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Scientific Journal for Damietta Faculty of Science **14**(3) 2024, 42-56
doi: 10.21608/sjdfs.2025.339574.1199
ISSN Print 2314-8594
ISSN Online 2314-8616

https://sjdfs.journals.ekb.eg/

Identification and Optimization of Culture Conditions of a Marine AgarDegrading Bacterium *Pseudoalteromonas agarivorans* EGPS36 Isolated from Egypt

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Received: 26 November 2024 /Accepted: 08 December 2024

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Abstract

A collection of 44 differently looking colonies of marine bacteria were isolated from the Mediterranean Sea Coast of Port Said City, Port Said Governorate, Egypt. The basal salt solution (BSS) medium was utilized to evaluate the agarase production in all originated bacteria. The solution of Lugol's iodine was employed to evaluate the agarase formation qualitatively. 8 bacterial isolates out of the 44 revealed the capability of agarase formation. Quantitatively, agarase was assessed by employing the 3,5-Dinitrosalycilic acid (DNS) methodology. The most active isolate of the agarase enzyme was identified by assessing the morphological, biochemical, and genetic traits. The biochemical tests and the 16S rRNA gene sequencing confirmed that the marine bacterial isolate EGPS36 was grouped in a manner with *Pseudoalteromonas agarivorans* and assigned an accession number of (PQ203725) in the GenBank. The optimum *P. agarivorans* EGPS36 growth and agarase potency were compatible with an inoculum size, agitation speed, incubation duration, temperature, pH, and sodium chloride level of 2%,
150 rpm, 48 hr, 35℃, 8, and 3.5% concentration, respectively. The higher proliferation and agarase potency were accomplished via the implementation of agar as a provider of carbon via a level of 0.4%. The proliferation and agarase formation were concealed when the galactose, glucose, rhamnose, sucrose, arabinose, raffinose, cellulose, xylan, and xylose were applied. The higher proliferation and agarase potency were accomplished by implementing yeast extract as a nitrogen source at 0.3%. The proliferation and agarase formation were concealed when the tryptophan and tryptone were applied.

Keywords: Identification, Agar-degrading bacterium, Marine, Optimization, *Pseudoalteromonas agarivorans* EGPS36.

Introduction

Marine ecosystems, which make up over 71% of the planet's surface, may contain useful enzymes that are currently mostly unknown (**Ghanbarzadeh et al., 2018**). The wide-ranging industrial uses of marine microbial

enzymes have garnered tremendous attention, but very few have been successfully isolated, purified, and characterized for their properties and uses. It is well known that microbial enzymes have certain advantages over enzymes derived from animal or plant sources, including high catalytic capacity, ease of genetic discovery, mass culture ability, broad biodiversity, cost-effectiveness, process efficacy, and sustainability **(Nguyen & Nguyen, 2017)**. According to the cleavage pattern, agarases are classified as either β-agarase (E.C. 3.2.1.81) or α-agarase (E.C. 3.2.1.158). The enzymes known as agarases are also known as glycoside hydrolases (GH) because they break the glycosidic bonds of complex polysaccharides, such as agar, to make it easier for them to be hydrolyzed **(Barbeyron et al., 2001)**. Agarose (70%) and agaropectin (30%), two distinct sorts of polysaccharides, are combined to generate the polymer termed agar **(Kolhatkar & Sambrani, 2018)**. According to **Li et al. (2020)**, agarases are denominated into two kinds via their potency to split the β-1,4 and α-1,3 glycosidic bonds of the polymer agarose: α-agarases (EC 3.2.1.1.158) and β-agarases (EC 3.2.1.81). As **Park et al. (2020)** cited, β-agarases dissolve β-1,4 glycosidic connections in agarose to generate neoagarooligosaccharides with D-galactose residues at their reducing ends, but α-agarases dissolve α1,3 glycosidic connections in agarose to generate agarooligosaccharides with 3,6-anhydro-L-galactose residues at their reducing ends. The largest number of agarase enzymes that have been characterized are categorized as β-agarase **(Song et al., 2016)**. Based on the similarity in the sequence of their amino acids, microbial agarases are denominated into diverse glycoside hydrolase (GH) families. In the case of α-agarases, they are associated with the families GH96 and GH117 as **Jang et al. (2021)** pointed out, whereas those that belong to β-agarases are associated with the families GH16, GH50, GH86, and GH118 as **Chi et al. (2012)** revealed. The bacteria that disintegrate the agar are split into two diverse denominations corresponding to how they implicate rigid agar. As discovered by **Kobayashi et al. (1997),** bacteria in class one induced the agar to become soft resulting in a depression surrounding it, but bacteria in class two induced the agar to liquefy. Numerous agar-degrading bacteria have been recognized and extracted from the marine region due to agar is an established element of the cell walls of red algal cells that are prevalent in the coastal and aquatic environments **(An et al., 2018)**.

Biologically active molecules, antimicrobial agents, and diverse exoenzymes that assist in the dissolution of polysaccharides originated via marine algae comprising alginate, agar, and carrageenan are manufactured through an assortment of strains in the genus of *Pseudoalteromonas*. As pointed out by **Al Khudary et al. (2008)**, *Pseudoalteromonas* strains are often found in marine debris and seawater. One of the major genera in the class of gammaproteobacteria is *Pseudoalteromonas* bacterium **(Zhao et al., 2014)**. **Gauthier et al. (1995)** made the recommendation in 1995 to divide the genus *Alteromonas* into *Pseudoalteromonas* under the studies of phylogeny. Within the genus *Pseudoalteromonas*, several aquatic agarases were recovered. *P. antarctica* N-1 was isolated from marine algae **(Vera et al., 1998)**.

Pseudoalteromonas sp. CKT1 from sea mud **(Chiura & Kita-Tsukamoto, 2000).** *P. issachenkonii* sp. Nov. was gathered from brown algae **(Ivanova et al., 2002)**. *P. hodoensis* H7 was gathered from seawater **(Chi et al., 2014)**. *Pseudoalteromonas* sp. MHS was gathered from marine algae as revealed by **Sharabash et al. (2022).** Removal of DNA through an agarose gel is one of the most valuable applications of agarase as **Jung et al. (2017)** revealed. Agarase is implemented to generate the protoplast from algae. **Khambhaty et al. (2008)** identified protoplasts from *Gelidiella acerosa*, employing agarose gel. Agarooligosaccharides and neoagarooligosaccharides, the originated products of agar disintegration via agarases, are exceptionally valuable in the medicinal, dietary, and cosmetic industries **(Park et al., 2020)**. A key component of the fermentation process that produce agarase enzyme is the standardization of the media. The media's structure has a significant impact on production levels. In addition to helping to maximize output, optimizing media composition also helps to prevent the waste of leftover components. A variety of physiological factors, including temperature, pH, carbon, and nitrogen supplies in the medium, are implicated in the development and durability of the agarase enzyme. According to **Shah & Mishra (2020)**, among the approaches implemented to evaluate the ideal variables and their significant impacts

is the one factor-at-time (OFAT) approach. The current work aimed to optimize the productivity of agarase by marine *P. agarivorans* EGPS36 which locally isolated from the seawater samples obtained from Mediterranean Sea Coast, Egypt.

Material and methods

Isolation and purification of marine bacteria

Seawater samples were collected from several locations and depths in cleaned plastic bottles from the Mediterranean Sea Coast of Port said City, Port said Governorate, Egypt. At once all the samples were transported to the Microbiology lab for isolation of agar-digesting bacteria. The marine bacteria were isolated by plating dilutions in sterile distilled water via the mixing of 10 ml of seawater in a 250 ml conical flask with 90 ml of sterilized distilled water and agitating for 15 min, and the resultant suspensions were constituted 10^{-1} dilution. 10^{-2},10^{-3}, 10^{-4}, and 10^{-5} were executed as described previously. About 1 ml from every dilution was added to a sterile Petri dish. The isolation of bacteria was conducted in Artificial Seawater agar medium (ASW) that comprised (g/L dist. H_2O): NaCl (24.7 g), $MgSO_4.7H_2O$ (6.3 g), $MgCl_2.2H_2O$ (4.6 g), KCl (0.7 g), NaH_2CO_3 (0.2 g), and Agar (15 g) **(Atlas, 2010)**. The ASW agar medium that was adjusted to pH 7.5 was poured into Petri dishes and combined properly within 1 ml of the seawater sample. After solidification of the agar in the dishes, they were inverted and incubated at 30 °C for 2 days in the static incubator. After the incubation, the developed single colonies of bacteria that diverged in color and appearance were picked up and streaked on the same isolation medium for purification. The purified bacterial isolates were stored on agar slants at 4 °C for future work.

