



Phylogenetic Diversity and Enzymatic Activities of Cultivable Actinomycetes Isolated from Marine Sediment at Ain Achir (Gulf of Annaba, Algeria)

Ilhem Meriane¹ · Stuart McMillan² · Andrew P. Desbois² · Mahmoud Kitouni¹

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Abstract

Algerian marine sediments are an underexplored and potentially rich source of microorganisms that may produce useful metabolites and enzymes. Here, we investigated the phylogenetic diversity and enzymatic activities of suspected *Actinomycetia* bacteria isolated from seabed sediment at Ain Achir, Gulf of Annaba, Algeria. Morphological, physiological and taxonomical properties were characterized and their potential to produce enzyme activities was studied. Phylogenetic analysis of partial 16S rRNA gene sequences assigned isolates into five main genera: *Rhodococcus* (21/35), *Microbacterium* (5/35), *Streptomyces* (3/35), *Brevibacterium* (2/35) and *Agromyces* (2/35). Screening for 14 enzymatic activities demonstrated their abilities to metabolize a wide range of substrates including DNA, L-asparagine, and L-glutamine (each degraded by all 35 isolates). Other substrates degraded by most isolates included uric acid and urea (both 33/35), starch (25/35) and tyrosine (24/35). Moreover, the number of enzymatic activities possessed by the isolates ranged from six (one isolate) to 10 (four isolates). This study is the first to investigate the phylogenetic diversity and enzymatic activities of actinomycetes isolated from sea sediment in the Gulf of Annaba and it confirms that actinomycetes from the marine environment are a valuable source of enzymes, with further investigation required to unlock their potential commercial applications.

Keywords Actinomycetota · Biodiscovery · Chitinase · Proteases · Streptomycetes

1 Introduction

Actinomycetia bacteria are free-living, Gram-positive bacteria with high guanosine-cytosine (GC) content in their DNA (70–80%). They are morphologically and phylogenetically diverse and exhibit great metabolic diversity, which makes them an important source of novel enzymes (Lewin et al. 2016; Trujillo 2016). Actinomycetes mainly occur as saprophytes and are widely distributed in natural habitats,

including soil, composts and in association with plants, as well as freshwater, marine and arid environments (Bhatti et al. 2017; Lewin et al. 2016; Trujillo 2016). Moreover, actinomycetes have been isolated from the water and sediment of various marine ecosystems, including the deep sea (Anandan et al. 2016), and from various marine organisms including fish, mollusks, algae, corals, sponges, shrimps, ascidians and sea cucumbers (Chen et al. 2021). Marine actinomycetes are a potential source of enzymes, enzyme inhibitors, pigments and bioactive molecules for future biotechnological applications (Kuo et al. 2023; Selim et al. 2021). Moreover, enzymes from marine organisms may be capable of performing reactions under conditions distinct from terrestrial strains, thus making them suitable for additional applications. However, compared to terrestrial actinomycetes, marine counterparts have been relatively underexplored despite the unique metabolic properties such strains possess (Girão et al. 2022). Indeed, these special bacteria have shown potential for various agricultural

✉ Andrew P. Desbois
ad54@stir.ac.uk

¹ Laboratory of Microbiological Engineering and Applications, Department of Microbiology, Faculty of Nature and Life Sciences, University of Constantine 1, Frères Mentouri, 25000 Constantine, Algeria

² Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK

and environmental applications, including bioremediation of toxic heavy metals, polycyclic aromatic hydrocarbons, pesticides and radioactive wastes, and they also exhibit anti-biofouling, biosurfactant and phytopathogen control properties (Ettoumi et al. 2016; Jagannathan et al. 2021; Jeddi et al. 2022). Cellulases produced by a *Gordonia* sp. BPSGA4 demonstrated potential to hydrolyze biomass of *Cymodocea* spp. seagrasses for bioethanol production (Rajkumar et al. 2021), whilst chitinases produced by another marine actinomycete, *Curtobacterium* sp. CBMAI 2942, exerted antifungal activity against the tomato pathogen, *Aspergillus* sp. *Nigri* series CBMAI 1846, which therefore could be developed as a biocontrol agent (Vasquez et al. 2024). Furthermore, *Rhodococcus ruber* MSA14, isolated from marine sediments in Baja California, Mexico, was found to contain more than 50 genes that encode enzymes capable of catabolizing aromatic hydrocarbons, thus offering promise for application in the bioremediation of oil-polluted sites (Embarcadero-Jiménez et al. 2024).

In Algeria, actinomycetes for biodiscovery purposes have been mainly isolated from terrestrial environments, particularly different soils (Djinni et al. 2019; Yekkour et

al. 2022), although some studies have been performed with strains from aquatic habitats, including hypersaline wetlands (Ayari et al. 2016; Bezuidt et al. 2016; Djinni et al. 2019; Menasria et al. 2022; Oussadi and Kitouni 2015). Still, relatively little is known for strains that inhabit sediment in the Mediterranean Sea off the coast of Algeria (Boublenza et al. 2024; Matmoura et al. 2023; Ouchene et al. 2022). Thus, the aim of the present study was to investigate the phylogenetic diversity and enzymatic activities of culturable marine actinomycetes isolated from sediment collected from the coast of Ain Achir located in the Gulf of Annaba, Algeria.

