Isolation and phylogenetic characterisation of an antimicrobially active bacterial strain from the *Telescopium telescopium*

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Abstract
Antimicrobial resistance is a global concern that diminishes the options for treating diseases caused by microbes. The WHO global report indicates a rise in mortality from infectious diseases attributable to antimicrobial resistance, leading to a global economic loss of up to 100 trillion USD by 2050. Consequently, the identification of novel antibacterial agents having clinical relevance is imperative. The diversity of marine ecosystems has led to the discovery of a wide range of metabolites with bioactive qualities. Conversely, numerous bioactive compounds linked to marine invertebrates are synthesised by their symbiotic microbes. Thus, the current work focused on the identification of bacterial strains from Telescopium telescopium exhibiting antimicrobial potential. This investigation isolated 10 distinct bacterial colonies, designated as ZTS-01 to ZTS-10. Among the 10 isolates, ZTS-08 showed superior antimicrobial activity against the examined bacterial and fungus species except C. albicans.

The PCR-amplified 16s rRNA gene of ZTS-08 comprises of 1500 bp in length and its G+C proportion is 58.46%. The multiple sequence alignments reveal variance, conserved sites and parsimony sites of 0.88, 0.06 and 0.021 respectively, with an overall mean distance of 0.01. According to molecular phylogenetics and morphological analyses, the bacterial isolate ZTS-08 is a member of the N. dassonvillei species.

Keywords: Antimicrobial resistance, Molluscs, *Telescopium telescopium*, 16s rRNA, Phylogeny.

Introduction

Antimicrobial resistance (AMR) poses a significant global challenge that limits the options available for treating infectious diseases caused by viruses, bacteria and fungi[18]. A global surveillance report from the World Health Organization highlighted a rise in morbidity and mortality due to infectious diseases attributed to AMR. This trend could lead to an estimated worldwide economic loss of up to 100 trillion USD by 2050, corresponding to a 2%–3% decrease in gross domestic product[37]. Current estimates indicate that antimicrobial resistance is responsible for approximately 700,000 deaths globally each year, with projections suggesting a rise to 10 million by the year 2050[18]. Consequently, it is imperative to identify novel antimicrobial compounds that hold clinical relevance[2].

Previous studies have thoroughly examined terrestrial resources to identify novel bioactive compounds[7]. However, the world's oceans make up over 70% of the planet's surface and provide a vast resource for the development of chemotherapeutic agents, the isolation of new sources of antimicrobial and other bioactive compounds from marine environments is growing quickly these days[8]. Moreover, the diversity of marine organisms and habitats results in marine natural products that include a broad range of chemical classes such as terpenes, polyketides, acetogenins, peptides and alkaloids which exhibit a variety of structures reflecting an impressive array of biosynthetic pathways[38].

In the last 3 decades, marine organisms have been the subject of a global initiative aimed at discovering novel natural products. A limited variety of marine organisms, including plants, animals and microbes, have produced over 12,000 novel chemicals, leading to the identification of numerous new compounds annually[14]. A significant barrier to the advancement of many marine natural products is that the concentrations of compounds are frequently minimal, constituting less than one millionth of the wet weight[27]. Conversely, increasing evidence indicates that numerous compounds initially linked to the biomass of marine invertebrates are not produced by the organisms themselves but rather synthesised by symbiotic or associated microorganisms or originate from a diet consisting of microorganisms[3].

Hence, microorganisms linked with marine invertebrates are therefore regarded as significant suppliers of marine bioactive compounds. Marine invertebrates including sponges, tunicates, soft and hard corals, bryozoans and sea slugs, are significant sources of natural bioactive products[9]. Molluscs represent the largest marine phylum among marine invertebrates, comprising of 23% of marine organisms. The systematic structural analysis of specific secondary metabolites derived from molluscs indicates that these compounds are produced by symbiotic bacteria rather than by molluscs[6]. For example, the peptide antibiotic Bacicyclin was isolated from *Bacillus* sp. BC028 and this bacterial strain was obtained from the common mussel (*Mytilus edulis*)[34].

S. sampsonii SCSIO 054 is a marine bacterium that is linked to gastropods and molluscs. It has a biosynthetic gene cluster that makes julichromes, an antibacterial compound[19]. Hence,

the present study focused on systematically characterising the bioactive potential of marine bacteria associated with *Telescopium telescopium*. *Telescopium telescopium* (Linne, 1758) represents the largest snail inhabiting the muddy substratum of midlittoral mud floors of mangrove forests, classified within the Potamidiae family and is commonly referred to as Rodong or Berongan[35]. The shells of *T. telescopium* measure approximately 8–15 cm in length. These robust conical shells exhibit a colouration that ranges from black to a very dark reddish-brown, though their distinctive markings are often obscured by mud and various encrusting organisms[5].

This species exhibits a broad geographical range across the Indo-West Pacific region, extending from Madagascar to the Philippines and the northern coast of Australia[10]. Chan et al[11] noted that *T. telescopium* possesses bioactive secondary metabolites in their bodies, serving as a defense mechanism that frequently holds medicinal value. Researchers have reported the presence of potent antimicrobial and immune-contraceptive substances in the spermathecal gland of *T. telescopium*[25,26]. However, to date, there remains a significant lack of information regarding the antimicrobial potentials of microbial species associated with *Telescopium*. This study aims to isolate and identify *Telescopium* associated bacterial species that exhibit antimicrobial properties.

Material and Methods

Sample collection: Young and healthy *T. telescopium* samples were collected from the estuary regions of Korangi mangroves, which are situated in northeastern Andhra Pradesh, bordering to the Bay of Bengal (Long: 18°33' 52" to 18°32' 11"; N; Lat: 84°21' 26" E to 84°18' 22" E). The samples were carefully transferred to sterile zip pouches and subsequently transported to the laboratory for analysis. The sample was cleaned with tap water and then with distilled water. Following that, the *T. telescopium* was carefully dissected and the tissue was separated. The isolated tissue was preserved at -20°C for subsequent studies.

