

16S RRNA PROFILING OF THE GUT MICROFLORA IN EUTHYNNUS AFFINIS AND THUNNUS ALBACARES COLLECTED FROM THE SOUTHERN PART OF INDIA

A. Nasreem^{1*}, D. Deleep Packia Raj²

^{1*}Department of Zoology and Research centre, Scott christian College (Autonomous) Nagercoil 629001 (Affiliated to Manonmaniam Sundaranar University), Tirunelveli, TamilNadu, India. Email: anasreem@gmail.com

²Department of Zoology and Research centre, Scott christian College (Autonomous) Nagercoil 629001 (Affiliated to Manonmaniam Sundaranar University), Tirunelveli, TamilNadu, India.

Received date: 04/04/2026

Accepted date: 08/05/2026

ABSTRACT

The intestinal microflora of fishes is essential for digestion, absorption of nutrients, immunity, and general well-being of the hosts. Gut microbiota composition depends on both host and environmental conditions. It greatly contributes to fish physiology and adaptation. This study focused on determining the bacterial diversity of two economically relevant tuna fish species, i.e., *Euthynnus affinis* (Kawakawa) and *Thunnus albacares* (Yellowfin tuna) that were sourced from different geographic regions of the south-west coast of India, which includes Thoothoor, Kanyakumari, and Trivandrum. Intestinal tissue was dissected aseptically, homogenized, serially diluted, and inoculated in Nutrient Agar plates for isolation of bacteria. Isolates were then subjected to DNA extraction and 16S rRNA PCR amplification followed by Sanger sequencing. The six isolated bacteria included *Microbacterium* sp. and *Bacillus cereus* (Trivandrum), *Bacillus cereus* and *Lacrimispora amygdalina* (Thoothoor), and *Escherichia coli* and *Oceanisphaera ostreae* (Kanyakumari). The results offer baseline information regarding composition of the gut microbiota associated with *E. affinis* and *T. albacares* from the Indian south coast and also provide knowledge on host-microbe relationships in natural environments.

KEYWORDS: gut bacteria, microorganisms, microflora, 16S rRNA, PCR amplification, tuna fish

1. INTRODUCTION

Microbiomes in fish guts play a vital role in the physiology of the host, helping in digestion, nutrient uptake, metabolism, immune system, and resisting diseases (Egerton et al., 2018). The microbial composition and diversity of fish gut microbiomes depend on various host factors as well as environmental factors. The host factors include fish species, genetic makeup, and age, while environmental factors comprise geographical location, water chemistry, temperature, and habitat type (Lokesh & Kiron, 2016). As fishes are continuously exposed to their environment, any change in environmental conditions causes significant alterations in the gut microbial community composition that ultimately influences fish health and physiology (Sylvain et al., 2020).

Tuna species constitute one of the most ecologically and economically valuable groups of marine fish, playing a critical role in marine ecosystems and global fisheries. These highly migratory pelagic fishes are widely distributed in tropical and temperate oceans, where they act as apex predators and play an important role in marine food webs. *Euthynnus affinis* and *Thunnus albacares* are two commercially viable fishes that commonly found in the Indian Ocean. The body of *E. affinis* is streamlined and fusiform in shape with distinctive dark oblique stripes on its dorsal side, while *T. albacares* is easily distinguished by its metallic blue colored dorsum, silver belly, and extended yellow dorsal and anal fins. Both species serves as commercially valuable fish resources and provide a good source of protein for human consumption. Due to their wide geographic distribution, migratory behaviour, and ecological significance, these species provide suitable model for studying gut microbiota and host-microbiome relationships.

The development of molecular biology technology has greatly contributed to research on microbial diversity and taxonomy. In the existing range of molecular technologies, DNA sequencing of the 16S ribosomal RNA gene is one of the most popular and successful methods for bacterial identification and phylogenetic investigation. The gene is characterized by highly conserved and highly variable areas, allowing precise differentiation between various bacteria taxa at the genus and species levels. Hence, the DNA sequencing of the 16S rRNA gene became a standard method for bacterial isolate description and diversity assessment. Bacterial identification and taxonomic classification can be significantly refined through sequence comparisons utilizing database searches, including NCBI BLAST databases (Janda & Abbott, 2007; Clarridge, 2004).