Qualitative and quantitative evaluations of agarase

The basal salt solution (BSS) medium was used to screen the agarase production rates in all of the bacterial isolates according to the method described by **Hofsten & Malmqvist (1975)**. In brief, BSS medium was prepared (pH 7.5), autoclaved, and poured into sterile Petri dishes. After solidification, all of the bacterial isolates were cultivated using a streak plate technique. The inoculated agar plates were incubated for 48 hr at 30°C. After incubation, plates were covered with Lugol's solution of iodine to qualitatively detect agarase production. Agar dissolution was indicated by the existence of a yellow transparent zone encircling the bacterial growth against a brown background **(Liu et al., 2016)**. For later use, the bacteria displaying agarolytic function were preserved at -80 °C in 30% (v/v) glycerol.
Quantitative evaluation for agarase production was done according to **Miller (1959)**. The selected agarolytic isolates were cultivated into 250 ml Erlenmeyer flasks holding 100 ml of BSS and 0.2% (w/v) agar as the sole form of carbon. The flasks then were incubated for 48 hr at 30 °C in a vibrating incubator at 150 rpm. The supernatant was employed as a source of extracellular agarase after the cultures were centrifuged for 15 min at 7000 rpm and 4 °C to discard the bacterial cells. The reducing sugar amounts released from agar hydrolysis were evaluated using 3,5-Dinitrosalycilic acid (DNS).

Assay of agarase activity

1 ml of supernatant was mixed with 3 ml of 20 mM, pH 8 Tris HCl buffer solution containing 0.2% (w/v) agarose as the substrate. The combined reaction was incubated at 40 °C for 30 min. 1 ml of the reaction was withdrawn and 1 ml of DNS was introduced to it. The reaction was subsequently exposed to heating for 10 min until it reached boiling water. Spectrophotometric measurements of the optical density absorbance values (λ=540nm) of the reducing sugar result were evaluated after it was chilled in a temperature room. The same procedures were applied to blanks of substrate devoid of enzyme and enzyme devoid of substrate. Considering D-galactose as the standard reducing sugar in a spectrum of 0–10µmole/mL, the standard curve was constructed. **Baleta et al. (2024)** revealed that one unit of enzyme activity (U) is the level of enzyme sufficient for the release of one µmole of the reducing sugar (D-galactose) per minute depending on the assay parameters.

Phenotypic characterization and molecular diagnosis of the selected agar-degrading isolate

The colony form, elevation, margin, texture, color, and transparency were taken into consideration during the identification. The purified bacteria were stained employing Gram and endospore stains. According to the principles outlined by **Romanenko et al. (2003)**, the physiological and biochemical investigations were evaluated and defined for the prospective manufacturer isolate of the agarase enzyme.

As **Ausubel et al. (2003)** published, the phenol/chloroform methodology was employed for extracting the DNA of bacterial cells. The genomic DNA purity was assessed via a 1% agarose gel electrophoresis.

As revealed by **Lane (1991)**, the universal prokaryotic primers 27F (5'AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') for amplifying the 16S rRNA gene. Macrogen, Korea, sequenced the product of PCR amplification assuming the previously established primers.

Altschul et al. (1997) reported that the generated 16S rDNA sequence was aligned via the BLASTn system of the GenBank database (https://www.ncbi.nlm.nh.gov.blast) to identify the greatest matches with comparable sequences in the database. The most suitable DNA sequences comparable to our 16S rDNA segment were retrieved from NCBI GenBank and CLUSTAL Omega was implemented for alignment. Sequences of identical and unidentifiable species were deleted, and non-alienable sections were manually dismissed. Lastly, MEGA version 4 was implemented to display and interpret phylogenetic tree evaluations **(Tamura et al., 2021)**. Applying maximum composite likelihood approaches, neighbor-joining was conducted **(Tamura & Nei, 1993)**. The confidence levels were established via 1000 bootstrap replicas, and only values 30 or above were selected and represented next to the phylogenetic branches.

Factors influencing the growth of bacteria and the production rate of agarase enzyme

250 ml Erlenmeyer flasks containing 100 ml of sterilized BSS medium supplemented with 0.2% (w/v) agar as the substrate were inoculated individually with varying inoculum concentrations that range from 0.5%- 3% (v/v) via 0.5% (v/v) interval to explore the consequence of the amount of inoculum. Spectrophotometric monitoring of the fluid cultural optical density at λ=600nm was implemented to assess the growth of bacteria. A previously established agarase enzyme assay was conducted. Triplicate runs of the test were made.

Different optimizing factors such as agitation rate (100, 150, 200, and 250 rpm), incubation times (1-5 days), temperature (25, 30, 35, 40, 45, 50, and 55 °C), pH (3-12), NaCl content (0-0.5% w/v), multiple carbon (agarose, fructose, galactose, rhamnose, glucose, raffinose, lactose, sucrose, maltose, mannitol, arabinose, trehalose, xylan, xylose, and cellulose, 0.2% (w/v)) and nitrogen suppliers (peptone, tryptone, beef extract, tryptophane, casein, yeast extract, glycine, ammonium chloride, ammonium sulfate, ammonium dihydrogen phosphate, ammonium nitrate, and diammonium hydrogen phosphate, 0.2% (w/v)) were also assessed in this research during the optimizing of agarase production. The appropriate carbon and nitrogen source concentration was established by introducing various amounts to the BSS medium from 0.1-1% (w/v) through 0.1% intervals **(Baleta et al., 2024)**.

Statistical assessment

Version 18 of the SPSS program was implemented to conduct the statistical evaluation. A one-way variance assessment (ANOVA) was applied to analyze the data. A lower than 0.05 constituted the *p*value chosen to calculate significance in statistics. Each test was performed three times. The data received was mean ± standard error **(O'Connor, 2000)**.

Results

Seawater bacterial isolation and agarolytic capability assessment

Through the utilization of an ASW agar medium, 44 bacterial isolates were obtained. Microscopical and morphological traits were employed to differentiate these isolates. The assessment for production of agarase was evaluated for each isolate. A total of eight of the isolated bacteria proved to be able to secrete the agarase as revealed in **Fig. 1**. For eight bacterial isolates, the agarase capability was quantitatively assessed. Agarase could

potentially be generated via isolate EGPS36 with activity (5± 0.152 U/ml) as revealed in **Table 1**.

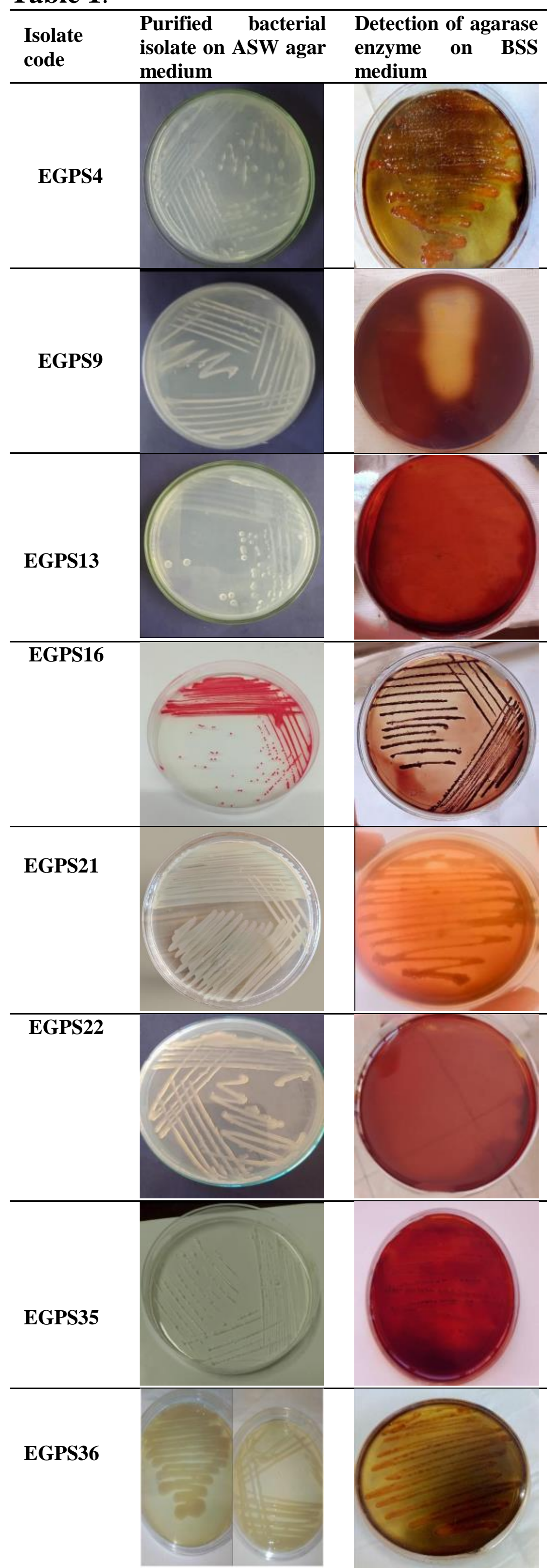

Fig. 1. The purified bacterial isolates from the Mediterranean Sea Coast of Port Said City on ASW agar medium and the detection of their agarolytic activity on BSS medium.