Preparation of *T. telescopium* tissue suspension: The complete organism of *T. telescopium* was fragmented into minute sections and gently macerated with 10 ml of sterilised marine water. The homogenate was centrifuged at 1000 rpm for 5 minutes and the supernatant was collected. Subsequent serial dilutions of the supernatant were prepared as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}.

Isolation of bacterial strains: The isolation of bacterial species was conducted by inoculating dilutions onto marine agar plates, which were then incubated for 48 hours at 35±2°C. Marine agar plates were made by dissolving 5.0 g of peptone, 3.0 g of beef extract, 15.0 g of yeast extract, 8.0 g of NaCl and 15.0 g of bacteriological-grade agar in 1 litre of sterilised marine water. To this medium, an additional 1% of NaCl was added to enhance bacterial growth. The pH of the medium was adjusted to 7.2 using 0.1 M NaOH. Then the medium was sterilised in an autoclave at 121°C for 15 minutes.

Following incubation, the morphological traits of colonies cultivated on each plate were recorded. The morphologically distinct bacterial strains were chosen and purified on 1% NaCl-enriched marine agar medium using the repetitive streak plate technique. The predominant bacterial colonies exhibiting varied characteristics were selected to evaluate their antibacterial capability and the most effective isolates were chosen for future investigation.

Production of crude extract: The preparation of crude extract commenced by culturing the purified bacterial isolates in a 500-mL marine nutrient broth. The cultures were agitated at 110 rpm for three days at ambient temperature (29±2°C). Then, the medium was centrifuged at 6,000 rpm for 10 minutes to isolate the cells from the media. The obtained liquid supernatant was mixed with ethyl acetate (EtOAc) in a 1:1 (v/v) ratio and the suspension was agitated for 15 minutes. The organic layer was subsequently recovered from the aqueous phase by using a separating funnel. The organic layer was evaporated with a rotary evaporator at 40°C and the concentrated extract was collected into a sterile container.

Screening of antimicrobial activity: The antibacterial capacity was assessed using the well diffusion method against Gram-positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*, as well as Gram-negative bacteria, such as *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. The antifungal efficacy was assessed utilising *Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevisiae* as test fungi. Erythromycin and nystatin were used as positive controls to assess antibacterial and antifungal efficacy. DMSO was used as a negative control for both antibacterial and antifungal activities.

Nutrient agar and potato dextrose agar media were prepared for the cultivation of bacteria and fungi. Subsequently, 0.5 mm wells were made and filled with positive control, negative control and crude extracts of bacterial isolates. Then, the plates were sealed with parafilm and incubated at 37°C for 24 hours. Following incubation, the zone of inhibition for the extracts was quantified in millimetres utilising an antibiotic zone scale.

Extraction of genomic DNA: The CTAB lysozyme technique was employed to extract the genomic DNA from the purified bacterial isolate, demonstrating significant antibacterial activity. To isolate DNA, 2 mL of a 48-hour-old pure culture was transferred to a 2 mL Eppendorf tube and centrifuged at 2000 rpm for 10 minutes at ambient temperature. Following centrifugation, the cell pellet was re-suspended in 450 µL of Tris EDTA solution. 50 µL of 10 mg/mL concentrated lysozyme were added to the resultant cell solution, which was then gently vortexed and incubated

at 37°C overnight. Subsequent to incubation, 150 µL of a 2:1 mixture of 10% SDS and 10 mg/mL proteinase K solution was added to the cell suspension with gentle agitation and incubated at 55°C for 30 minutes. Subsequently, 200 µL of 5 M NaCl and 160 µL of CTAB solutions were added, mixed gently and incubated at 65°C for 10 minutes.

Following incubation, an equivalent amount of a 24:24:1 mixture of phenol, chloroform and isoamyl alcohol solution was introduced to the cell suspension, agitated briskly and subsequently centrifuged at 8000 rpm for 5 minutes. The upper aqueous layer was transferred to a new 2 mL Eppendorf tube and an equal volume of ice-cold ethanol was added, followed by centrifugation at 6000 rpm for 25 minutes to precipitate DNA. Subsequent to precipitation, the DNA was rinsed with 70% ethanol and air dried. The resultant DNA pellet was reconstituted in 100 µL of TE buffer. The quality of the isolated DNA was assessed using 1% agarose gel electrophoresis.

Amplification and sequencing of 16S rRNA gene: PCR amplification and 16S rRNA gene sequencing were conducted as outlined by Li et al[21]. Primers 27F (5'AGAGTTTGATCMTGGCTCAG 3') and 2490R (5'GACATCGAGGTGCCAAAC3') were used to amplify the 16S rRNA gene from a potent bacterial isolate. The PCR reaction mixture comprised of 2 µL of template DNA, 2 µL of each 10 pmol primer, 2 µL of 0.2 mM dNTP, 2 µL of 2 mM $MgCl_2$ and 2 µL of 2U Taq polymerase, with the final volume adjusted to 25 µL using nuclease-free water. DNA amplification was performed in a thermocycler using the following protocol: an initial denaturation phase at 94°C for 5 minutes 40 cycles consisting of 30 seconds at 94°C (denaturation), 45 minutes at 52°C (annealing) and 90 seconds at 72°C (extension), followed by a final elongation phase at 72°C for 5 minutes and subsequently cooled to 4°C.

Amplified PCR products were resolved by electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide and subsequently photographed. The standard DNA samples (100 bp DNA ladder marker) were used as a molecular size reference. The purified PCR products underwent Sanger di-deoxy sequencing in both forward and reverse orientations, utilising the Big Dye Terminator v3.1 cycle sequencing kit on the ABI Prism 3700 DNA Analyser (Applied Biosystems Inc., USA).

Sequence alignment and phylogenetic analysis: The nucleotide composition of 16s rRNA gene was computed by Biologics International Crop server (https:// www.biologicscorp.com/tools/GCContent/). The molecular identification of pure bacterial isolates was performed by creating a phylogenetic tree using analogous bacterial species.