Despite significant advances in studies on the fish gut microbiota, data concerning culturable bacterial diversity of wild tuna from the coastal region of India is relatively sparse. Previous studies on the bacterial microbiota have concentrated

on farmed species or employed a non-culture method in regions like the Pacific Ocean, Atlantic Ocean, and South China Sea. Hence, there is a relative dearth of information regarding the composition and geographical variations in culturable gut bacteria associated with *E. affinis* and *T. albacares* from the south-west coast of India is limited. Isolation and molecular characterization of culturable bacteria are crucial since some of these bacteria may have probiotic characteristics, facilitate nutrient digestion, improve fish wellbeing, and produce industrially relevant molecules (Gupta et al., 2020). Thus, the present study was designed to isolate and characterize the culturable gut bacteria associated with *E. affinis* and *T. albacares* collected from three geographically distant coasts of South-Western coastal region of India, i.e., Thoothoor, Kanyakumari, and Trivandrum. The bacterial strains isolated were characterized based on the identification of 16S rRNA sequences through BLAST analysis. This investigation will contribute to the existing literature with baseline information on the culturable gut microbiota of tuna fish.

2. MATERIALS AND METHODS

2.1 Sample Collection and Bacterial Isolation

Samples of tuna fish were collected from three geographically different regions located in the south-west coastal region of India, such as Trivandrum, Thoothoor, and Kanyakumari. The samples collected were taken to the lab and processed on the very same day. The gut tissues were aseptically removed and sterilized with sterile distilled water and 70% ethanol, followed by homogenization in 1 mL of sterile distilled water. Serial dilution was performed up to 10⁻⁵ using sterile distilled water.

The aliquots of 10⁻³ and 10⁻⁴ dilutions were spread on Nutrient Agar plates and incubated at 37°C for 24 hours. Colonies with morphological difference were isolated and sub-cultured and further grown in Nutrient Broth at 37°C overnight.

2.3 Isolation of Genomic DNA

Extraction of genomic DNA was done from overnight cultures of bacteria through the sodium dodecyl sulfate (SDS) extraction method but with minor adjustments. Pelleting of the bacterial cells was achieved by centrifugation at 21,500 × g for 5 minutes at 4°C then suspension in TE buffer followed by lysozyme and RNase A treatment to allow cell lysis and RNA degradation, respectively.

The DNA extraction procedure involved phenol:chloroform alcohol (25:24:1, v/v/v) and chloroform alcohol (24:1, v/v) extractions to purify the cell lysate. Precipitation was carried out through the addition of sodium acetate and ice-cold ethanol, washed with 70% ethanol, dried using an air stream, and finally resuspended in deionized water for further processing.

2.4 Agarose Gel Electrophoresis of Genomic DNA

To determine the purity and integrity of the isolated genomic DNA, agarose gel electrophoresis analysis was done according to existing protocols. For this process, an 0.8% agarose gel was made by mixing 0.4 g agarose with 50 mL of 1× Tris-Acetate-EDTA (TAE) buffer solution. The mixture was warmed to dissolve the agarose completely followed by cooling and addition of ethidium bromide.

The cooled agarose was poured into a gel mold containing a comb. After the agarose had been solidified, the gel was transferred to an electrophoresis unit containing 1× TAE buffer solution. DNA samples were loaded onto individual wells after mixing with loading dye. Electrophoresis was performed at 100 V for migration of the tracking dye to about two-thirds of the total distance covered by the gel.

2.5 Polymerase Chain Reaction (PCR) Amplification of the 16S rRNA Gene

Identification of the bacteria was achieved through PCR amplification of the 16S ribosomal RNA (rRNA) gene, which is conserved within all bacterial genera and serves as a basis for bacterial taxonomy and classification.