2 Materials and Methods

2.1 Study Area and Sampling

The Gulf of Annaba is located on the east coast of Algeria. It is open to the Mediterranean Sea to the north and stretches for 40 km between Cape Rosa in the east ($36^{\circ} 58' \text{ N}$, $8^{\circ} 15' \text{ E}$) and Cape Guard in the west ($7^{\circ} 47' \text{ E}$, $36^{\circ} 58' \text{ N}$) (Fig. 1), with a maximum water depth of 65 m (Boutabia-Trea et al.

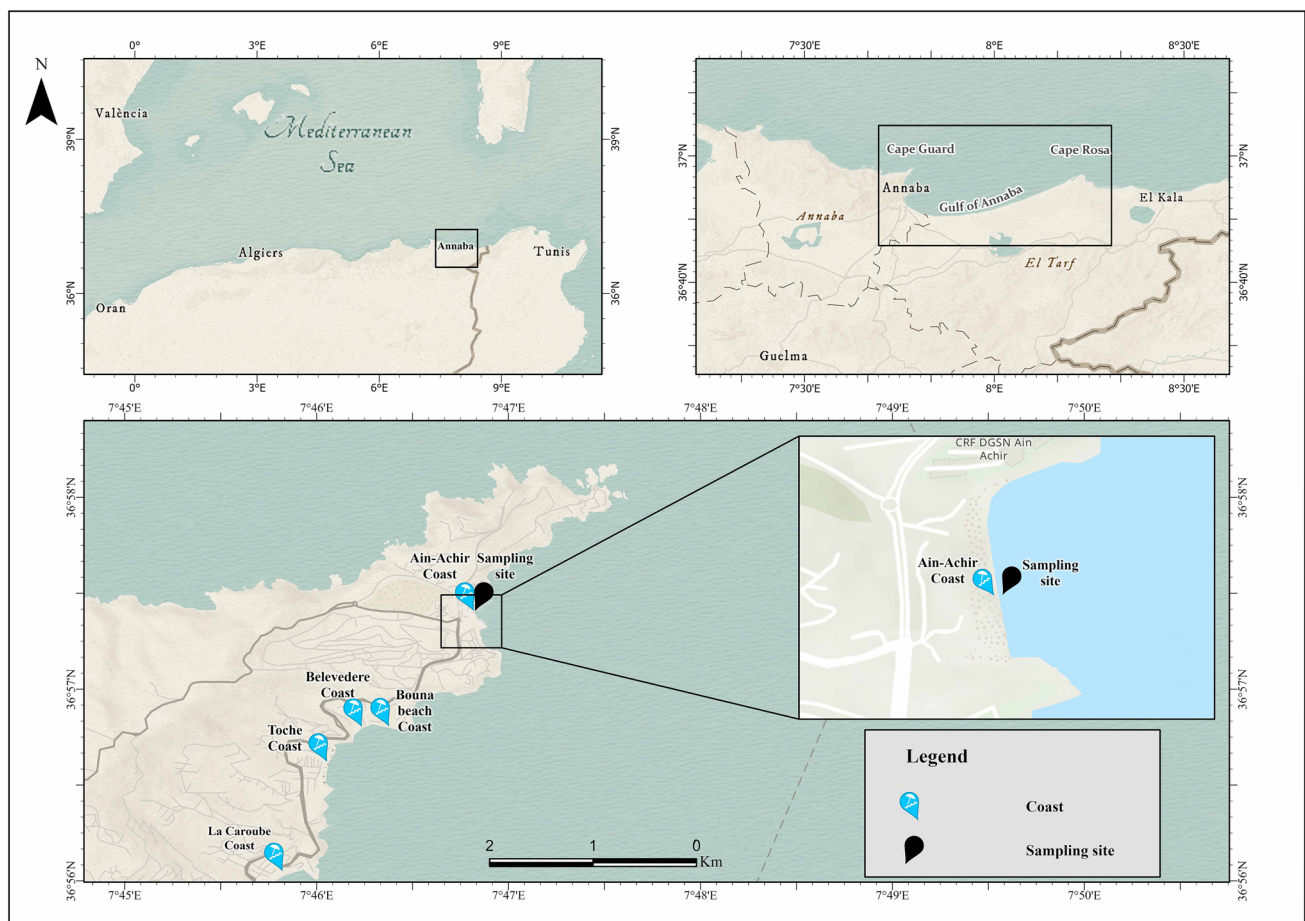


Fig. 1 Location of the sampling site in Ain-Achir, Gulf of Annaba, Algeria

2015). A sediment sample was collected on 18th September 2014 from the coast at Ain-Achir (36° 57' 28" N, 7° 46' 50" E) at a depth of 10 m and a distance of 120 m from the shore (Fig. 1), using a Van Veen sampler (Mohseni et al. 2013). The sample consisted of a fine dark brown sandy sediment without any stones or vegetation, and it was placed in a sterile plastic box and transported to the laboratory and stored at 4 °C before analysis.