Table 1. Quantitative evaluation of agarase

The bacterial isolate	Agarase activity (U/ml)
EGPS4	1.73± 0.185
EGPS9	2.56± 0.233
EGPS13	0.5± 0.057
EGPS16	3.56± 0.233
EGPS21	1.1± 0.208
EGPS22	0.9± 0.173
EGPS35	1.43± 0.066
EGPS36	5± 0.152

The selected isolate EGPS36 identification

The morphological and cultural characteristics of the isolate EGPS36 are represented in **Table 2**. In line with the biochemical investigation listed in **Table 3** and **Table 4**, isolate EGPS36 was almost certainly affiliated with *P. agarivorans*. Molecular investigations validated the identity. **Fig. 2** revealed some representative biochemical tests for the marine bacterial isolate EGPS36.

Table 2. Morphological and cultural characteristics of the isolate EGPS36

Morphological and cell characteristics	Isolate EGPS36
Pigmentation	Absence
Form	Circular
Elevation	Convex
Margin	Entire
Texture	Smooth
Color	Beige
Transparency	Opaque
Gram stain	Negative/rods
Endospore stain	Negative

Table 3. The biochemical investigation of the seawater bacterial isolate EGPS36.

Biochemical tests	Result
Catalase	Positive
Oxidase	Positive
Motility test	Positive
Growth at 6.5% NaCl	Positive
Indole production	Negative
Methyl red	Positive
Voges Proskauer	Negative
Citrate utilization	Negative
Urea hydrolysis	Negative
Casein hydrolysis	Positive
Starch hydrolysis	Positive
Gelatin hydrolysis	Positive
Lipase production	Positive
Nitrate reduction	Positive
Esculin hydrolysis	Negative
H2S production	Negative

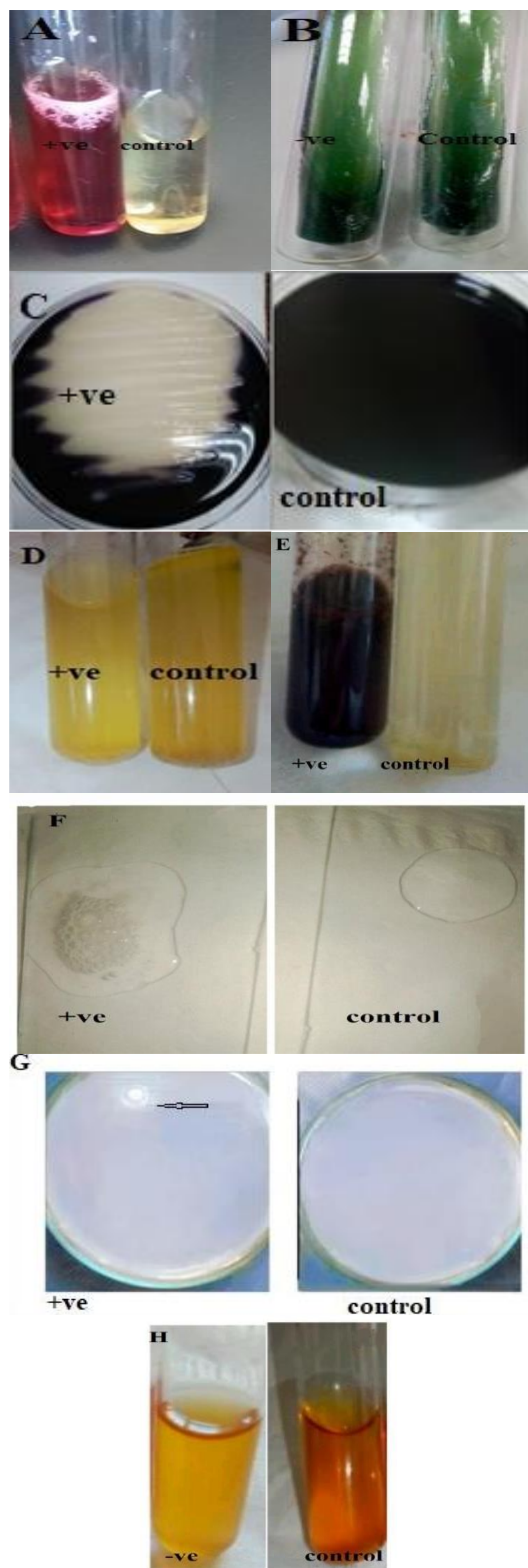

Fig. 2. Some representative biochemical tests for the marine bacterial isolate EGPS36. **(A)** Methyl red test. **(B)** citrate utilization test. **(C)** Starch hydrolysis test. **(D)** Gelatin hydrolysis test. **E** Nitrate reduction test. **(F)** Catalase test. **(G)** Casein hydrolysis test. **(H)** Urea hydrolysis test.

Table 4. The fermentation of various sugars by the bacterial isolate EGPS36.

Sugar	Production of acid	Production of gas
D-glucose	Negative	Negative
D-galactose	Negative	Negative
Glycerol	Negative	Negative
D-trehalose	Positive	Negative
D-mannitol	Positive	Positive
D-raffinose	Negative	Negative
L-arabinose	Negative	Negative
D-xylose	Negative	Negative
L-rhamnose	Negative	Negative
D-sorbitol	Negative	Negative
Sucrose	Negative	Negative
Maltose	Positive	Positive
D-Lactose	Negative	Negative
D-mannose	Negative	Negative
D-ribose	Negative	Negative

Phylogenetic analysis

With a similarity degree of 99.44%, the 16S rRNA gene sequencing confirmed that the marine bacterial isolate EGPS36 was grouped in a manner with *P. agarivorans*. **Table 5** reveals the similarity in the ratio of alignment between marine bacterial isolate EGPS36 and other corresponding sequences in the database via the BLASTn approach. The evolutionary relationships tree for *P. agarivorans* EGPS36 is presented in **Fig. 3**. The 16S rDNA sequence is being assigned a GenBank accession number of (PQ203725).

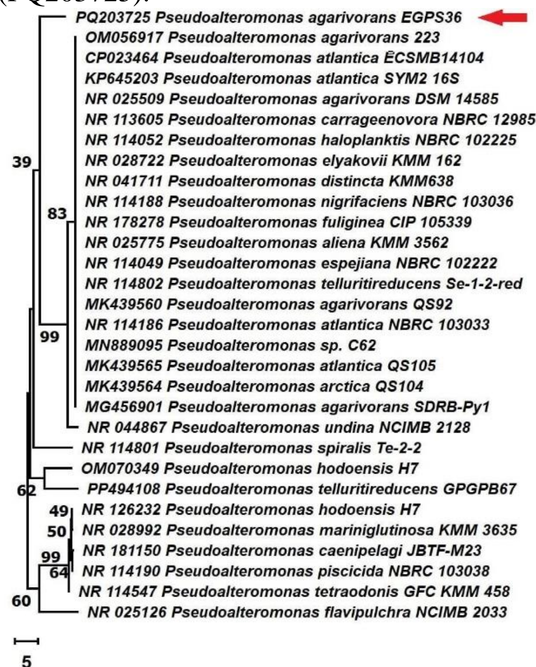

Fig. 3. The phylogenetic tree of *P. agarivorans* EGPS36. At the tree branches (>30), the bootstrap results (1000 replicates) were recorded in percentage. Five substitutions of nucleotides were displayed for each nucleotide site on the scale bar.