PCR amplification was done using universal primers for 16S rRNA of bacteria:

Forward primer: AGAGTTTGATCMTGGCTC

Reverse primer: AAGGAGGTGWTCCARCC

PCR amplification mix contained 25 µL Red Taq Master Mix, 2 µL forward primer, 2 µL reverse primer, 10 µL template DNA (50 ng µL⁻¹), and 11 µL nuclease-free water in a total volume of 50 µL.

The PCR thermocycling parameters were as follows: initial denaturation step at 94° C for 5 min; then 35 cycles with 94° C for 30 s, 55° C for 30 s, and 72° C for 45 s; followed by 72° C for 7 min.

2.6 Identification of PCR Products

PCR products obtained in the study were analyzed through gel electrophoresis using agarose gels. A 1% agarose gel was prepared by mixing 0.5 g of agarose in 50 mL of 1x TAE buffer. After solidifying the agarose gel, the PCR products were run together with a 1 kb DNA ladder at 80 volts for 45 minutes. Amplified DNA fragments of about 1500 bp in size were detected under UV light and photographed through a gel documentation system.

2.7 DNA Sequencing and Sequence Analysis

PCR products displaying positive amplification reactions were isolated and sequenced by Sanger sequencing methods. Approximately 1500 bp internal regions of the bacterial 16S rDNA gene were sequenced using the same primer pair utilized for amplifying the genes.

The sequenced nucleotide sequences were then subjected to BLAST analyses using the Basic Local Alignment Search Tool found in the NCBI database. Bacteria were classified based on the highest homology between the obtained sequence and the sequences available in the GenBank database. Identified bacteria were used to evaluate the culturable diversity of the gut bacteria associated with tuna fish collected from various geographic origins.

3. RESULTS AND DISCUSSION

3.1 Isolation of Gut Bacteria from Tuna Fish

Tuna specimens belonging to *E. affinis* and *T. albacares* were collected from three different geographical sites, viz. Thoothoor, Kanyakumari, and Trivandrum, which were used right away after being collected in order to maintain the indigenous microbiome of the fish samples. Samples of the tuna fish used in the present study are depicted in Figure 1. After performing aseptic dissection and sterilizing the surface, gut tissues were homogenized and subjected to serial dilution in order to isolate bacteria.