2.2 Measurement of Physicochemical Parameters of the Seawater and Sediment

At the time of sampling, the temperature, salinity and pH of the seawater were measured using a multiparameter portable meter (HI9828; HANNA Instruments, France). Electrical conductivity, salinity and pH measurements, along with analysis of organic and mineral content of the marine sediment, were conducted by Laboratoire Horizon (Annaba, Algeria). Further details of physicochemical properties of the seawater, the sampling site, and sediment sample are provided in Supplementary Table S1.

2.3 Isolation, Purification and Maintenance of Suspected Marine Actinomycetes

2.3.1 Isolation Media

All chemicals and reagents were purchased from Sigma-Aldrich (UK and Germany), unless stated. Fourteen agar media were used for the selective isolation of marine actinomycetes (Supplementary Table S2). These media were chosen as they offer a range of different carbohydrates, proteins and amino acids, and inorganic salts in their compositions, which provide conditions to encourage the isolation of a diversity of species. These media were prepared with natural seawater collected from the coast at Ain-Achir. All media components were sterilized by autoclaving at 121 °C for 20 min before use. To inhibit fungal contaminants the media were supplemented with 10 µg/mL amphotericin B, dissolved and diluted in sterile distilled water (Remya and Vijayakumar 2008).

2.3.2 Pretreatment and Isolation

First, the sediment sample was air-dried aseptically in a laminar flow hood (room temperature [RT], 24 h) before being used to inoculate the various agar media by each of the following five methods, in an attempt to increase the likelihood of success for recovering actinomycetes. For Method 1 (dry/stamp), the dried sediment was ground lightly with an alcohol-sterilized mortar and pestle, before being taken up on a sterile glass rod and stamping around the surface of

the agar plate to create a serial dilution effect (Jensen et al. 2005; Mincer et al. 2002). For Method 2 (dry/dilute), 0.5 g of sediment was suspended in 5 mL of sterile (autoclaved) seawater (SSW). The diluted sample was vigorously mixed by vortex, allowed to settle for 10 min, and then 100 µL of suspension was inoculated onto the surface of an agar plate and spread (Jensen et al. 2005). For Method 3 (dry/dilute/heat), 1 g of sediment was suspended in 3 mL SSW, mixed vigorously by vortex and heated to 55 °C for 6 min. The suspension was allowed to settle for 15 min and then 75 µL was inoculated and spread across the agar surface (Jensen et al. 2005). For Method 4 (dry/dilute/sonicate), 0.5 g of sediment was suspended in 5 mL of SSW, mixed by vortex, allowed to settle for 10 min, and then disrupted using a 750-W sonicator (Bioblock Scientific, France) for 10 min (50%-pulsed). Next, 100 µL of suspension was inoculated onto the surface of an agar plate and spread (Jensen et al. 2005; Ouhdouch et al. 2001). For Method 5 (dry/stamp/dilute/heat), the dried sediment was ground lightly with an alcohol-sterilized mortar and pestle, before an autoclaved glass rod was pressed onto the sediment and then repeatedly onto the surface of an agar plate, in a clockwise direction, to create a serial dilution effect (Jensen et al. 2005; Mincer et al. 2002). Following this, a further 1 g of the dried sediment was diluted with 3 mL of SSW, mixed vigorously by vortex, and heated at 55 °C for 6 min. The suspension was allowed to settle for 15 min, and then 50 µL was inoculated and spread onto that same agar plate (Jensen et al. 2005). Inoculated agar plates were incubated at 28 °C for 7–30 days.

2.3.3 Purification and Maintenance

Individual colonies of suspected marine actinomycetes were picked with a sterile loop and sub-cultured onto fresh plates of the same medium. Only one colony with the same visual characteristics (i.e., color, morphology, reverse side pigments and size) was selected from the same medium. For short-term storage, cultures were maintained on agar slants at 4 °C, whilst for long-term storage cultures were stored at – 20 °C in broth supplemented with glycerol (20% v/v).

2.4 Initial Characterization and Identification of Isolates

Colony characteristics and the presence of aerial and substrate (reverse side) mycelium were observed after culture at 28 °C for up to 21 days on yeast extract–malt extract agar (ISP 2). Colors were determined using the RAL-code, a standardized universal system that uses a numerical code to indicate a defined color (<https://www.ralcolorchart.com/ral-classic>). Microscopic characterization was carried out using the cover slip culture technique, where a sterile cover slip

was inserted at 45° into the agar media. A colony was then streaked along the interface where the cover slip met the agar and the plate incubated at 28 °C for up to 7 days (Williams and Cross 1971). Gram staining was performed (Attimarad et al. 2012) and each isolate examined under a light microscope (BH-25; Olympus, Japan). Phenotypic characteristics were used for tentative identification to genus level using several resources (Bergey 1994; Goodfellow 2012; Rosenberg et al. 2014). Melanin pigment production of each isolate was determined on ISP 6 and ISP 7 agar for up to 20 days of incubation at 28 °C (Shirling and Gottlieb 1966). The ability of each isolate to grow at different temperatures (4, 20, 30, 37 and 45 °C; at pH 7 and 0% NaCl), pH (3, 5, 7 and 12; at 28 °C and 0% NaCl) and NaCl concentrations (7, 10, 12 and 15%; at pH 7 and 28 °C) was tested on ISP 2 agar, with plates observed for growth for up to 10 days.

