the oligosaccharide forming abilities of the alginate lyase of *Paenibacillus* sp. OM980630

The liberated sugars were subjected to TLC on silica plates, utilizing a mobile phase of 1-butanol, acetic acid, and water (4:6:1, v:v:v), and a 5 µL sample of the reaction mixture. Following a spray with 10% sulphuric acid in ethanol, the enzymatic products were visualized via heating at 110°C for 5 minutes.

2.9. Assessment of optimal medium for alginate lyase production by *Paenibacillus* sp. OM980630

Twelve reported media (Table 1) were evaluated for their potential in promoting alginate lyase production by *Paenibacillus* sp. OM980630. All media were pH-adjusted to 7.5 and inoculated with a 24-hour old 10% (v/v) inoculum prepared in M-7 medium. The flasks were then incubated at 30°C for 48 hours in an orbital shaker under constant agitation (150 rpm).

Table 1 Composition of media used for the production of alginate lyase by *Paenibacillus* SP. OM980630

Media No.	Composition (%, w/v)	Reference
M-1	Sodium alginate 0.5, Peptone 0.1, Yeast extract 0.5, NaCl 0.5	[27]
M-2	Sodium alginate 0.5, NaCl 0.5, Peptone 0.5, $MgSO_4$ 0.1, Yeast extract 0.1, KH_2PO_4 0.1	[27]
M-3	Yeast extract 0.05, Na_2HPO_4 0.4, KH_2PO_4 0.1, $(NH_4)_2SO_4$ 0.1, $MgSO_4.7H_2O$ 0.05, Sodium alginate 0.4	[28]
M-4	Peptone 0.5, Yeast extract 0.1, Sodium alginate 0.5, NaCl 3.0	[29]
M-5	NaCl 3.0, KCl 0.2, Yeast extract 0.01, Peptone 0.05, $MgSO_4.7H_2O$ 0.01, Sodium alginate 2.0	[30]
M-6	NH_4NO_3 1.0, K_2HPO_4 1.5, NaH_2PO_4 0.5, $MgSO_4.7H_2O$ 0.1, Sodium alginate 0.1	[31]
M-7	Yeast extract 0.5, Polypeptone 0.5, NaCl 2.0, $MgSO_4.7H_2O$ 0.02, K_2HPO_4 0.01, $MnCl_2$ .002, Sodium alginate 0.2	[32]
M-8	Yeast extract 0.03, Peptone 0.05, $(NH_4)_2SO_4$ 0.2, KH_2PO_4 0.1, $MgSO_4.7H_2O$ 0.05, Sodium alginate 0.2	[33]
M-9	Sodium alginate 0.5, Tryptone 1.3, KH_2PO_4 0.2, $CaCl_2$ 0.01, $FePO_4$ 0.001, $FeSO_4$ 0.001, $MgSO_4.7H_2O$ 0.05, NaCl 3.0	[34]
M-10	K_2HPO_4 0.7, KH_2PO_4 0.2, $(NH_4)_2SO_4$ 0.1, Sodium citrate 0.05, Sodium alginate 0.2	[35]
M-11	Polypeptone 0.05, Yeast extract 0.03, $(NH_4)_2SO_4$ 0.2, KH_2PO_4 0.1, $MgSO_4.7H_2O$ 0.05	[36]
M-12	$(NH_4)_2SO_4$ 0.12, NaCl 2.4, KH_2PO_4 0.05, K_2HPO_4 0.1, KCl 0.03, $FeCl_2.4H_2O$ 0.001, $MgSO_4.7H_2O$ 0.05, $CaCl_2.2H_2O$ 0.02, Glucose 0.03, Sodium alginate 0.03	[37]

2.10. Evaluation of inducer's role in seed and production medium for alginate lyase production by *Paenibacillus* sp. OM 980630