To find a homologous sequence or species, the 16S rRNA gene sequence of the purified bacterial isolates was run via the NCBI server's BLASTn program. From the NCBI database, species that were similar to the target 16s rRNA gene sequence, were chosen based on the degree of similarity. The 16S rRNA gene sequences of the selected species were obtained from the NCBI database and subjected to multiple sequence alignment using UPGMA. A distance matrix was established to examine the variances among the sequences and a maximum-parsimony tree was created using MEGAX based on the differences indicated in the distance matrix. The assessment of phylogenetic tree topologies was conducted using the bootstrap method with 1000 replicates for all nodes[15].

Results and Discussion

Isolation of bacterial strains: The present study encompassed the complete tissue of *T. telescopium* for the purpose of isolating bacterial strains, resulting in the identification of a total of 10 distinct bacterial colonies. The ten bacterial isolates have been labelled as ZTS-01, ZTS-02, ZTS-03, ZTS-04, ZTS-05, ZTS-06, ZTS-07, ZTS-08, ZTS-09 and ZTS-10. Figures 1 and 2 present the isolation and purification plates of the bacterial isolates. Researchers have thoroughly investigated extreme environments over the past few decades, revealing the presence of bacteria across diverse settings. Korangi mangrove forest represents one of the largest coastal regions in India, indicating significant potential for bacterial diversity. The current study indicates that the *T. telescopium* from the Korangi mangrove forest serves as a significant source of bacteria.

Environmental factors directly influence the type, quantity and metabolic activities of microflora within any ecosystem[1]. Isolation is essential for acquiring novel microbes and their physiological characteristics, which aid in understanding their physiological and environmental functions as well as their potential applications[31]. Numerous bacteria and archaea have been isolated from marine invertebrates, leading to the identification of several new taxa[32].

Screening of antimicrobial activity of isolated bacterial colonies: Table 1 and figure 3 show the antibacterial activity of the crude extracts of 10 bacterial isolates. Based on the findings, the ZTS-08 had stronger antibacterial activity against the tested bacterial and fungal species. The crude extract of ZTS-08 displayed the highest antimicrobial activity against *Micrococcus luteus* (28 mm) followed by *Bacillus subtilis* (27 mm), *Staphylococcus aureus* (24 mm), *Pseudomonas aeruginosa* (22 mm), *Escherichia coli* (21 mm), *Salmonella typhi* (30 mm), *Aspergillus niger* (16 mm) and *Saccharomyces cerevisiae* (9 mm) whereas the ZTS-08 extract does not exhibit any effect on *Candida albicans*. In comparison to other bacterial strains, ZTS-08 demonstrates superior activity, leading to its molecular identification through 16s rRNA analysis. Potential antimicrobial species can be found through the assessment of antimicrobial activity from crude extracts, which is also used to isolate and identify the secondary metabolites that give rise to bioactivities.

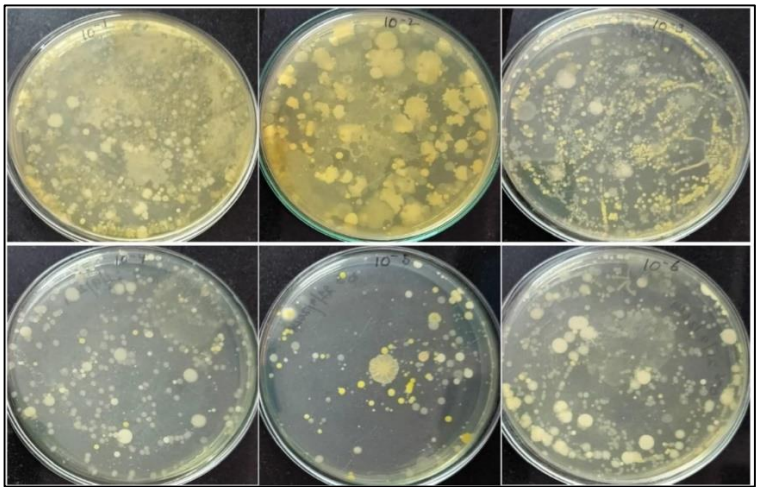

Figure 1: Isolation of bacterial strains from *T. telescopium* on marine agar plates

Figure 2: Pure bacterial strains isolated from *T. telescopium* on marine agar plates.