2.5 16S rRNA Gene Amplification and Phylogenetic Analysis

Amplification of the 16S rRNA gene was completed as described by Berdolt et al. (2008) with some modifications. Genomic DNA was extracted from fresh colonies grown on actinomycete isolation agar (AIA) and transferred to 100 µL of Milli-Q ultra-pure water (Millipore Ltd, Watford, UK). Samples were mixed by vortex and heated to 98 °C for 10 min before centrifugation (14,500×g, RT, 5 min). Supernatants were transferred to fresh tubes and then 300 µL absolute ethanol was added before mixing by inversion. Samples were centrifuged (14,500×g, RT, 5 min), supernatants discarded, and each pellet dissolved in 20 µL Tris–EDTA (TE) buffer (pH 8.0), before mixing, centrifuging (3000×g, RT, 2 s), and storing at 4 °C. Genomic DNA was quantified by spectrophotometry (Nano Drop ND-1000; Thermo Fisher Scientific Inc, Wilmington, DE, USA) and each sample adjusted to 50 ng/mL with Milli-Q water.

Polymerase chain reaction (PCR) amplification was completed with primers targeting the V3–V5 region of the 16S rRNA gene: S-C-Act-235-S-20 (5′-CGCGGCCTATCAGCTTGTTG-3′) and S-C-Act-878-A-19 (5′-CCGTACTCCCCAGCGGGG-3′) (Eurofins Genomics, Ebersberg, Germany; Stach et al. 2003) using a gradient thermocycler (Biometra T; Biometra, Göttingen, Germany). Reaction mixtures consisted of 2 µL genomic DNA (5 ng), 1 µL of each primer (10 µM), 10 µL of 2×Q5 HotStart High Fidelity master mix (New England Biolabs Inc, Ipswich, USA), and made up to 20 µL with Milli-Q water. The PCR began with preheating at 98 °C for 1 min, then 30 cycles of denaturing at 98 °C for 15 s, annealing at 70 °C for 15 s and extension at 72 °C for 20 s, before a final extension at 72 °C for 2 min. PCR products were visualized by gel electrophoresis (1% agarose with 50 ng/mL ethidium bromide) with a 100-bp

DNA ladder (Thermo Scientific). PCR products in bands were purified using GeneJet PCR-purification kit (Thermo Scientific) according to the manufacturer's instructions and sequenced (Eurofins Genomics).

The partial 16S rRNA gene sequences (503–617 bp) were analyzed using Bioedit software (<https://bioedit.software.informer.com/7.2>) and deposited into the NCBI GenBank database (accession numbers MK130802 to MK130834). The sequences were compared with available 16S rRNA gene sequences by BLASTn, with multiple sequence alignments then performed with MUSCLE (Madeira et al. 2024) and a maximum likelihood tree constructed by neighbor-joining method using MEGA 10.1.8 software (Kumar et al. 2018). Genera-level identities were proposed for each isolate based on sequence similarity.

2.6 Screening of Isolates for Enzyme Activities

Isolates were screened for their abilities to degrade substrates using qualitative agar plate assays, specifically starch, cellulose, chitin, casein, gelatin, tyrosine, DNA, pectin, Tween 80, lecithin, uric acid, L-glutamine, L-asparagine and urea, which thus detected various enzyme activities. The assay methods for detecting the various enzyme activities and the supporting references can be found in Supplementary Table 3. Typically, each isolate was streaked in a straight line or spotted onto the agar containing the appropriate test substrate. Inoculated plates and tubes were incubated at 28°±2 °C and checked for activities during 3–30 days.

3 Results

3.1 Isolation of Bacteria from Marine Sediment

Thirty-five colonies resembling actinomycetes were isolated and these were designated ID 1 to ID 35. Of the 14 isolation media tested, nine yielded actinomycete-like colonies, with seven isolates collected from SGIA, followed by GGA (6 isolates), then AIA and RHA (5 isolate each), GAA (4 isolates) and finally M1, M2, SCN and KA (2 isolates each) (see Supplementary Table S2 for abbreviations; Supplementary Table S4). Of the sample pre-treatment methods, all yielded suspected actinomycete isolates, with Method 2 leading to the isolation of 10 isolates, followed by Method 4 (9 isolates) and Method 3 (7 isolates) (Supplementary Table S4).

3.2 Identification by Colony Morphology

After inoculation onto ISP 2 agar media, three isolates formed well-developed aerial mycelium after 5 days that

was either white (ID 11 and ID 15) or grey (ID 33) in color, whilst ID 8 formed a yellow-orange aerial mycelium after 7 days (Supplementary Table S5). On ISP 2, most isolates formed colonies of different sizes and colors (mostly whites, yellows and oranges) typically after 48–72 h, with three isolates (ID 11, ID 15 and ID 33) taking up to 5 days to form colonies. A detailed description of the colony morphology after culture on ISP 2 is given in Supplementary Table S5.

3.3 Micromorphological Identification

Suspected actinomycete cells were observed as various shapes by microscopic examination (Supplementary Table S5). Gram stain results showed that the isolates were Gram positive to Gram variable (Supplementary Table S4). Four isolates produced brown melanoid pigments, specifically ID 4 on ISP 7 medium, ID 11 on ISP 6 and ISP 7 media, ID 20 on ISP 6 medium, and ID 21 on ISP 6 and ISP 7 media (Supplementary Table S4).