Table 5. Alignment homology ratio of the marine bacterial isolate EGPS36 and other corresponding sequences in the NCBI database applying the BLASTn tool.

Strain number	Strain	Query cover %	Similarity %	Accession number
1	*P. agarivorans* strain 223	99	100	OM056917.1
2	*P. atlantica* strain ECSMB14104	99	99.72	CP023464.1
3	*P. atlantica* strain SYM2	99	99.72	KP645203.1
4	*P. agarivorans* DSM 14585	99	99.44	NR_025509.1
5	*P. carrageenovora* strain NBRC 12985	99	99.3	NR_113605.1
6	*P. haloplanktis* strain NBRC 102225	99	99.88	NR_114052.1
7	*P. elyakovii* strain KMM 162	99	99.23	NR_028722.1
8	*P. distincta* strain KMM638	99	99.16	NR_041711.1
9	*P. nigrifaciens* strain NBRC 103036	99	98.95	NR_114188.1
10	*P. fuliginea* strain CIP 105339	99	98.59	NR_178278.1
11	*P. aliena* strain KMM 3562	99	98.17	NR_025775.1
12	*P. espejiana* strain NBRC 102222	99	99.44	NR_114049.1
13	*P. telluritireducens* strain Se-1-2-red	99	99.02	NR_114802.1
14	*P. agarivorans* strain QS92	99	99.65	MK439560.1
15	*P. atlantica* strain NBRC 103033	99	99.37	NR_114186.1
16	*Pseudoalteromonas* sp. strain C62	98	99.93	MN889095.1
17	*P. atlantica* strain QS105	98	99.93	MK439565.1
18	*P. arctica* strain QS104	98	99.93	MK439564.1
19	*P. agarivorans* strain SDRB-Py1	98	99.86	MG456901.1
20	*P. undina* strain NCIMB 2128	99	97.75	NR_044867.1
21	*P. spiralis* strain Te-2-2	99	99.22	NR_114801.1
22	*P. hodoensis* strain H7	99	99.86	OM070349.1
23	*P. telluritireducens* strain GPGPB67	98	99.93	PP494108.1
24	*P. hodoensis* strain H7	99	99.51	NR_126232.1
25	*P. mariniglutinosa* strain KMM 3635	99	96.77	NR_028992.1
26	*P. caenipelagi* strain JBTF-M23	99	96.28	NR_181150.1
27	*P. piscicida* strain NBRC 103038	99	95.71	NR_114190.1
28	*P. tetraodonis* GFC strain KMM 458	99	98.66	NR_114547.1
29	*P. flavipulchra* strain NCIMB 2033	99	95.84	NR_025126.1

Elements impacting the P. agarivorans EGPS36 proliferation and the formation of agarase enzyme

The observations exhibited in **Fig. 4(I)** proved that at 2% inoculum size, there was higher *P. agarivorans* EGPS36 proliferation and agarase efficiency, showing growth (0.547±0.003) and activity (4.68 U/ml±0.089) that significantly plummeted (p<0.05) once the inoculum amount progressed until it attained the lowest limit at 3%, revealing activity (2.38 U/ml±0.053) and proliferation (0.208±0.001).

The observations exhibited in **Fig. 4(II)** revealed that the superior *P. agarivorans* EGPS36 growth (0.466±0.003) and agarase potency (4.91 U/ml±0.045) were compatible with an agitation rate of 150 rpm. Agarase performance dropped considerably (p<0.05) at agitation rates exceeding 200 rpm.

The observations exhibited in **Fig. 4(III)** proved that after 2 days, there was higher *P. agarivorans*
EGPS36 proliferation and agarase potency, showing growth (0.521±0.004) and activity (5.7 U/ml±0.053) that significantly decreased (p<0.05) when the incubation duration progressed until it attained the lowest limit after 5 days, revealing activity (2.86 U/ml±0.037) and proliferation (0.248±0.003).

The observations exhibited in **Fig. 4(IV)** proved that the higher *P. agarivorans* EGPS36 proliferation and agarase potency were evaluated at a temperature scale of (30-45°C), attaining superior growth (0.658±0.003) and activity (5.37 U/ml±0.055) at 35°C. The proliferation and activity significantly plummeted (p<0.05) when the temperature progressed until it attained the lowest limit at 55°C, revealing activity (2.7 U/ml±0.074) and proliferation (0.277±0.002).

The observations exhibited in **Fig. 4(V)** proved that the higher *P. agarivorans* EGPS36 proliferation and agarase potency were evaluated via the pH scale of 7 to 10, attaining superior growth (0.621±0.002) and activity (4.97 U/ml±0.062) at pH 8. The proliferation and activity significantly plummeted (p<0.05) as the pH level progressed until it attained the lowest limit at pH 12, revealing activity (2.68 U/ml±0.073) and proliferation (0.252±0.003).

The observations exhibited in **Fig. 4(VI)** proved that there was no *P. agarivorans* EGPS36 proliferation and agarase potency at a zero concentration of NaCl. The higher *P. agarivorans* EGPS36 proliferation and agarase potency were evaluated via a level of 2% to 4% NaCl, attaining superior growth (0.492±0.002) and activity (5.02 U/ml±0.009) at a level of 3.5% NaCl. The proliferation and activity significantly plummeted (p<0.05) when the salt level progressed until it attained the lowest limit at 5%, revealing activity (2.24 U/ml±0.038) and proliferation (0.255±0.005).

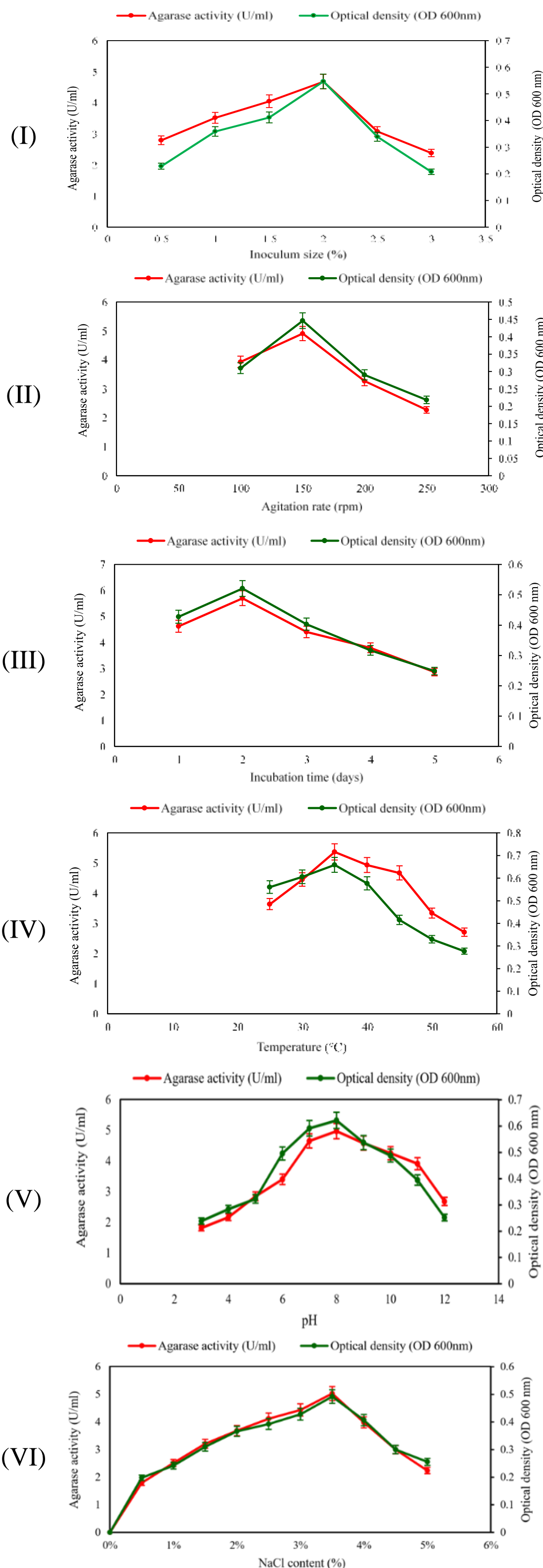

Fig. 4. The implication of various culture conditions on the proliferation and agarase manufacturing of *P. agarivorans* EGPS36; **(I)**, Inoculum size; **(II)**, Agitation rate (rpm); **(III)**, Incubaion time; **(IV)**,

Temperature; **(V)**, pH; **(VI)**, NaCl content (%). Measurements are documented as the average (n = 3) ±SE.