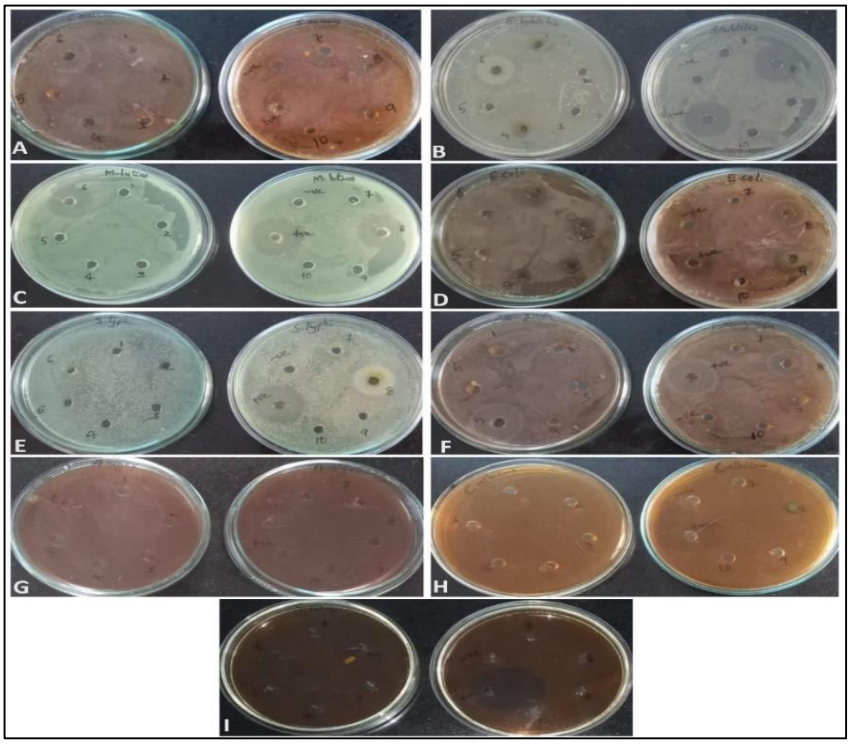

Figure 3: Antimicrobial activity of crude extracts from *the ten bacterial isolates*.
A) Inhibitory zones of *Staphylococcus aureus*, B) Inhibitory zones of *Bacillus subtilis*,
C) Inhibitory zones of *Micrococcus luteus*, D) Inhibitory zones of *Escherichia coli*,
E) Inhibitory zones of *Salmonella typhi*, F) Inhibitory zones of *Pseudomonas aeruginosa*,
G) Inhibitory zones of *Aspergillus niger*, H) Inhibitory zones of *Candida albicans*,
I) Inhibitory zones of *Saccharomyces cerevisiae*.

Table 1
Antimicrobial activity of crude extracts from *the ten bacterial isolates.*

Name of the Strain	Zone of Inhibition (mm) of ZTS isolates											
	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**+Ve**	**-Ve**
Staphylococcus aureus	-	-	-	18	-	16	15	24	-	-	28	-
Bacillus subtilis	21	-	11	14	-	12	-	27	-	-	30	-
Micrococcus luteus	-	-	-	16	-	-	-	28	-	-	28	-
Escherichia coli	11	8	9	10	-	11	11	21	-	-	26	-
Salmonella typhi	-	-	-	-	-	-	-	20	-	-	26	-
Pseudomonas aeruginosa	-	-	-	11	-	-	-	22	-	-	24	-
Aspergillus nigra	12	-	-	8	-	-	-	16	-	-	22	-
Candida albicans	-	-	-	-	-	-	-	-	-	-	18	-
Saccharomyces cerevisiae	-	-	-	-	-	-	-	9	-	-	18	-

Bacteria are one of the finest natural resources for antimicrobial chemicals because they may compete with other microorganisms to maintain the health of their colonies and minimise potentially hazardous microbes[4]. As demonstrated by the current results, Lotfy et al[22] found that the fungal isolate *Aspergillus awamori* produced a bioactive chemical called DEHP, which demonstrated strong antibacterial activity against Gram-negative bacteria and *Candida albicans*. The presence of secondary metabolites in the extracts is typically linked to their antimicrobial properties.

Molecular and Phylogenetic characterization of ZTS-08
Genomic DNA quality and quantity: The findings were reported as mean ± standard deviation (SD) derived from three separate experiments. The extraction of genomic DNA from bacterial isolate ZTS-08 was accomplished, yielding a total DNA content of 426±8 µg/gm. The absorbance ratio of the extracted DNA at A260/280 is measured at 1.9±0.01. The molar percentage of G+C in ZTS-08 genomic DNA was determined to be 54.68%. The melting temperature of DNA correlates directly with the molar percentage of guanine and cytosine content. The Marmur equation's slope of regression is 0.41 and the number 69.3 is obtained by estimating the DNA with 100 mol% A+T intersecting the temperature axis at 69.3°C. The genomic DNA extracted from ZTS-08 appeared as a distinct band on 1% agarose gel. Figure 4A displays the genomic DNA band on a 1% agarose gel.

PCR amplification and sequencing of 16s rRNA gene: The high discriminating strength and primer specificity of the 16s rRNA gene have made it an effective tool for phylogenetic research[28]. The primers used for the amplification of the 16S rRNA from the bacterial isolate ZTS-08 yielded distinct single bands measuring 1500 bp, demonstrating high quality. Figure 4B illustrates the PCR amplification of the 16s rRNA gene as observed in 1.5% agarose gel. The amplified PCR gene product from ZTS-08 was sequenced using Sanger's di-deoxy method and the results were deposited in the NCBI database under the accession number PQ621652.

The development of molecular methods, such as 16s rRNA isolation and sequencing procedures, has made it possible to discover various kinds of microbial communities with great efficacy. The 16S rRNA gene, due to its high degree of conservation and functional consistency, has emerged as a crucial evolutionary indicator that can identify the three facets of life[36]. The bacterial isolate ZTS-08 was identified in this study using the 16S rRNA technique sequencing and the molecular characterisation aids in understanding the evolutionary relationships between ZTS-08 and related species. Numerous instances have extensively used the 16s rRNA gene sequence to distinguish between Actinomycetes and non-Actinomycetes bacteria, as well as anaerobic Gram-positive bacilli, a task often challenging in clinical microbiology[12].

Tazi et al[30] noted that direct sequencing of 16s rRNA genes from environmental samples has emerged as a prevalent and efficient method for analysing microbial community prevalence, organisation and function. The 16s rRNA gene was used because its coding region is easy to amplify and make a sequence. This has a big effect on phylogenetic studies because it makes it possible to reliably put taxa into groups and genera.

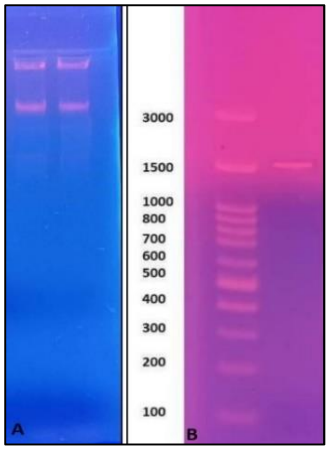

Figure 4: Photograph showing A) bacterial isolate ZTS-08 genomic DNA in 1% agarose gel B) PCR amplified 16S rRNA gene band in 1.5% agarose gel.

Sequence analysis: The sequence properties, including nucleotide composition and %GC, were assessed using the GC content calculator from Biologics International Crop

(https://www.biologicscorp.com/tools/GCContent/). The nucleotide makeup of the ZTS-08 16S rRNA gene is presented in table 2. The 16s rRNA gene sequence consists of 333 bp of adenine (A), 283 bp of thymine (T), 500 bp of guanine (G) and 367 bp of cytosine (C). The G+C content was calculated to be 58.46%. Figure 5 illustrates the GC distribution across the amplified 16s rRNA gene sequence of ZTS-08.