3.4 Physicochemical Growth Characteristics

All isolates grew on ISP 2 at pH 5 and pH 7, and the majority (with the exception of isolate ID 14) on ISP 2 at pH 12 (Table 1). Only two isolates (ID 14 and ID 19) were unable to form colonies on ISP 2 at pH 3 (Table 1). All isolates formed colonies at 20 °C, 30 °C, 37 °C and 45 °C, whilst at 4 °C only two isolates (ID 15 and ID 33) did not develop (Table 1). All isolates showed growth in the presence of 7% and 10% NaCl, whilst all exhibited growth at 12% NaCl (with the exception of ID 15); all but three isolates grew at 15% NaCl (Table 1).

3.5 Molecular Characterization and Phylogenetic Analysis

Of the 35 isolates, 33 yielded acceptable quantities of DNA to permit analysis of partial 16S rRNA gene sequences, but it was not possible to extract sufficient quantities of DNA for two isolates (ID 26 and ID 34). BLASTn of the partial 16S rRNA gene sequences indicated that these 33 isolates belonged to five genera of *Actinomycetia*: *Rhodococcus* (*Nocardiaceae*) (21/35 isolates), *Microbacterium* (*Microbacteriaceae*) (5/35 isolates), *Streptomyces* (*Streptomycetaceae*) (3/35 isolates), *Agromyces* (*Microbacteriaceae*) (2/35 isolates) and *Brevibacterium* (*Brevibacteriaceae*) (2/35 isolates) (Fig. 2; Supplementary Table 6).

3.6 Enzymatic Activities

All isolates showed at least six enzymatic activities among the fourteen different activities tested (Table 2). The isolate

with the fewest activities was ID 16, whereas four isolates possessed enzymatic activities to degrade 10 different substrates (ID 1, ID 4, ID 21 and ID 33). DNA, L-asparagine, and L-glutamine were degraded by all the isolates, with uric acid and urea degraded by 33/35 isolates (Table 2). Starch was degraded by 25/35 isolates, whilst tyrosine was degraded by 24/35 isolates (Table 2). Fewer isolates degraded lecithin (9/35) and gelatin (7/35) (Table 2). Tween 80 was degraded by isolate ID 1 only and none of the isolates could degrade the pectin (Table 2).

4 Discussion

In this study, 35 actinomycete isolates from various genera were isolated from seabed sediments in the Gulf of Annaba, and these revealed a considerable range of enzymatic capabilities, thereby confirming this habitat to contain a wealth of microbes worthy of further exploration. Most isolates (32/35) grew in the presence of 15% NaCl, which confirmed their halotolerance and supports the suggestion that these isolates are marine actinomycetes.

Several other studies have isolated actinomycetes from marine sediments around the world including Florida, USA (Christensen and Martin 2017), Tamil Nadu, India (Rao et al. 2017), Bengal Bay, India (Shaik et al. 2017), Turkey (Tuncer and Bizsel 2017), South Sinai, Egypt (Abdelfattah et al. 2016; Hegazy et al. 2023), and the Tyrrhenian Sea, Italy (Ettoumi et al. 2016). The genera identified in our study have been isolated from marine environments previously (Girão et al. 2022) including “rare actinomycetes”, which are defined as non-streptomycetes or actinomycete species whose isolation by culture methods is considerably lower than streptomycetes, such as *Rhodococcus*, *Microbacterium*, *Brevibacterium* and *Agromyces* strains (Suriya et al. 2016). *Rhodococcus*, *Streptomyces* and *Micromonospora* are typical indigenous genera in marine ecosystems (Jensen et al. 2005; Maldonado et al. 2005; Ward and Bora 2006) and we detected the first two of these genera in our isolate collection, with *Rhodococcus* isolates predominating (64% of isolates). Of note, rare halophilic actinomycetes, most abundantly *Actinopolyspora* and *Nocardiopsis* isolates, were studied after being collected from hypersaline wetlands (Sebkhas-Chotts) close to the Algerian coast (Menasria et al. 2022), whilst other studies in Algeria have also reported rare actinomycete genera from other environments (Djinni et al. 2019). In comparison to other studies of culturable actinomycetes from marine sediments, our study concurs with Claverías et al. (2015) who also reported a predominance of *Rhodococcus* isolates from sediments collected in Valparaíso Bay, Chile. Meanwhile, many other studies have reported *Streptomyces* to predominate,

Table 1 Growth of the actinomycete isolates in different conditions of pH, temperature and elevated NaCl concentrations. + : growth observed,—growth absent

Isolate	Culture condition														
	pH			Temperature (°C)					NaCl (%)						
	3	5	7	12	4	20	30	37	45	7	10	12	15		
ID 1	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 2	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 3	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 4	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 5	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 6	+	+	+	+	+	+	+	+	+	+	+	+	−		
ID 7	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 8	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 9	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 10	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 11	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 12	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 13	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 14	−	+	+	−	+	+	+	+	+	+	+	+	−		
ID 15	+	+	+	+	−	+	+	+	+	+	+	−	−		
ID 16	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 17	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 18	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 19	−	+	+	+	+	+	+	+	+	+	+	+	+		
ID 20	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 21	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 22	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 23	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 24	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 25	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 26	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 27	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 28	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 29	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 30	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 31	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 32	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 33	+	+	+	+	+	+	+	+	+	+	+	+	+		
ID 34	+	+	+	+	−	+	+	+	+	+	+	+	+		
ID 35	+	+	+	+	+	+	+	+	+	+	+	+	+		

including in sediments around Costa Rica (Solano et al. 2009), South Africa (Cosa and Okoh 2012), India (Augustine et al. 2013), Turkey (Özcan et al. 2013), China (Chang et al. 2015), Canada (Duncan et al. 2015), and sediments from the North Aegean Sea and Eastern Mediterranean (Tuncer and Bizsel 2017).