The observations exhibited in **Fig. 5(I)** evaluated the implication of diverse carbon suppliers on *P. agarivorans* EGPS36 proliferation and agarase potency. The higher proliferation and agarase potency were accomplished via the implementation of agar, attaining growth (0.437±0.003) and activity (5.44 U/ml±0.049). Agarose was established to be the second carbon source for achieving perfect proliferation (0.414±0.001) and agarase potency (5.02 U/ml±0.035). Fructose, maltose, lactose, mannitol, and trehalose, respectively were believed to be acceptable carbon suppliers for agarase synthesizing by *P. agarivorans* EGPS36, although the agarase potency significantly plummeted ($p<0.05$) when these carbon suppliers were introduced. The proliferation and activity were concealed when galactose, glucose, rhamnose, sucrose, arabinose, raffinose, cellulose, xylan, and xylose were utilized. The observations exhibited in **Fig. 5(II)** proved that the higher *P. agarivorans* EGPS36 proliferation and agarase potency were evaluated via the implementation of agar at a level of 0.4%, attaining growth (0.713±0.004) and activity (5 U/ml±0.074). The proliferation and activity significantly plummeted ($p<0.05$) as the agar level progressed until it attained the lowest limit at 1% level, revealing activity (1.56 U/ml±0.028) and proliferation (0.089±0.004).

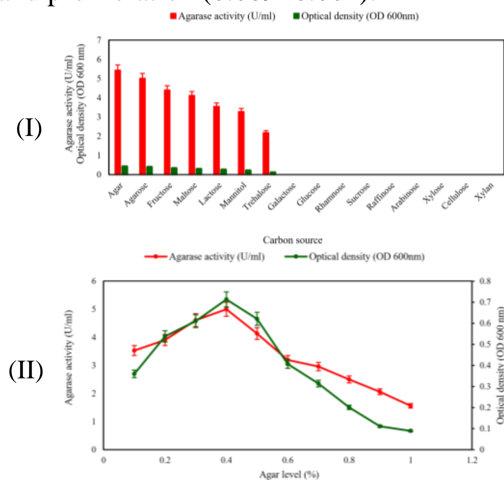

Fig. 5. The implication of various culture conditions on the proliferation and agarase manufacturing of *P. agarivorans* EGPS36; **(I)**, Carbon source; **(II)**, Agar level (%). Measurements are documented as the average (n = 3) ±SE.

The observations exhibited in **Fig. 6(I)** evaluated the implication of diverse nitrogen suppliers on *P. agarivorans* EGPS36 proliferation and agarase potency. The higher

proliferation and agarase potency were accomplished via the implementation of yeast extract, attaining growth (0.622±0.006) and activity (4.42 U/ml±0.024). Peptone and beef extract were established to be the second and third nitrogen suppliers, respectively for achieving perfect proliferation and agarase potency. The agarase activity and growth significantly plummeted ($p<0.05$) via the utilization of inorganic nitrogen suppliers. Tryptophan and tryptone exhibited the suppression of agarase production and growth. The observations exhibited in **Fig. 6(II)** proved that the higher *P. agarivorans* EGPS36 proliferation and agarase potency were evaluated via the implementation of yeast extract at a level of 0.3%, attaining growth (0.741±0.009) and activity (4.52 U/ml±0.024). The proliferation and activity significantly plummeted ($p<0.05$) as the yeast extract level progressed until it attained the lowest limit at 1% level, revealing activity (1.21 U/ml±0.097) and proliferation (0.106±0.003).

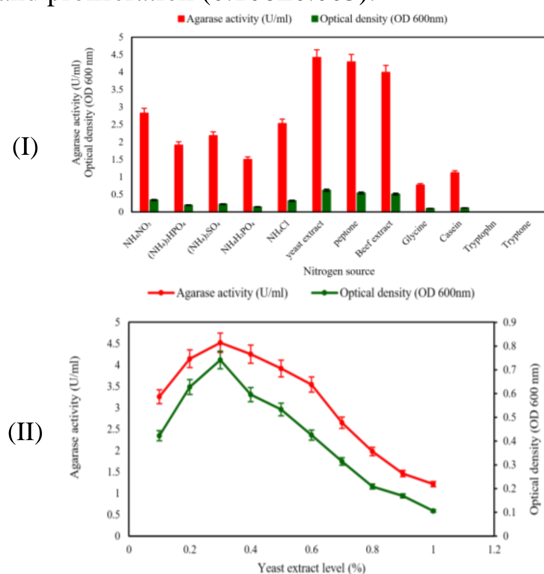

Fig. 6. The implication of various culture conditions on the proliferation and agarase manufacturing of *P. agarivorans* EGPS36; **(I)**, Nitrogen source; **(II)**, Yeast extract level (%). Measurements are documented as the average (n = 3) ±SE.

Discussion

Although the 16S rRNA genomic sequence of the marine isolate EGPS36 revealed an elevated homogeneity within *P. arctica*, *P. atlantica*, *P. haloplanktis*, and *P. hodoensis*, it differs from them in terms of physiology and cultural traits. For example, the colonies of *P. arctica* are slightly orange on the agar medium, while the colonies of isolate EGPS36 are beige on the agar medium. Also, *P. arctica* can utilize D-glucose, D-galactose, and sucrose **(Al Khudary et al., 2008)**, while the isolate EGPS36 is not able to utilize them. Colonies of *P. atlantica* may form melanin-like pigment in the culture medium, while the isolate EGPS36 does not form pigments in the culture medium. *P. atlantica* can utilize D-glucose and D-raffinose as carbon suppliers, while the isolate EGPS36 is not able to utilize them. In contrast to the isolate EGPS36, *P. atlantica* is capable of hydrolyzing urea and esculin as well as producing H_2S **(Garrity et al., 2007)**. *P. haloplanktis* can utilize citrate and D-mannose as carbon suppliers **(Garrity et al., 2007)**, while the isolate EGPS36 is not able to utilize them. Colonies of *P. hodoensis* are pale yellow on the agar medium, while the colonies of isolate EGPS36 are beige on the agar medium. *P. hodoensis* is positive for citrate utilization, while the isolate EGPS36 is negative. *P. hodoensis* is negative for methyl red and nitrate reduction tests **(Li et al., 2019)**, while the isolate EGPS36 is positive. With a similarity degree of 99.44%, the 16S rRNA gene sequencing confirmed that the marine bacterial isolate EGPS36 was grouped in a manner with *P. agarivorans* as they possessed considerable identity via the morphological and cultural features. On top of that, the bulk of the physiological and biochemical traits of the marine bacterial isolate EGPS36 and *P. agarivorans* were the same. Our decision to classify the marine bacterial isolate EGPS36 as *P. agarivorans* was ultimately validated by both molecular and conventional characterization.

Both the development of the bacteria and the sort of media have an immense impact on the generation and potency of enzymes. The research effort focused on investigating the association of crude agarase action and unique parameters that were adjusted to evaluate the highest quantity of agarase yield from *P. agarivorans* EGPS36. The length of the incubation stage, the volume of the inoculum, the level of agitation, the temperature, the pH, the extent of tolerance to salt, and the involvement of distinct suppliers of nitrogen and carbon are some of the parameters that influence the process. The maximum propagation of *P. agarivorans* EGPS36 and agarase potency were shown to be linked with the inoculum content. The appropriate

inoculum size for growth and agarase potency was evaluated to be 2%, which dropped once the inoculum size grew. Conversely, **Veerakumar & Manian (2022)** revealed that 1.5% was the perfect inoculum size for *Halomonas meridiana* agarase effectiveness. As **Ha et al. (2023)** cited, 10% was the optimal inoculum size for agarase potency from *Flavobacterium* sp. AT2. Increasing bacterial engagement with the surrounding nutrients could be a cause for the increased performance of enzymes noticed when the inoculum size rose. The interaction between them exhibited an encouraging impact on enzyme activity, which could support the metabolism of bacteria and protein generation **(Thi Nguyen & Tran, 2018)**.

The agarase created by *P. agarivorans* EGPS36 was predicted to be greatly implicated by the level of agitation. Insufficient proliferation developed, which could lead to weak agarase potency, as a consequence of insufficient nourishment under lower agitation levels. The superior rate of agitation for agarase efficiency and proliferation was 150 rpm, which facilitated adequate media aeration and absorption of nutrients. *Bacillus* sp. R2 possessed an equivalent outcome, as **Cheba (2022)** mentioned. Shaking at a greater speed provides a possibility to denature the enzyme.