One extremely variable feature of every species is its GC content. According to Lynch[23], the GC content of bacterial species might vary between 25 and 75 percent. In line with previous studies, the 16s rRNA gene from bacterial isolate ZTS-08 has a comparatively high GC content. Hildebrand et al[17] observed significant variability in GC content among bacterial species, with values ranging from 13% to 75% across different species. Mann and Chen[24] estimate that a number of factors, including evolutionary changes and environmental impacts, are implicated in the GC variation, even though the precise causes of these variations in GC content within and between species are unclear.

Sinden[29] proposed that high GC content may be favoured by evolution due to the increased resilience of DNA, as the assembling of guanine and cytosine requires more energy than the positioning of adenine and thymine. This is further supported by Zhang and Gao[39] findings, who showed that as amino acids are selectively adopted to be found in GC-rich species, rising percentages of amino acids translated by GC-rich codons favourably contribute to enhanced evolutionary trends. Therefore, elevated GC composition results in microbial genomes being less prevalent than AT nucleotides, indicating that bacterial genes possess a high GC content. In accordance with a study by Foerstner et al[16] microbial communities that live in the same environments typically have a comparable percentage of GC, irrespective of the taxonomic classification.

Multiple sequence alignment: The BLASTn report shows the percentage of identity for the sequences that are comparable to the ZTS-08 16s rRNA sequence. BLASTn screening of ZTS-08's 1483 bp 16s rRNA gene sequence against the GenBank database showed that ZTS-08 shares similarities with *Streptomyces* and *Nocardiopsis* species. The results indicate that the 16s rRNA gene sequence of ZTS-08 exhibits a 99% identity to *Nocardiopsis dassonvillei* strain OK-8, with an E value of 0.0. A total of 15 sequences from the *Nocardiopsis* and *Streptomyces* genera were retrieved from the GenBank database and used for multiple sequence alignment. The sequences chosen for the multiple sequence alignment and phylogeny are presented in table 3.

Multiple sequence alignments showed that 16s rRNA has a varying number of deletions and insertions. The variance, conserved and parsimony sites of 16s rRNA alignments are 0.88, 0.06 and 0.021 respectively, with a mean distance of 0.01 (Table 4). The evolutionary variation in sequences at the generic level ranges from 0.00068 to 0.02368. ZTS-08 has the greatest evolutionary divergence with *Nocardiopsis alborubida* strain OAct926 (0.02368) and the lowest with *Nocardiopsis synnemataformans* (0.00539). Table 5 illustrates the evolutionary divergence of selected species. High levels of conservation were found when multiple sequence alignments were performed on the 16s rRNA gene sequences.

Multiple sequence alignment (MSA) methods integrate computational techniques to align evolutionarily related sequences, considering all evolutionary events such as mutations, insertions, deletions and rearrangements[33]. Computing the distance matrix revealed the disparities among the selected sequences, the maximum-parsimony phylogenetic tree was constructed using MEGAX based on the distance matrix and the bootstrap method with 1000 repetitions for all nodes evaluated topologies[15].

Phylogeny: In the phylogenetic tree, there were two main clades and the first main clade consisted of two subclades. The first subclade of main clade consisted of two clades, which were composed of ZTS-08, *Nocardiopsis dassonvillei* strain OK-8 (KF543088.1), *Nocardiopsis halotolerance* strain OK-4 (KC759319.1), *Streptomyces* sp. VL61 (KC492827.1) and *Streptomyces flavidofuscus* strain 174510 (EU593716.1).

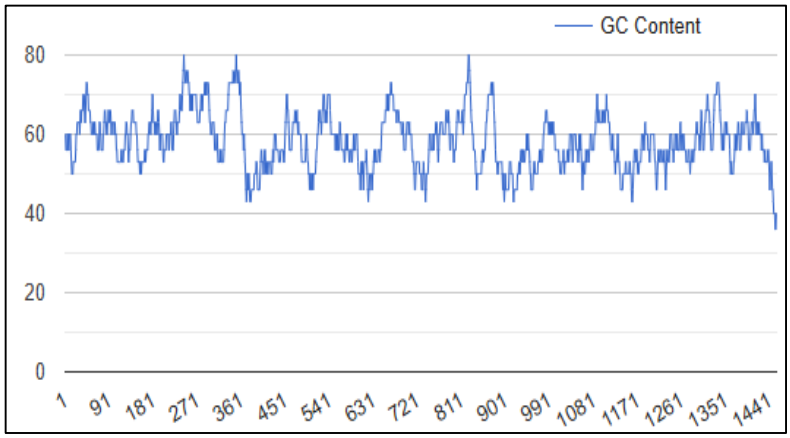

Figure 5: GC distribution over the amplified 16s rRNA gene sequence of ZTS-08.

Table 2
Nucleotide composition of ZTS-08 16s rRNA gene

S.N.	Parameter	Number of nucleotides	% of nucleotides
1	Total bases	1483	--
2	Adenine	333	22
3	Thymine	283	21
4	Guanine	500	33
5	Cytosine	383	24
6	G+C	367	58.46