The impact of the inducer, alginate, at a concentration of 0.2% (w/v), was scrutinized within both the seed and the production medium (pH 7.5) regarding the production of alginate lyase. This evaluation involved the creation of four distinct combinations of seed and production medium (M-7), with the presence or absence of alginate. The *Paenibacillus* sp. OM980630 cultures, under varying media conditions, were then incubated for a 48-hour period at 30°C in an orbital shaker maintaining a steady speed of 150 rpm

3. Results

3.1. Isolation, screening and selection of alginate lyase producing microorganisms

Seaweed specimens encompassing *Sargassum, Colpomenia, Iyengaria, Padina, Cystoseria,* and *Spatoglossum* were procured as sources for the potential extraction of alginate lyase-producing organisms. These samples were subjected to an enrichment phase lasting one month at 30°C in physiological saline that was supplemented with 1% sodium alginate. Thereafter, serial dilutions were carried out weekly, reducing the concentration to a factor of 10^8, using sterile physiological saline.

Aliquots of 100 µL from various dilutions were spread onto agar plates having a pH of 7.5. These plates were prepared with 2.0% (w/v) Luria broth powder, 0.4% (w/v) sodium alginate, and 2.0% agar. After two days of incubation at 30°C, 31 isolates, as enumerated in Table 2, emerged. These isolates exhibited diverse capacities for alginate degradation in a semi-solid medium, as demonstrated by the CPC method at different dilutions. This is depicted in Fig.1a. The isolates were further subcultured multiple times on semi-solid media to procure refined bacterial colonies, which were then subjected to an additional round of alginate digestion using CPC method (Fig. 1b). To confirm the previous findings, each isolate was cultured under submerged conditions in a medium with pH 7.4, containing 2.0% (w/v) Luria broth powder and 0.4% (w/v) sodium alginate, with alginate lyase activity quantified using the DNSA method.

Among the cohort, the SG4 isolate demonstrated optimal performance in terms of growth (1.94 ±0.097 mg/mL). and alginate activity (0.21±0.0105 IU) in both solid and liquid mediums, thus justifying its selection for subsequent investigations. Isolate SG4 was subjected to Gram staining in order to examine its morphology. Isolate SG4 was identified as a Gram-negative bacillus through Gram staining, as shown in Fig. 1c.

Table 2 Alginate lyase-producing microbial isolates: screening and selection through enzyme activity assessment

Sample	Name of sample	Zone of hydrolysis	Enzyme activity (IU)
Sargassum	SG-0	+	+
	SG-1	+	+
	SG-2	+ +	++
	SG-3	+ +	+ + + +
	SG-4	+ + + +	-
	SG-5	-	+ +
	SG-6	+ +	
Colpomenia	COL-1	+ +	+
	COL-2	-	-
	COL-3	+ +	+ +
	COL-4	-	-
	COL-5		
Iyengvaria	IGY-1	-	-
	IGY-2	-	-
	IGY-3	+ + +	+ +
	IGY-4	+ +	+ +
	IGY-5	-	-
	IGY-6	-	-
Padina	PAD-1	+ +	+
	PAD-2	+ +	+
	PAD-3	+	-
	PAD-4	+ + +	+ +
Cystoseria	CYS-0	+ +	+
	CYS-1	-	-
	CYS-2	+ + +	+ +
Spatoglossum	SPC-1	+ +	+
	SPC-2	-	-
	SPC-3	+ + +	+ +
	SPC-4	+	+
	SPC-5	+ +	+ +
	SPC-6	+ +	++