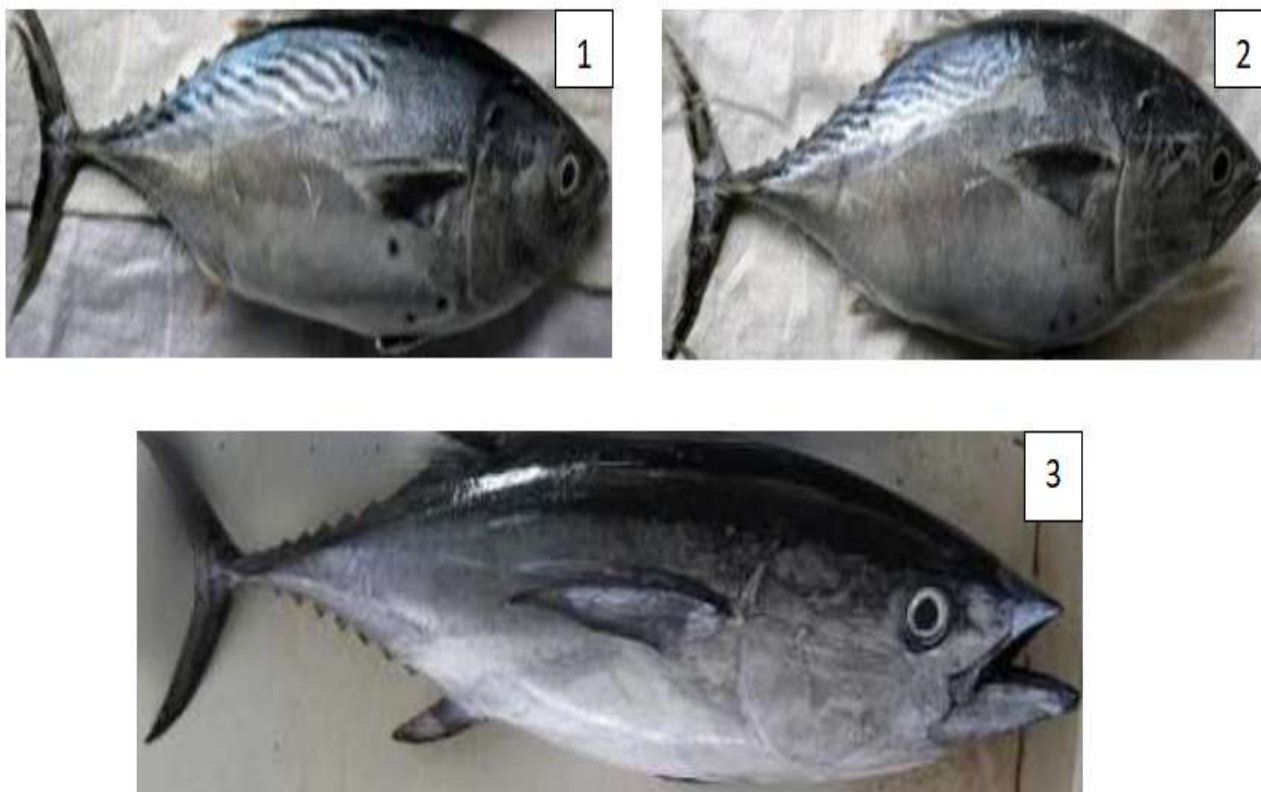


Figure 1. Samples of the tuna fish collected for the present study: (1) Thoothoor, (2) Kanyakumari, and (3) Trivandrum.

Bacterial colonies formed upon incubation in Nutrient Agar media and were characterized in terms of size, shape, elevation, and texture, thereby indicating that the samples contained diverse bacteria populations. Typical examples of isolated bacterial colonies are illustrated in Figure 2.

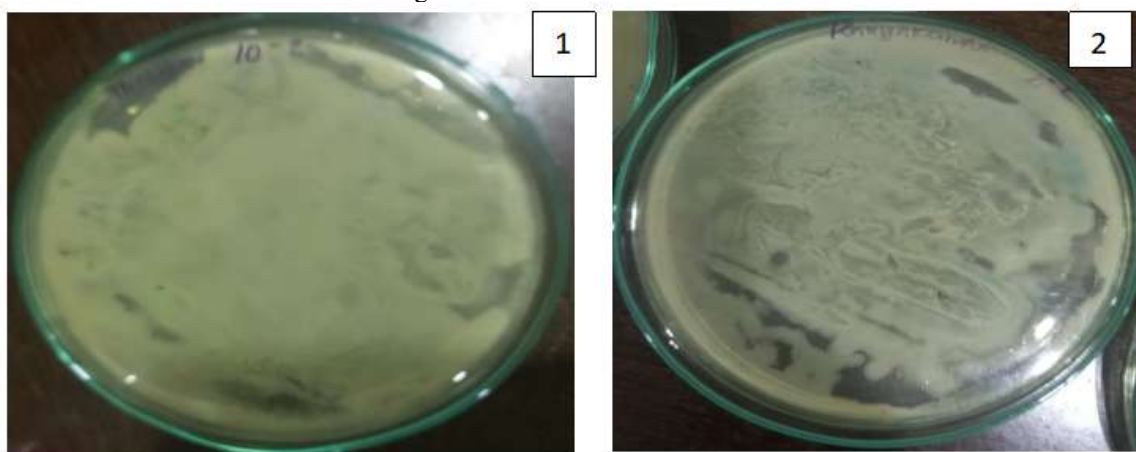




Figure 2. Isolated colonies of bacteria isolated from tuna gut after serial dilution. (1) Thoothoor, (2) Kanyakumari, and (3) Trivandrum.

The occurrence of colonies exhibiting different morphologies indicates a heterogeneous microbial population within the tuna gut. This finding is consistent with previous studies showing that the intestinal microbiota of marine fishes is shaped by dietary, environmental, and habitat-related factors (Egerton et al., 2018; Tarnecki et al., 2017). The highly migratory nature and diverse feeding habits of tuna may further influence the composition and diversity of their gut microbiota.