Table 3
List of selected species sequences used for MSA and phylogenetic study

Species	Max score	Total score	Query cover	E value	% Ident.	Length
KF543088.1 *Nocardiopsis dassonvillei* strain OK-8	2667	2667	99%	0.0	99.06	1494
MF321781.1 *Nocardiopsis* synnemataformans strain AIw2	2575	2575	97%	0.0	98.70	1614
KC492827.1 *Streptomyces* sp. VL61	2575	2575	99%	0.0	98.05	1526
MW448353.1 Actinomycetes bacterium strain P46	2569	2569	97%	0.0	98.63	1468
MH843116.1 *Nocardiopsis* synnemataformans strain KaS3	2567	2567	97%	0.0	98.69	1478
KM214828.1 *Streptomyces* sp. Ahbb4	2564	2564	97%	0.0	98.56	1523
KC759319.1 *Nocardiopsis halotolerance* strain OK-4	2564	2564	98%	0.0	98.36	1466
X97885.1 *Nocardiopsis antarctica*	2562	2562	97%	0.0	98.63	1461
ON8104321.1 *Nocardiopsis alborubida* strain DB	2558	2558	97%	0.0	98.49	1458
NR_112743.1 *Nocardiopsis alborubida* strain NBRC 13392	2558	2558	97%	0.0	98.49	1458
MG661750.1 *Nocardiopsis alborubida* strain OAct926	2553	2553	97%	0.0	98.42	1551
MH843133.1 *Nocardiopsis dassonvillei* strain KaW34	2551	2551	97%	0.0	98.62	1457
EU593716.1 *Streptomyces flavidofuscus* strain 174510	2542	2542	97%	0.0	98.48	1461
KU744854.1 *Nocardiopsis dassonvillei* strain YHSA42	2542	2542	97%	0.0	98.29	1467
OM033449.1 *Actinomycetes bacterium strain* MAF14	2540	2540	97%	0.0	98.29	1455

Table 4
Phylogenetic parameters of ZTS-08

S.N.	Parameter	Value
1	Conserved sites (%)	88.72
2	Variable sites (%)	6.27
3	Parsimony sites (%)	2.13
4	Singletons (%)	3.16
5	Overall mean distance	0.01

The second subclade of main clade 1 consisted of two clades, which were composed of *Nocardiopsis synnemataformans* strain AIw2 (MF321781.1), *Nocardiopsis dassonvillei* strain KaW34 (MH843133.1), *Nocardiopsis antarctica* (X97885.1), *Actinomycetes* bacterium strain P46 (MW448353.1) and *Nocardiopsis* synnemataformans strain KaS3 (MH843116.1). The second main clade of main clade was composed of two subclades.

The first subclade of main clade 2 consisted of two subclades, which were composed of *Nocardiopsis dassonvillei* strain YHSA42 (KU744854.1), *Actinomycetes bacterium strain* MAF14 (OM033449.1) and *Streptomyces* sp. Ahbb4 (KM214828.1) while the second subclade of main clade 2 is composed of *Nocardiopsis alborubida* strain DB (ON810432.1), *Nocardiopsis alborubida* strain NBRC 13392 (NR_112743.1) and *Nocardiopsis alborubida* strain OAct926 (MG661750.1). Molecular phylogenetics reveals that the bacterial isolate ZTS-08 belongs to the *Nocardiopsis dassonvillei* species. Figure 6 displayed the maximum-parsimony phylogenetic tree. According to DeGroot et al[13], generic level identification was considered successful when all hits concerned a single genus and the maximum percent identification scores were higher than 95%.

The identification of species was considered successful only when one species with the greatest percent identity score (greater than 95%) was included. The integration of various species and strains primarily arranges classes in the phylogenetic tree. Thus, creating a local barcode database is essential for many ecological applications, such as building a community phylogenetic tree[20].

Conclusion

The current findings indicate that the bacterial strain ZTS-08 demonstrated more effective antimicrobial potential compared to all 10 isolates from *T. telescopium*. The amplification of the 16S rRNA from the bacterial isolate ZTS-08 produced clear single bands measuring 1483 bp with the G+C content calculated at 58.46%. Additionally, ZTS-08 exhibits the highest similarity to *Nocardiopsis dassonvillei* strain OK-8, showing an identity of 99%. In terms of evolutionary divergence, ZTS-08 is most similar to *Nocardiopsis alborubida* strain OAct926 (0.02368) and least similar to *Nocardiopsis synnemataformans* (0.00539).

The phylogenetic analysis indicated that the bacterial isolate ZTS-08 is closely related to the *Nocardiopsis dassonvillei* strain OK-8 (KF543088.1). The current investigation revealed that *T. telescopium* serves as a significant source of bioactive bacteria. The findings concluded that the isolated bacterial strain ZTS-08 exhibits superior antimicrobial properties against a broad spectrum of microbial species.

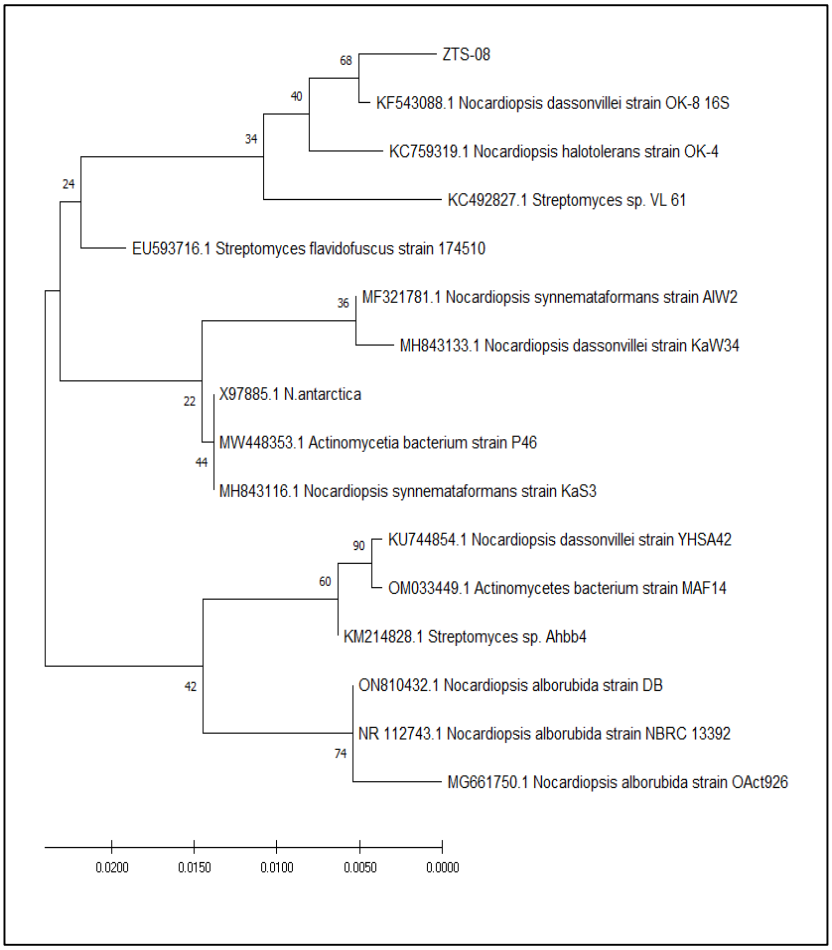

Figure 6: Maximum Parsimony tree of bacterial isolate ZTS-08 and other relative species.