++++ Maximum, +++ moderate, ++low, +minimum, -negative

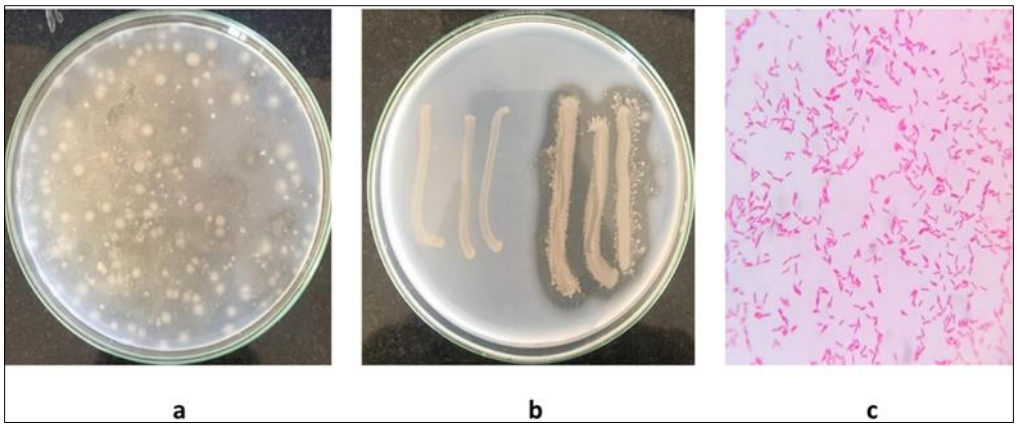

Figure 1 (a-c) Alginate-lyase producing microorganism, isolate SG4, screened via CPC method on alginate medium (a), evidenced by alginate hydrolysis (b), and further characterized by Gram staining (c)

3.2. Identification of the potent producer of alginate lyase

The selected isolated SG4 was sent to Biologia Research Pvt. Ltd.India for identification. According to the results of 16S rRNA nucleotide sequence analysis, the bacterium was identified as *Paenibacillus* sp OM980630 and a phylogenetic tree for knowing evolutionary relationship of taxa was constructed (Fig. 2). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite likelihood method and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 579 positions in the final dataset. Evolutionary analyses were conducted in MEGA11. The nucleotide sequence was deposited at NCBI GenBank under the accession number OM980630.

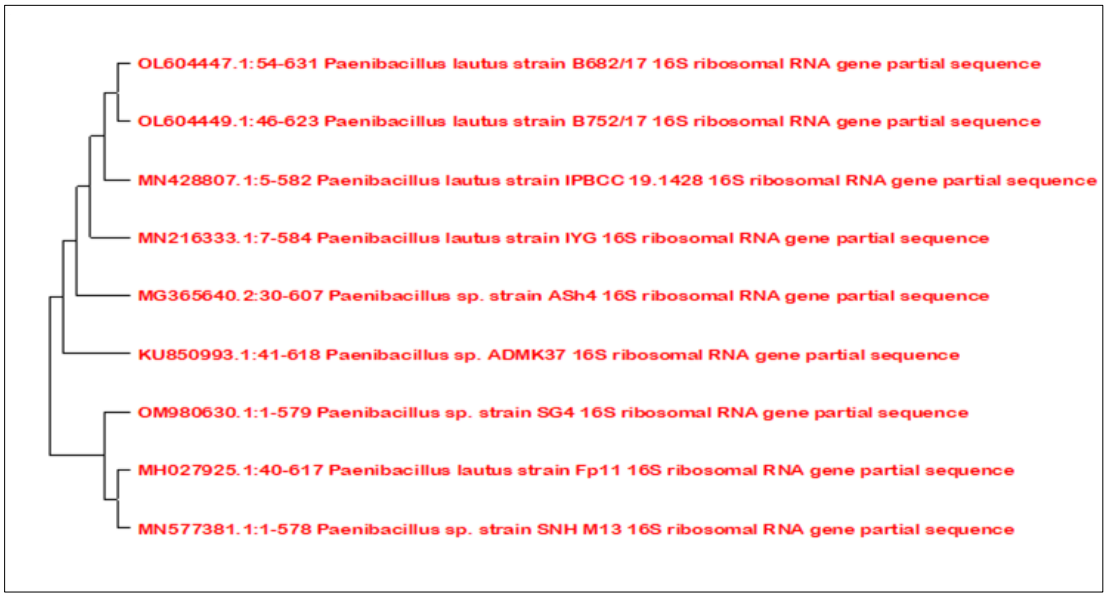

Figure 2 Evolutionary relationships of taxa

3.3. Assessment of capability of alginate lyase of *Paenibacillus* sp OM980630 for oligosaccharides formation

Alginate oligosaccharides, produced by alginate lyase from isolated *Paenibacillus* sp OM980630, was evaluated via Thin Layer Chromatography (TLC). This enables the identification of Alginate Oligosaccharides (AOS) of varying degrees of polymerization, indicative of alginate hydrolysis. The depiction of alginate depolymerization products via TLC is presented in Fig. 3.