Optimum *P. agarivorans* EGPS36 growth and agarase potency emerged after 48 hr, consistent with the findings. *Cellvibrio mixtus* SC-22 possessed an equivalent finding, as **Cha et al. (2009)** published. Long-term incubation periods are tied to the possibility of enzyme damage via interactions with multiple ingredients in the medium.

Temperature has a significant consequence on both the development of bacteria and agarase. The maximum propagation of *P. agarivorans* EGPS36 and agarase synthesis were evaluated at 35°C. *Microbacterium* sp. SS5 possessed an equivalent finding as **Labade et al. (2024)** cited. At elevated temperatures, agarase potency collapses due to enzyme denaturation, which hinders the generation of enzyme complex and substrate.

The level of pH has a major implication on the reproduction of *P. agarivorans* EGPS36 and agarase generation. The agarase is exceptionally dependent on pH since it impacts its ionic phase, which subsequently alters the tertiary structure and performance of the enzyme. Also, pH generates irreversible deactivation by altering the active sites. Any alteration from the perfect pH level could give rise to the denaturation of the enzymes and abrupt losses in their efficiency. Consistent with the findings, the ideal propagation and activity were cited at pH 8. *Aquimarina agarilytica* ZC1 documented an equivalent finding as **Dong et al. (2021)**.

Cell lysis usually originates from the administration of NaCl at a greater amount, which results in an osmotic imbalance in the microbial surroundings. The evaluation of the consequence of diverse levels of salt on the growth of *P. agarivorans* EGPS36 and agarase action would be necessary. Consistent with the findings, the ideal propagation and activity were cited at a dosage of 3.5% NaCl. Conversely, **Baleta et al. (2024)** cited that 3% was the perfect salt level for *Bacillus subtilis* agarase effectiveness. As findings revealed, there was no growth for *P. agarivorans* EGPS36 and no agarase function when sodium chloride was not available. Unlike *Pseudomonas aeruginosa* ZSL-2, which proliferated more efficiently and generated agarase in lack of salt, this strain did not require sodium chloride as a particular requirement as **Ziayoddin et al. (2014)** found.

The accessibility of a carbon supply is an aspect of the fermentation microbial media that is vital in modulating the overall proliferation and cell metabolism **(Nagar et al., 2010)**. The perfect propagation of *P. agarivorans* EGPS36 and agarase formation were evaluated via the application of agar as a carbon supply. *Alteromonas agarlyticus* GJ1B and *P. hodoensis* H7 represented an equivalent finding as **Chi et al. (2014)** documented. Fructose, maltose, lactose, mannitol, and trehalose respectively were believed to be acceptable carbon suppliers for agarase synthesizing by *P. agarivorans* EGPS36, but the agarase potency plummeted when these carbon suppliers were implemented. This might arise as a consequence of the bacterium consuming these sugars quickly, triggering the agarase formation. The proliferation and agarase performance were concealed when galactose, glucose, rhamnose, sucrose, arabinose, raffinose, cellulose, xylan, and xylose were implemented, and this reflected a catabolite inhibition. Microorganisms frequently demonstrate an action termed catabolite inhibition, which governs the manufacturing of enzymes associated with metabolic processes

and eventually evaluates the point to which the microbes metabolize a given molecule. *Pseudomonas aeruginosa* AG LSL-11 exhibited confirmation of catabolite inhibition for agarase creation, where agarase synthesis diminished while agar was co-supplemented via additional resources of carbon as **Lakshmikanth et al. (2006)** published. On the opposite, fructose was evaluated to elevate agarase manufacturing in the *Pseudomonas aeruginosa* ZSL-2, and the co-supplemented resources of carbon weren't hindering agarase formation in this organism as **Ziayoddin et al. (2014)** cited. Variations in the agar quantity were discovered to alter how much agarase was synthesized. The agar dosage chosen to assess the agarase potency was 0.1-1% (w/v). *P. agarivorans* EGPS36 created the potent yield of agarase via agar through a level of 0.4% (w/v). *Alteromonas macleoii* BC7 possessed an equivalent finding, as **Zilda et al. (2024)** mentioned. When the agar level grew over 0.4%, the agarase efficacy plummeted. **Fu et al. (2009)** postulate that this could be caused by introducing extra agar to the cultivation medium raising its viscosity, limiting proliferation of cells, and diminishing the creation of agarase.

The formation of agarase is severely implicated by the medium accessibility to nitrogen as a basic element. As stated by **You et al. (2023)**, nitrogen supplies are crucial for bacteria to manufacture nucleic acid and protein as key components. The organic nitrogen providers had an encouraging implication on culture propagation and enzyme potency. The perfect propagation of *P. agarivorans* EGPS36 and agarase formation were evaluated via the application of yeast extract as a nitrogen provider. *Acinetobacter* sp. PS12B represented an equivalent finding as **Roseline & Sachindra (2018)** revealed. The yeast extract dosage chosen to assess the agarase potency was 0.1-1% (w/v). Tryptophan and tryptone exhibited the suppression of agarase potency and growth and this reflected also a catabolite inhibition. Contrary, *Pseudoalteromonas* sp. JYBCL 1 and *Aquimarina agarilytica* NI125 exhibited optimum production of agarase enzyme via the implementation of tryptone as an organic nitrogen supplier as **Jung et al. (2012)** and **Farahat (2019)** published, respectively. *P. agarivorans* EGPS36 created the potent yield of agarase via yeast extract through a level of 0.3% (w/v). When the yeast extract level grew over 0.3%, the agarase potency plummeted. **Mehta et al. (2006)** claimed that the release of enzymes was impeded by rising the levels of yeast extract, attributable to its rich protein and amino acid profile.

Conclusion

A marine bacterium that consumes agar as its fundamental supply of energy and carbon was isolated from seawater. The biochemical investigation and the 16S rRNA gene sequencing confirmed that the marine bacterial isolate EGPS36 was grouped in a manner with *P. agarivorans* within a similarity degree of 99.44%. The 16S rDNA sequence is being assigned a GenBank accession number of (PQ203725). Elements impacting the proliferation of *P. agarivorans* EGPS36 and the formation of agarase were researched. The optimum *P. agarivorans* EGPS36 growth and agarase potency were compatible with an inoculum size, agitation speed, incubation duration, temperature, pH, and sodium chloride level of 2%, 150 rpm, 48 hr, 35°C, 8, and 3.5%, respectively. The higher proliferation and agarase potency were accomplished via the implementation of agar through a level of 0.4%. The higher proliferation and agarase potency were accomplished via the implementation of yeast extract through a level of 0.3%.

References

Al Khudary, R., Stoßer, N. I., Qoura, F., & Antranikian, G. (2008). *Pseudoalteromonas arctica* sp. nov., an aerobic, psychrotolerant, marine bacterium isolated from Spitzbergen. International Journal of Systematic and Evolutionary Microbiology, 58(9), 2018-2024. https://doi.org/10.1099/ijs.0.64963-0

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research, 25(17), 3389-3402. https://doi.org/10.1093/nar/25.17.3389

An, K., Shi, X., Cui, F., Cheng, J., Liu, N., Zhao, X., & Zhang, X. H. (2018). Characterization and overexpression of a glycosyl hydrolase family 16 beta-agarase YM01-1 from marine bacterium *Catenovulum agarivorans* YM01T. Protein Expression and Purification, 143, 1-8. https://doi.org/10.1016/j.pep.2017.10.002

Atlas R. M. (Ed.) (2010). Media, alphabetical. In: Handbook of microbiological media 4th ed. Chemical Rubber Company Press. pp.11-1879, Florida, USA.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (2003). Current protocols in molecular biology supplement 59. John Wiley & Sons. pp. 122-220. New York, USA. https://www.wiley.com/enau/Current+Protocols+in+Molecular+Biology+-p-9780471503385

Baleta, Z. A., Abou-Dobara, M. I., & El-Sayed, A. K. (2024). Isolation and optimization of agarase enzyme by marine bacterium *Bacillus subtilis*. Scientific Journal for Damietta Faculty of Science, 14(1), 129-141.