Table 5
Estimates of evolutionary divergence among the selected species.

S.N.	Species (Accession No)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	ZTS-08																
2	KF543088.1	0.00539															
3	MF321781.1	0.02165	0.01948														
4	KC492827.1	0.01624	0.01343	0.02259													
5	MW448353.1	0.00964	0.00547	0.00205	0.00615												
6	MH843116.1	0.00555	0.00138	0.00068	0.00745	0.00000											
7	KM214828.1	0.02233	0.01948	0.01919	0.02900	0.00205	0.00543										
8	KC759319.1	0.00964	0.00547	0.00821	0.00753	0.00890	0.00551	0.00821									
9	X97885.1	0.01034	0.00616	0.00205	0.00685	0.00000	0.00000	0.00205	0.00893								
10	ON810432.1	0.00830	0.00412	0.00206	0.00343	0.00274	0.00275	0.00137	0.00755	0.00275							
11	NR_112743.1	0.00830	0.00412	0.00206	0.00343	0.00274	0.00275	0.00137	0.00755	0.00275	0.00000						

12	MG661750.1	0.02368	0.02082	0.03320	0.02895	0.00341	0.00542	0.01709	0.00958	0.00342	0.00069	0.00069				
13	MH843133.1	0.00558	0.00139	0.00206	0.00618	0.00138	0.00412	0.00481	0.00554	0.00139	0.00138	0.00138	0.00549			
14	EU593716.1	0.01447	0.01027	0.00548	0.01164	0.00412	0.00416	0.00479	0.01032	0.00412	0.00414	0.00414	0.00616	0.00418		
15	KU744854.1	0.01241	0.00822	0.00409	0.00750	0.00342	0.00343	0.00273	0.01095	0.00343	0.00412	0.00412	0.00477	0.00414	0.00619	
16	OM033449.1	0.00901	0.00482	0.00412	0.00413	0.00481	0.00482	0.00275	0.00825	0.00483	0.00412	0.00412	0.00481	0.00415	0.00622	0.00137	1

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References
1. Adebusoye S.A., Ilori M.O., Amund O.O., Teniola O.D. and Olatope S.O., Microbial degradation of petroleum hydrocarbons in a polluted tropical stream, *World journal of Microbiology and Biotechnology*, **23**,1149-1159 **(2007)**

2. Aminov R.I., A brief history of the antibiotic era: lessons learned and challenges for the future, *Frontiers in microbiology*, **8(1)**,134, doi: http://dx.doi.org/10.3389/ fmicb.2010.00134 **(2010)**

3. Anjum K., Abbas S.Q., Shah S.A., Akhter N., Batool S. and ul Hassan S.S., Marine sponges as a drug treasure, *Biomolecules & Therapeutics*, **24(4)**, 347 **(2016)**

4. Arango R.A., Carlson C.M., Currie C.R., McDonald B.R., Book A.J. and Green III F., Antimicrobial activity of actinobacteria isolated from the guts of subterranean termites, *Environmental Entomology*, **45(6)**, 1415-1423 **(2016)**

5. Atlas of Living Australia Year in Review, Atlas of Living Australia, Publication series No. 5, Canberra, Australia, 24, https://doi.org/10.54102/ala.49192 **(2021–22)**

6. Benkendorff K., Molluscan biological and chemical diversity: Secondary metabolites and medicinal resources produced by marine molluscs, *Biological Reviews*, **85(4)**, 757-775 **(2010)**

7. Bibi F., Faheem M.I., Azhar E., Yasir M., Alvi A.S. and Kamal A.M., Bacteria from marine sponges: a source of new drugs, *Current Drug Metabolism,* **18(1)**, 11-15 **(2017)**

8. Biswas K., Paul D. and Sinha S.N., Marine bacteria: a potential tool for antibacterial activity, *J Appl Environ Microbiol*, **4(1)**, 25-29, doi: http://dx.doi.org/ 10.12691/jaem-4-1-3 **(2016)**

9. Blunt J.W., Copp B.R., Keyzers R.A., Munro M.H. and Prinsep M.R., Marine natural products, *Natural Product Reports*, **34(3)**, 235-294, doi: http://dx.doi. org/10.1039/b603047p **(2017)**

10. Brandt R.A., The non-marine aquatic Mollusca of Thailand, *Archiv fur Molluskenkunde*, **105**, pls-1 **(1974)**

11. Chan M.W., Ali A., Ullah A., Mirani Z.A. and Balthazar-Silva D., A size-dependent bioaccumulation of metal pollutants, antibacterial and antifungal activities of *Telescopium telescopium*, *Nerita albicilla* and *Lunella coronata*, *Environmental Toxicology and Pharmacology*, **87**, 103722, https://doi.org/10.1016/ j.etap.2021.103722 **(2021)**

12. Colmegna I., Rodriguez-Barradas M., Young E.J., Rauch R. and Clarridge J., Disseminated *Actinomyces meyeri* infection resembling lung cancer with brain metastases, *The American Journal of the Medical Sciences*, **326(3)**, 152-155 **(2003)**

13. DeGroot H., Al-Omari A.A. and Ghazaly S.A.E., Outcomes of suture button repair of the distal tibiofibular syndesmosis, *Foot & ankle International*, **32(3)**, 250-256 **(2011)**