Marine actinomycetes produce a wide variety of extracellular hydrolytic enzymes that helps to explain their selective distribution in different marine niches (Cappelletti et al. 2019; Cheng et al. 2020; Kim 2019; Li et al. 2017; Ul-Hassan and Wellington 2009), as these proteins provide a competitive advantage for space and nutrients (Cheng et al. 2020). Moreover, actinomycetes play a role in the breakdown and recycling of organic compounds and diverse biopolymers such as cellulose, chitin and agar, not only in soils but also sediments (Bhatti et al. 2017; Hazarika and Thakur 2020). Consistent with this, the actinomycetes isolated in this present study presented a range of biological activities, indicating the production of enzymes with potential industrial relevance. Whilst the individual biological activities determined by the assays applied in this study are not in themselves novel, the range of activities assessed is performed relatively rarely in an individual study, although similar examples are available (e.g., Menasria et al. 2022).

DNA is an important source of carbon, nitrogen and phosphorus in marine systems and all the isolates in this study demonstrated DNase activity, which could be developed into useful molecular tools (Valsala and Sugathan 2017). All the isolates showed L-asparaginase and L-glutaminase activities, whilst most also showed uricase activity (33/35 isolates). These three enzymes are therapeutically important, with L-asparaginase and L-glutaminase used to treat leukemias due to their antioxidant activities. Uricase helps to reduce uric acid accumulation and supports its elimination in several parts of the human body, thus helping to prevent pathologies, such as gout, hyperuricemia and osteoporosis (Barzkar et al. 2021; Tandon et al. 2021). Many isolates (33/35) showed urease activity, an enzyme that has been used clinically to prevent kidney failure and in dental care (Arias et al. 2017; Phang et al. 2018). In agriculture, urease can be applied with fertilizers to provide adequate nitrogen in soils, while it may also remove excess urea from fermented drinks and alcoholic beverages, and from wastewater (Arias et al. 2017; Phang et al. 2018).

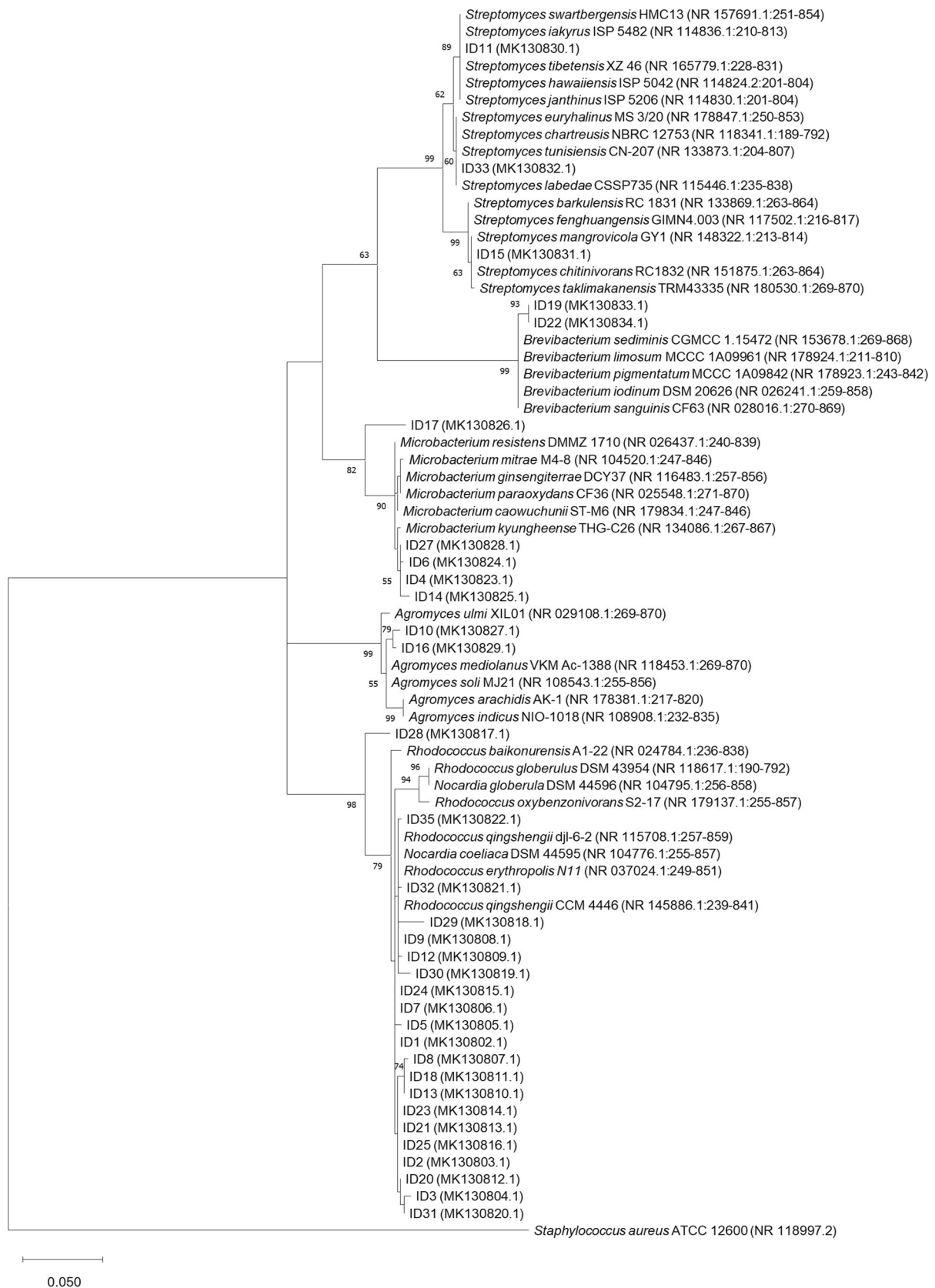
In our study, 25/35 isolates showed amylase activity, which has applications in medical and analytical chemistry, and in food, textile, paper, brewing and baking industries (Farooq et al. 2021). Twenty-four of 35 isolates showed tyrosinase activity, a copper-dependent enzyme that is applied for many purposes including as a detoxifying agent for phenol-containing waters (Roy et al. 2014) and in biosensors for clinical diagnostics and to detect aromatic