https://doi.org/10.21608/sjdfs.2024.250619.1146

Barbeyron, T., Flament, D., Michel, G., Potin, P., & Kloareg, B. (2001). The sulphated-galactan hydrolases, agarases and carrageenases: structural biology and molecular evolution. Cahiers de Biologie Marine, 42(1-2), 169-183. https://www.vliz.be/imisdocs/publications/289157.pdf

Cha, J. A., Kim, Y. J., Seo, Y. B., & Yoon, M. H. (2009). Isolation of an agarolytic bacteria, *Cellvibrio mixtus* SC22 and the enzymatic properties. Journal of Applied Biological Chemistry, 52(4), 157-162. https://doi.org/10.3839/jabc.20099.027

Cheba, B. A. (2022). *Bacillus* sp. R2 Agarase Production: Medium Composition and Fermentation Conditions

Optimization. Science Nature, 5(1), 1-6. http://www.worldascience.com/journals/index.php/wassn/article/view/35

Chi, W. J., Chang, Y. K., & Hong, S. K. (2012). Agar degradation by microorganisms and agar-degrading enzymes. Applied Microbiology and Biotechnology, 94(4), 917-930. https://doi.org/10.1007/s00253-012-4023-2

Chi, W. J., Park, J. S., Kang, D. K., & Hong, S. K. (2014). Production and characterization of a novel thermostable extracellular agarase from *Pseudoalteromonas hodoensis* newly isolated from the west sea of South Korea. Applied Biochemistry and Biotechnology, 173(7), 1703-1716. https://doi.org/10.1007/s12010-014-0958-3

Chiura, H. X., & Kita-Tsukamoto, K. (2000). Purification and characterisation of a novel agarase secreted by a marine bacterium, *Pseudoalteromonas* sp. strain CKT1. Microbes and Environments, 15(1), 11-22.

https://doi.org/10.1264/jsme2.2000.11

Dong, C., Lin, B., Song, Y., Peng, T., Zhong, M., Li, J., & Hu, Z. (2021). Characterization and activity enhancement of a novel exo-type agarase Aga575 from *Aquimarina agarilytica* ZC1. Applied Microbiology and Biotechnology, 105(21-22), 8287-8296. https://doi.org/10.1007/s00253-021-11553-y

Farahat, M.G. (2019). Enhanced anti-oxidant activity of neoagarooligosaccharides produced by β-agarase derived from *Aquimarina agarilytica* NI125. Novel Research in Microbiology Journal, 3(6), 511-525. https://dx.doi.org/10.21608/nrmj.2019.66744

Fu, X. T., Lin, H., & Kim, S. M. (2009). Optimization of medium composition and culture conditions for agarase production by *Agarivorans albus* YKW-34. Process Biochemistry, 44(10), 1158-1163. https://doi.org/10.1016/j.procbio.2009.06.012

Garrity, G. M., Staley, J. T., Boone, D. R., De Vos, P., Goodfellow, M., Rainey, F. A., Schleifer, K. H., Brenner, D. J. & Krieg N. R. (Eds.) (2007). Bergey's Manual® of Systematic Bacteriology: Second Edition: Volume Two: The

Proteobacteria: Part B: The Gammaproteobacteria. Springer Science and Business Media. Springer, New York, USA.https://books.google.com/books/about/Bergey_s_Manual_of_Systematic_Bacteriolo.html?id=5zSYmcq0G dgC

Gauthier, G., Gauthier, M., & Christen, R. (1995). Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. International Journal of Systematic and Evolutionary Microbiology, 45(4), 755-761. https://doi.org/10.1099/00207713-45-4-755

Ghanbarzadeh, M., Golmoradizadeh, A., & Homaei, A. (2018). Carrageenans and carrageenases: Versatile polysaccharides and promising marine enzymes. Phytochemistry Reviews, 17(3), 535-571. https://doi.org/10.1007/s11101-018-9548-2

Ha, D., Thao, T. T. P., THOA, L., Dung, T. T., Dung, T. Q., Lan, T. T. P., & Huy, N. D. (2023). Isolation of an agricultural soil agarolytic *Flavobacterium* sp. AT2 and its agarase characterization. Applied Ecology and Environmental Research, 21(5), 3911-3922. http://dx.doi.org/10.15666/aeer/2105_39113922

Hofsten, B. V., & Malmqvist, M. (1975). Degradation of agar by a Gram-negative bacterium. Journal of General Microbiology, 87(1), 150-158. https://doi.org/10.1099/00221287-87-1-150

Ivanova, E. P., Sawabe, T., Alexeeva, Y. V., Lysenko, A. M., Gorshkova, N. M., Hayashi, K., & Mikhailov, V. V. (2002). *Pseudoalteromonas issachenkonii* sp. nov., a bacterium that degrades the thallus of the brown alga *Fucus evanescens*. International Journal of Systematic and Evolutionary Microbiology, 52(1), 229-234.

https://doi.org/10.1099/00207713-52-1-229

Jang, W. Y., Kwon, M. J., Kim, K. Y., & Kim, Y. H. (2021). Enzymatic characterization of a novel recombinant 1, 3-α-3, 6-anhydro-L-galactosidase specific for neoagarobiose hydrolysis into monosaccharides. Applied Microbiology and Biotechnology, 105(11), 4621-4634. https://doi.org/10.1007/s00253-021-11341-8

Jung, C., Kwon, H., Park, C., & Lee, J. (2012). Optimization of *Pseudoalteromonas* sp. JYBCL 1 culture conditions, medium composition and extracellular β-Agarase activity. Biotechnology and Bioprocess Engineering, 17(5), 937-945. https://doi.org/10.1007/s12257-012-0009-2

Jung, S., Lee, C. R., Chi, W. J., Bae, C. H., & Hong, S. K. (2017). Biochemical characterization of a novel coldadapted GH39 β-agarase, AgaJ9, from an agar-degrading marine bacterium *Gayadomonas joobiniege* G7. Applied Microbiology and Biotechnology, 101(5), 1965-1974. https://doi.org/10.1007/s00253-016-7951-4

Khambhaty, Y., Mody, K., & Jha, B. (2008). Purification, characterization and application of a novel extracellular agarase from a marine *Bacillus megaterium*. Biotechnology and Bioprocess Engineering, 13(5), 584-591.

https://doi.org/10.1007/s12257-008-0026-3

Kobayashi, R., Takisada, M., Suzuki, T., Kirimura, K., & Usami, S. (1997). Neoagarobiose as a novel moisturizer with whitening effect. Bioscience, Biotechnology, and Biochemistry, 61(1), 162-163. https://doi.org/10.1271/bbb.61.162

Kolhatkar, N., & Sambrani, S. (2018). Isolation and identification of agar degrading bacteria from marine environment. IOSR Journal of Pharmacy and Biological Sciences, 13(3), 1-7. https://doi.org/10.9790/30081303030107

Labade, D., Sevamani, S., Tabassum, H., Madhyastha, H., & Wani, M. (2024). Statistical optimization of process variables for agarase production using *Microbacterium* sp. SS5 strain from non-marine sources. Preparative Biochemistry and Biotechnology, 54(3), 393-406.

https://doi.org/10.1080/10826068.2023.2245866

Lakshmikanth, M., Manohar, S., Patnakar, J., Vaishampayan, P., Shouche, Y., & Lalitha, J. (2006). Optimization of culture conditions for the production of extracellular agarases from newly isolated *Pseudomonas aeruginosa* AG LSL-11. World Journal of Microbiology and Biotechnology, 22(5), 531-537. https://doi.org/10.1007/s11274005-9068-2

Lane, D.J. (1991). 16S/23S rRNA sequencing. In: nucleic acid techniques in bacterial systematics (ed. by Stackebrandt E., Goodfellow M.). Chichester, Wiley. pp. 115-175.

https://cir.nii.ac.jp/crid/1570291225428119552

Li, A., Luo, H., Hu, T., Huang, J., Alam, N. U., Meng, Y., & Li, O. (2019). Screening and enzymatic activity of high-efficiency gellan lyase producing bacteria *Pseudoalteromonas hodoensis* PE1. Bioengineered, 10(1), 240249. https://doi.org/10.1080/21655979.2019.1628882

Li, R. K., Ying, X. J., Chen, Z. L., Ng, T. B., Zhou, Z. M., & Ye, X. Y. (2020). Expression and characterization of a GH16 family β-agarase derived from the marine bacterium *Microbulbifer* sp. BN3 and its efficient hydrolysis of agar using raw agar-producing red seaweeds *Gracilaria sjoestedtii* and *Gelidium amansii* as substrates. Catalysts, 10(8), 885. https://doi.org/10.3390/catal10080885