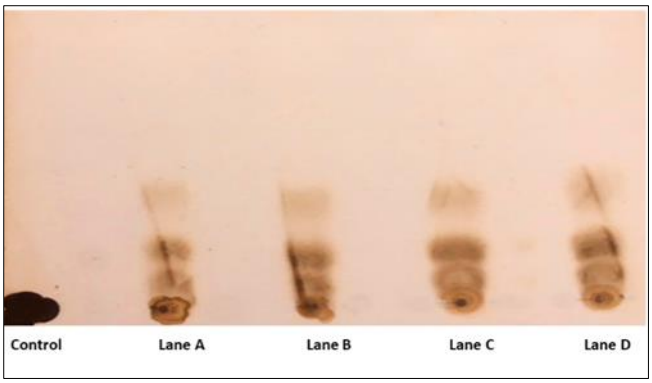

Figure 3 TLC of oligosaccharides released by the action of alginate lyase from *Paenibacillus* sp. OM980630 Lane A shows unhydrolysed alginate. Lane A to D represents the hydrolysate obtained from enzymatic reaction for 15, 20, 25 and 30 minutes respectively

3.4. Medium choice and inducer role in extracellular alginate lyase production from *Paenibacillus* sp. OM 980630

Paenibacillus sp. OM980630 was cultured in twelve unique media known for promoting alginate lyase production (Table 3). Among these, medium M-7 emerged as the most effective, achieving the highest alginate lyase activity (0.31±0.0155 IU) and a significant cell mass of 2.1±0.105mg/mL. Medium M-3 was the next best, whereas M-4 and M 9 led to the least cellular growth and enzyme production, respectively.

To investigate the effects of the inducer (alginate; 0.2%, w/v) on alginate lyase production by *Paenibacillus* sp. OM980630, four unique seed and production medium combinations were utilized (Table 4). The combination of alginate-positive seed and production medium demonstrated the most favourable results for both growth (0.218± 0.01mg/mL) and alginate lyase production (3.36 ±0.168 IU). Consequently, alginate was used as a carbon source and inducer in all subsequent experiments.

Table 3 Comparative analysis of growth media for improved alginate lyase production by *Paenibacillus* sp. OM980630

Medium No.	alginate lyase activity (IU)	Cell mass (mg/mL)	Final pH
M-1	0.069 ± 0.00345	1.58 ± 0.079	7.6 ± 0.38
M-2	0.071 ± 0.00355	1.48 ± 0.074	7.7 ± 0.385
M-3	0.25 ± 0.0125	1.80 ± 0.090	8.2 ± 0.41
M-4	0.065 ± 0.00325	0.66 ± 0.033	7.5 ± 0.375
M-5	0.073 ± 0.00365	1.39 ± 0.069	7.5 ± 0.375
M-6	0.19 ± 0.0095	1.22 ± 0.061	7.9 ± 0.395
M-7	0.31 ± 0.0155	2.1 ± 0.105	8.6 ± 0.43
M-8	0.041 ± 0.00205	1.13 ± 0.057	7.5 ± 0.375
M-9	0.042 ± 0.0021	1.07 ± 0.0535	7.34 ± 0.367
M-10	0.074 ± 0.0037	0.14 ± 0.007	8.2 ± 0.41
M-11	0.088 ± 0.0044	1.59 ± 0.0795	7.6 ± 0.38
M-12	0.069 ± 0.00345	1.52 ± 0.076	7.7 ± 0.385