compounds in food (Min et al. 2019). Caseinase activity was shown by 23/35 isolates while 7/35 isolates showed gelatinase activity: Marine proteases are used in many industries such as the manufacture of detergents and pharmaceuticals, leather processing and waste treatment (Barzkar 2020).

Marine cellulases are used in biofuel production and in the leather, textile, detergent, agriculture, food, medicine, animal feed and paper sectors (Barzkar and Sohail 2020; Trivedi et al. 2016), and 15/35 isolates in this present study showed cellulase activity. Fifteen of 35 isolates showed chitinase activity and chitin can be an important energy source for marine actinomycetes (Jahromi and Barzkar 2018; Muffler et al. 2015). Moreover, chitinases can help control phytopathogens due to antifungal, insecticidal, and antiparasitic activities (Beygmoradi et al. 2018). Only 9/35 isolates showed lecithinase activity, while only one isolate could degrade Tween 80, indicating a low preponderance of lipolytic activity. Lipases have wide applications in scientific research, medicine, food and chemical industries (Merkulyeva et al. 2021) and may be used as biocontrol agents against nematodes (Sheetal et al. 2019).

Our study concerned only actinomycetes culturable in the specific conditions provided, and this means the isolates collected represent a fraction of the biodiversity in the study area. Indeed, the similarity in partial 16S rRNA gene sequences between the isolates provides support for this suggestion, particularly for the *Rhodococcus* spp. where very high similarity was detected. The limitations of partial sequencing of the 16S rRNA gene are well recognized and more accurate identification of the actinomycete isolates would have been achieved through further sequencing efforts, with full-length 16S rRNA, multilocus approaches and whole-genome sequencing offering more accurate species- and strain-level classifications (Guo et al. 2008; Johnson et al. 2019; Jørgensen et al. 2024; Nouiouei et al. 2018). Furthermore, whilst the sediment sample was collected from the marine environment, the evidence available means it is not possible to conclude with absolute certainty that the isolates cultured are truly distinct from terrestrial counterparts, given that none showed an obligate requirement for salt as all could grow on agar lacking salt supplementation (Mincer et al. 2002). Though beyond the scope of this study, metagenomics approaches provide a more powerful means to gain a deeper insight into the biodiversity of actinomycetes in samples, whilst such methods can also be applied to discover sequences encoding enzymes of interest in non-cultured species (Beaudry et al. 2021; Yang et al. 2024).

There are many future directions that can be taken to develop one or more of the actinomycete isolates towards commercial application, with additional research required to identify and value the major market opportunities for the bacterium itself or specific enzymes it produces.



0.050

◀ **Fig. 2** Neighbor-joining phylogenetic tree constructed with partial 16S rRNA sequences of the isolates collected in this present study (referred to in format 'IDxx') and the species and strain names of the closest matches in GenBank database after BLASTn searches. Evolutionary history was inferred by the maximum likelihood method and Kimura 2-parameter model (Kimura, 1980), and percentage of trees in which the associated taxa clustered together is shown beside each branch (where $\geq 50\%$; 1000 bootstraps). GenBank accession numbers for the partial 16S rRNA sequences, including the ID isolates collected in this present study, are provided in brackets

Engagement with businesses in relevant sectors is an important next step and this could provide the means and impetus for follow-up studies. Further developments may include investigation of the genetic basis of the bioactivities, optimization of bacterial growth and production conditions for selected metabolites and enzymes, purification of these enzymes, and characterization of the conditions under which the bacterium or its products remain efficacious for its intended purpose.