Liu, G., Wu, S., Jin, W., & Sun, C. (2016). Amy63, a novel type of marine bacterial multifunctional enzyme possessing amylase, agarase and carrageenase activities. Scientific Reports, 6(18726), 1-12.

https://doi.org/10.1038/srep18726

Mehta, V. J., Thumar, J. T., & Singh, S. P. (2006). Production of alkaline protease from an alkaliphilic actinomycete. Bioresource Technology, 97(14), 1650-1654. https://doi.org/10.1016/j.biortech.2005.07.023

Miller, G. L. (1959). Use of Dinitrosalicylic Acid reagent for determination of reducing sugar. Analytical Chemistry 31(3), 426-428. https://doi.org/10.1021/ac60147a030

Nagar, S., Gupta, V. K., Kumar, D., Kumar, L., & Kuhad, R. C. (2010). Production and optimization of cellulasefree, alkali-stable xylanase by *Bacillus pumilus* SV-85S in submerged fermentation. Journal of Industrial Microbiology and Biotechnology, 37(1), 71-83. https://doi.org/10.1007/s10295-009-0650-8

Nguyen, T. H., & Nguyen, V. D. (2017). Characterization and applications of marine microbial enzymes in biotechnology and probiotics for animal health. Advances in Food and Nutrition Research, 80, 37-74. https://doi.org/10.1016/bs.afnr.2016.11.007

O'Connor, B. P. (2000). SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. Behavior Research Methods, Instruments, and Computers, 32(3), 396–402.

https://doi.org/10.3758/BF03200807

Park, S. H., Lee, C. R. & Hong, S. K. (2020). Implications of agar and agarase in industrial applications of sustainable marine biomass. Applied Microbiology and Biotechnology, 104(7), 2815- 2832. https://doi.org/10.1007/s00253-020-10412-6

Romanenko, L. A., Zhukova, N. V., Rohde, M., Lysenko, A. M., Mikhailov, V. V., & Stackebrandt, E. (2003). *Pseudoalteromonas agarivorans* sp. nov., a novel marine agarolytic bacterium. International Journal of Systematic and Evolutionary Microbiology, 53(1), 125-131. https://doi.org/10.1099/ijs.0.02234-0

Roseline, T. L., & Sachindra, N. M. (2018). Purification and characterization of agarase from marine Bacteria *Acinetobacter* sp. PS12B and its use for preparing bioactive hydrolysate from agarophyte red seaweed *Gracilaria verrucosa*. Applied Biochemistry and Biotechnology, 186(1), 66-84. https://doi.org/10.1007/s12010-018-2726-2

Shah, F., & Mishra, S. (2020). In vitro optimization for enhanced cellulose degrading enzyme from *Bacillus licheniformis* KY962963 associated with a microalgae *Chlorococcum* sp. using OVAT and statistical modeling.

SN Applied Sciences, 2(1923), 1-10. https://doi.org/10.1007/s42452-020-03697-9

Sharabash, M. M., Abouelkheir, S. S., Mabrouk, M. E., Ghozlan, H. A., & Sabry, S. A. (2022). Agarase production by marine *Pseudoalteromonas* sp. MHS: Optimization, and purification. Journal of Marine Science, 4(1), 11-22. https://doi.org/10.30564/jms.v4i1.3875

Song, T., Xu, H., Wei, C., Jiang, T., Qin, S., Zhang, W., & Cao, Y. (2016). Horizontal transfer of a novel soil agarase gene from marine bacteria to soil bacteria via human microbiota. Scientific Reports, 6(34103), 1-10. https://doi.org/10.1038/srep34103

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution, 10(3), 512-526.

https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution, 38(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

Thi Nguyen, H. Y., & Tran, G. B. (2018). Optimization of fermentation conditions and media for production of glucose isomerase from *Bacillus megaterium* using response surface methodology. Scientifica, 2018(1), 1-11.

https://doi.org/10.1155/2018/6842843

Veerakumar, S., & Manian, R. (2022). Agarase, amylase and xylanase from *Halomonas meridiana*: A Study on optimization of coproduction for biomass saccharification. Fermentation, 8(10), 479. https://doi.org/10.3390/fermentation8100479

Vera, J., Alvarez, R., Murano, E., Slebe, J. C., & Leon, O. (1998). Identification of a marine agarolytic

Pseudoalteromonas isolate and characterization of its extracellular agarase. Applied and Environmental Microbiology, 64(11), 4378-4383. https://doi.org/10.1128/AEM.64.11.4378-4383.1998

You, Y., Xie, W., Li, C., Gu, Z., Ban, X., Zhang, F., & Li, Z. (2023). Characterization and efficient production of an α-agarase from marine bacterium *Catenovulum maritimum* STB14. Food Bioengineering, 2(1), 3-14. https://doi.org/10.1002/fbe2.12037

Zhao, C. H., Luo, J. J., Gong, T., Huang, X. L., Ye, D. Z., & Luo, Z. H. (2014). *Pseudoalteromonas xiamenensis* sp. nov., a marine bacterium isolated from coastal surface seawater. International Journal of Systematic and Evolutionary Microbiology, 64(Pt_2), 444-448. https://doi.org/10.1099/ijs.0.050229-0

Ziayoddin, M., Lalitha, J., & Shinde, M. (2014). Optimization of agarase production by alkaline *Pseudomonas aeruginosa* ZSL-2 using Taguchi experimental design. International Letters of Natural Sciences, 12(2), 180-193. https://doi.org/10.56431/p-i3fum7

Zilda, D. S., Patantis, G., Dewi, A. S., Khatulistiani, T. S., Sibero, M. T., & Li, J. (2024). Characterization of βagarase produced by *Alteromonas macleodii* BC7. 1 and its oligosaccharide products. Case Studies in Chemical and Environmental Engineering, 10, 100865. https://doi.org/10.1016/j.cscee.2024.100865

الملخص العربي

عنوان البحث: تعريف وتحسين الظروف المزرعية للبكتيريا البحرية المحللة للأجار *Pseudoalteromonas agarivorans* EGPS36 المعزولة من مصر

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تم جمع ٤٤ عزلة بكتيرية مختلفة الشكل من البكتيريا البحرية من ساحل البحر الأبيض المتوسط بمدينة بورسعيد، مصر. تم استخدام وسط محلول الملح القاعدي لتقييم تكوين الأجاريز في جميع البكتيريا المعزولة. تم استخدام محلول لوجول اليود لتقييم تكوين الأجاريز نوعيا .كشفت ٨ عزلات بكتيرية من أصل ٤٤ عن قابلية تكوين الأجاريز. من الناحية الكمية، تم تقييم عمل الأجاريز باستخدام طريقة ٥،٣- داي نيترو سالسيلك أسيد. تم تعريف العزلة الأعلى المنتجة لإنزيم الأجاريز من خلال تقييم الخصائص المورفولوجية والكيميائية الحيوية والجينية. أكدت الإختبارات الكيميائية والتسلسل الجيني16 S rRNA أن العزلة البكتيرية البحرية EGPS30 تم تجميعها بطريقة مع *Pseudoalteromonas agarivorans* وعينت برقم انضمام (PQ203725) في بنك الجينات. كان نمو *Pseudoalteromonas agarivorans* EGPS36 الأعلى وفعالية الأجاريز متوافقين مع حجم اللقاح ، سرعة اهتزاز، فترة التحضين، درجة الحرارة، الأس الهيدروجيني، ومستوي كلوريد الصوديوم وكانت ٢ في المائة، ١٥٠دورة في الدقيقة، ٤٨ ساعة، ٣٥ درجة مئوية، ٨، و ٣،٥ في المائة على التوالى. تم تحقيق النمو الأعلى وفاعلية الأجاريز من خلال استخدام الأجار كمصدر للكربون من خلال مستوي ٠،٤ في المائة. في حين انخفض النمو وتكوين الأجاريز عند تطبيق الجالكتوز، الجلوكوز، الرامنوز، السكروز، الأرابينوز، الرافينوز، السليولوز، الزيلان، والزيلوز .تم تحقيق النمو الأعلى وفاعلية الأجاريز من خلال استخدام مستخلص الخميرة كمصدر للنتيروجين من خلال مستوي ٠،٣ في المائة .على الجانب الاخر انخفض النمو وتكوين الأجاريز عند استخدام التريبتوفان والتريبتون.