Table 4 The significance of inducer (alginate) in seed and production medium for alginate lyase production

Seed medium	Production medium	Cell mass (mg/mL)	Enzyme activity (IU)	Final pH
A +ve	A –ve	1.16 ± 0.058	0.024 ± 0.0012	7.9 ± 0.395
A –ve	A +ve	1.48 ± 0.074	0.19 ± 0.0095	8.4 ± 0.42
A +ve	A +ve	2.2 ± 0.11	3.36 ± 0.168	8.7 ± 0.435
A –ve	A –ve	0.58 ± 0.029	0.006 ± 0.0003	7.5 ± 0.375

A +ve: Supplemented with 0.2%, (w/v) alginate; A –ve: Without alginate.

4. Discussion

Alginate lyase producing microorganisms were isolated from seaweed samples collected from various locations from India, specimen provided by Agar Supreme, a premier alginate production industry in TamilNadu. The incorporation of seaweeds like *Sargassum* and *Gracilaria* as the exclusive carbon source in our enrichment cultures facilitated the isolation of a varied microbial microbiota, reflecting the methodologies adopted by Wang *et al*., (2017) [38].

Simultaneously, the scientific community has seen substantial advancements, such as the isolation of *Alteromonas portus* sp. nov. from marine soil by Haung *et al*., (2020) [39], the detection of alginate lyase-producing bacteria in the gastrointestinal tracts of hybrid abalone*, Haliotis rubra* x H. *laevigata* by Amin *et al*., (2017) [40], isolation of the alginate lyase-producing *Bacillus litoralis* strain in the kelps of *Laminaria japonica* in Shandong province, China by Wang *et al*., (2016) [41], and Chen *et al*., (2018) [42] identifying *Bacillus* sp. Alg07, a marine bacterium employing alginate as the lone carbon source.

The outcome of our endeavours was the acquisition of 31 isolates capable of digesting alginate to varying extents in a semi-solid medium. From this array, one isolate, SG4, stood out, demonstrating significant growth and alginate activity across solid and liquid media, which prompted its selection for further exploration. Following a 16S rRNA nucleotide sequence analysis, the isolate was submitted to GenBank and identified with the accession number OM980630.

The phylogenetic pathway was established using the Neighbor-Joining method, as detailed by Saitou and Nei, (1987) [43]. The phylogenetic tree was created and displayed with bootstrap percentages (from 500 replications) next to the branches, a technique suggested by Felsenstein, (1952) [44]. These percentages reflect the frequency of replicate trees where the related taxa clustered together. The evolutionary distances were computed using the Maximum Composite Likelihood method, a procedure outlined by Tamura *et al*, (2004) [45]. The units signify the number of base substitutions per site. The analysis involved nine nucleotide sequences and all ambiguous positions were omitted for each sequence pair (following pairwise deletion). The final dataset comprised a total of 579 positions. This comprehensive evolutionary study was conducted using MEGA11, as per Tamura *et al*., 2021[46] work.

Thin Layer Chromatography (TLC) analysis confirmed that alginate lyase from the *Paenibacillus* sp. OM980630 strain can produce alginate oligosaccharides of various lengths, corroborating findings from a study by Wang *et al*, (2017) [47].

5. Conclusion

In conclusion, this study successfully isolates and characterizes a potent strain of *Paenibacillus* sp., producing alginate lyase, and cataloged under gene bank accession number OM980630. Utilizing established methodologies like 16S rRNA gene sequencing and Medium M-7 optimization, the research lays a solid groundwork for future investigations. The isolated strain shows promising enzymatic activity, with potential applications in medical treatments such as cystic fibrosis and industrial processes like bioremediation and biofuel production. Future work will focus on optimizing production parameters and evaluating efficacy against *Pseudomonas aeruginosa* biofilms. Therefore, the study holds implications that extend beyond its immediate findings, with the potential to benefit both medical and industrial applications of alginate lyase.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare that they have no conflict of interest with each other or with the parent institute.

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