In conclusion, this study adds to the growing knowledge of actinomycetes in Algeria (Djinni et al. 2019; Menasria et al 2022) by demonstrating isolates collected from sediment in the Ain Achir Sea, Gulf of Annaba produce a wide range of enzymes, which could be developed towards applications in medicine, agriculture and food production, textile manufacture, waste treatment and other sectors.

Table 2 Observations on the actinomycete isolates in assays to detect different enzymatic activities through the degradation of various substrates. + : positive observation, —negative observation; n/a, no genus proposed

Isolate	Proposed genus	Substrate														No. of positives
		Starch	Cellulose	Chitin	Casein	Gelatin	Tyrosine	DNA	Pectin	Tween 80	Lecithin	Uric acid	L-glutamine	L-asparagine	Urea	
ID 1	<i>Rhodococcus</i>	+	—	+	+	—	+	+	—	+	—	+	+	+	+	10
ID 2	<i>Rhodococcus</i>	+	—	—	—	—	+	+	—	—	—	+	+	+	+	7
ID 3	<i>Rhodococcus</i>	—	—	—	+	—	+	+	—	—	—	+	+	+	+	7
ID 5	<i>Rhodococcus</i>	—	+	+	—	—	+	+	—	—	—	+	+	+	+	8
ID 7	<i>Rhodococcus</i>	+	—	—	+	—	+	+	—	—	—	+	+	+	+	8
ID 8	<i>Rhodococcus</i>	—	+	—	+	—	+	+	—	+	+	+	+	+	+	9
ID 9	<i>Rhodococcus</i>	+	—	—	—	+	+	+	—	—	—	+	+	+	+	8
ID 12	<i>Rhodococcus</i>	+	—	—	—	—	+	+	—	—	—	+	+	+	+	7
ID 13	<i>Rhodococcus</i>	+	—	—	—	—	+	+	—	+	+	+	+	+	+	8
ID 18	<i>Rhodococcus</i>	+	+	—	+	—	—	+	—	—	—	+	+	+	+	8
ID 20	<i>Rhodococcus</i>	+	+	+	—	—	—	+	—	—	—	+	+	+	+	8
ID 21	<i>Rhodococcus</i>	+	+	—	+	+	+	+	—	—	—	+	+	+	+	10
ID 23	<i>Rhodococcus</i>	+	+	—	+	—	—	+	—	—	—	+	+	+	+	8
ID 24	<i>Rhodococcus</i>	+	—	—	—	—	+	+	—	+	+	+	+	+	+	9
ID 25	<i>Rhodococcus</i>	—	+	+	+	—	—	+	—	+	+	+	+	+	+	9
ID 28	<i>Rhodococcus</i>	+	—	+	+	—	—	+	—	—	—	+	+	+	+	8
ID 29	<i>Rhodococcus</i>	+	+	+	+	—	—	+	—	—	—	+	+	+	+	9
ID 30	<i>Rhodococcus</i>	+	+	—	—	+	+	+	—	—	—	+	+	+	+	9
ID 31	<i>Rhodococcus</i>	+	+	+	+	—	+	+	—	—	—	+	+	+	+	9
ID 32	<i>Rhodococcus</i>	+	—	—	+	—	+	+	—	+	+	+	+	+	+	9
ID 35	<i>Rhodococcus</i>	—	—	+	+	+	+	+	—	—	—	+	+	+	+	9
ID 4	<i>Microbacterium</i>	+	—	+	—	+	+	+	—	+	+	+	+	+	+	10
ID 6	<i>Microbacterium</i>	—	—	+	+	+	—	+	—	—	—	+	+	+	—	7
ID 14	<i>Microbacterium</i>	—	—	+	+	—	+	+	—	+	+	+	+	+	+	9
ID 17	<i>Microbacterium</i>	—	+	+	+	—	+	+	—	—	—	+	+	+	+	9
ID 27	<i>Microbacterium</i>	+	—	+	+	—	+	+	—	—	—	+	+	+	+	9
ID 10	<i>Agromyces</i>	—	—	+	—	—	+	+	—	—	—	+	+	+	+	7
ID 16	<i>Agromyces</i>	—	+	—	—	—	—	+	—	—	—	+	+	+	+	6
ID 11	<i>Streptomyces</i>	+	+	—	+	—	+	+	—	—	—	+	+	+	+	9
ID 15	<i>Streptomyces</i>	+	+	—	—	—	+	+	—	—	—	+	+	+	—	7
ID 33	<i>Streptomyces</i>	+	—	—	+	+	+	+	—	+	+	+	+	+	+	10
ID 19	<i>Brevibacterium</i>	+	—	—	+	—	—	+	—	—	—	+	+	+	+	7
ID 22	<i>Brevibacterium</i>	+	+	—	—	—	—	+	—	—	—	+	+	+	+	7
ID 26	n/a	+	—	+	+	—	—	+	—	+	+	+	+	+	+	9
ID 34	n/a	+	—	—	+	—	+	+	—	—	—	+	+	+	+	8
No. of positives		25	15	15	23	7	24	35	0	1	9	33	35	35	33	

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Data Availability All data supporting the findings of this study are either available within the paper and its Supplementary Information or will be made available by request to the Corresponding Author.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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