14. Donia M. and Hamann M.T., Marine natural products and their potential applications as anti-infective agents, *The Lancet Infectious Diseases*, **3(6)**, 338-348 **(2003)**

15. Felsenstein, J., Confidence limits on phylogenies: an approach using the bootstrap, *Evolution*, **39(4)**, 783-791 **(1985b)**

16. Foerstner K.U., Von Mering C., Hooper S.D. and Bork P., Environments shape the nucleotide composition of genomes, *EMBO Reports*, **6(12)**, 1208-1213 **(2005)**

17. Hildebrand F., Meyer A. and Eyre-Walker A., Evidence of selection upon genomic GC-content in bacteria, *PLoS Genetics*, **6(9)**, e1001107 **(2010)**

18. Indraningrat A.A., Smidt H. and Sipkema D., Bioprospecting sponge-associated microbes for antimicrobial compounds, *Marine*

Drugs, **14(5)**, 87, doi: http://dx.doi.org/10.3390/md14050087 **(2016)**

19. Ji X., Dong Y., Ling C., Zhou Z., Li Q. and Ju J., Elucidation of the tailoring steps in julichrome biosynthesis by marine gastropod mollusk-associated *Streptomyces sampsonii* SCSIO 054, *Organic Letters*, **22(17)**, 6927-31 **(2020)**

20. Kress W.J., Erickson D.L., Jones F.A., Swenson N.G., Perez R., Sanjur O. and Bermingham E., Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama, Proceedings of the National Academy of Sciences, **106(44)**, 18621-18626 **(2009)**

21. Li M., Hong Y., Klotz M.G. and Gu J.D., A comparison of primer sets for detecting 16S rRNA and hydrazine oxidoreductase genes of anaerobic ammonium-oxidizing bacteria in marine sediments, *Applied Microbiology and Biotechnology*, **86**, 781-790 **(2010)**

22. Lotfy M.M., Hassan H.M., Hetta M.H., El-Gendy A.O. and Mohammed R., Di-(2-ethylhexyl) Phthalate, a major bioactive metabolite with antimicrobial and cytotoxic activity isolated from River Nile derived fungus *Aspergillus awamori*, *Beni-Suef University Journal of Basic and Applied Sciences*, **7(3)**, 263-269 **(2018)**

23. Lynch J.P., Roots of the second green revolution, *Australian Journal of Botany*, **55(5)**, 493-512 **(2007)**

24. Mann S. and Chen Y.P.P., Bacterial genomic G+C composition-eliciting environmental adaptation, *Genomics*, **95(1)**, 7-15 **(2010)**

25. Pakrashi A., Datta U. and Choudhury A., A search for immunocontraceptive agent from marine sources-- role of antispermatheca globulin of *Telescopium telescopium* on fertility regulation in male rat, *Indian J. Exp. Biol*, **30**, 1066-1074 **(1992)**

26. Pakrashi A., Roy P. and Datta U., Antimicrobial effect of protein(s) isolated from a marine mollusk Telescopium Telescopium, *Indian J. Physiol. Pharmacol*, **45**, 249-252 **(2000)**

27. Proksch P., Edrada R. and Ebel R., Drugs from the seas–current status and microbiological implications, *Applied Microbiology and Biotechnology*, **59**, 125-134 **(2002)**

28. Schneider T., Fetscher C., Krege S. and Michel M.C., Signal transduction underlying carbachol-induced contraction of human urinary bladder, *Journal of Pharmacology and Experimental Therapeutics*, **309(3)**, 1148-1153 **(2004)**

29. Sinden R.R., DNA bending, DNA structure and function, Gulf Professional Publishing, 58-88 **(1994)**

30. Tazi C., Crestani B., Tazi A., Hervier B., Adam-Marchand S., Nunes H., Cohen-Aubart F., Wislez M., Cadranel J., Housset B. and Lloret-Linares C., Pulmonary fibrosis in antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis: a series of 49 patients and review of the literature, *Medicine*, **93(24)**, 340-349 **(2014)**

31. Vandamme P., Pot B., Gillis M., De Vos P., Kersters K. and Swings J., Polyphasic taxonomy, a consensus approach to bacterial systematics, *Microbiological Reviews*, **60(2)**, 407-438 **(1996)**

32. Vreeland R.H., Rosenzweig W.D. and Powers D.W., Isolation of a 250-million-year-old halotolerant bacterium from a primary salt crystal, *Nature*, **407(6806)**, 897-900 **(2000)**

33. Wallace R.B. and Whitt W., A staffing algorithm for call centers with skill-based routing, *Manufacturing & Service Operations Management*, **7(4)**, 276-294 **(2005)**

34. Wiese J., Abdelmohsen U.R., Motiei A., Humeida U.H. and Imhoff J.F., Bacicyclin, a new antibacterial cyclic hexapeptide from Bacillus sp. strain BC028 isolated from *Mytilus edulis*, *Bioorganic & Medicinal Chemistry Letters*, **28(4)**, 558-561 **(2018)**

35. Willan R.C., A key to the potamidid snails (longbums, mudcreepers and treecreepers) of northern Australia, *Northern Territory Naturalist*, **24**, 68-80 **(2013)**

36. Woese C.R., Winker S. and Gutell R.R., Architecture of ribosomal RNA: constraints on the sequence of" tetra-loops", Proceedings of the National Academy of Sciences, **87(21)**, 8467-8471 **(1990)**

37. World Health Organization, Antimicrobial Resistance: Global Report on Surveillance, World Health Organization, Geneva, Switzerland **(2014)**

38. Wright A.E., Isolation of marine natural products, In Cannell R.J.P., ed., Methods in Biotechnology, Humana Press, New Jersey, USA, **4**, 365–408 **(1998)**

39. Zhang G. and Gao F., Quantitative analysis of correlation between AT and GC biases among bacterial genomes, *PLoS One,* **12(2)**, e0171408 **(2017)**.

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