3.2 Genomic DNA Extraction

The genomic DNA was successfully extracted from chosen bacterial isolates using the SDS extraction technique. The extracted genomic DNA was then subjected to analysis by agarose gel electrophoresis. The electrophoresis apparatus employed in analysing the DNA is illustrated in Figure 3.



Figure 3. The set-up for agarose gel electrophoresis for DNA analysis.

Clear and distinct DNA bands were observed following agarose gel after electrophoresis, indicating the successful extraction of the bacterial genomic DNA. The agarose gel picture captured during bacterial genomic DNA isolation is provided in Figure 4.

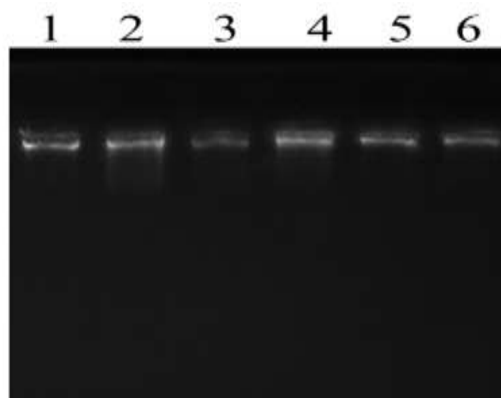


Figure 4. Agarose gel picture depicting bacterial genomic DNA extracted from tuna gut bacteria. Lanes 1-2: Thoothoor isolates; Lanes 3-4: Kanyakumari isolates; Lanes 5-6: Trivandrum isolates.

Identification of clear DNA bands without much degradation suggested that the genomic DNA extracted was good enough for PCR amplification and sequencing analysis. Good quality genomic DNA is required for bacterial identification through molecular techniques (Teletchea, 2009).

3.3 Amplification of the 16S rRNA Gene

Amplification of the 16S rRNA gene from the extracted genomic DNA was carried out using universal bacterial primers. Sequences of the primers used in this study are shown in Table 1.

Table 1. Universal bacterial 16S rRNA primers for amplification

Primer	Sequence (5'-3')
Forward Primer	AGAGTTTGATCMTGGCTC
Reverse Primer	AAGGAGGTGWTCCARCC

The PCR products were amplicons of ~1500 bp in all bacterial isolates. The amplicons were resolved by running the samples on 1% agarose gels and stained with ethidium bromide followed by UV observation.

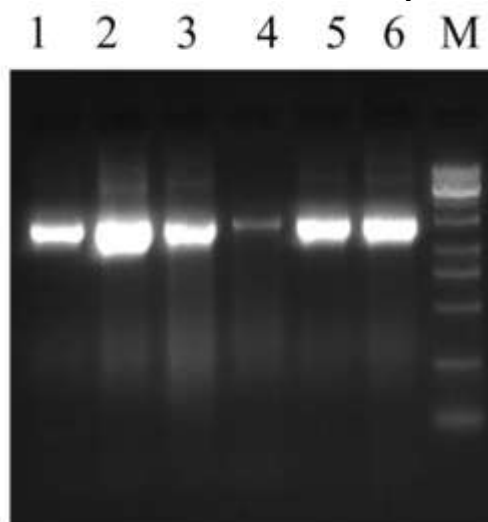


Figure 5. PCR products (~1500 bp) amplified by universal bacterial 16S rRNA primers. Lane 1-2: Thoothoor isolates; Lane 3-4: Kanyakumari isolates; Lane 5-6: Trivandrum isolates.

This result indicated that the purified genomic DNA was ready for further sequencing and taxonomic characterization. The use of 16S rRNA is one of the best methods for identification of bacteria due to the conserved character of this gene (Alikunhi et al., 2017).

3.4 Sequence Analysis and Identification of Bacterial Isolates

The amplified PCR products were subjected to Sanger sequencing and the sequences generated were analyzed via NCBI BLAST database. Sequence analysis revealed that Trivandrum isolate 1 had a close resemblance with *Microbacterium* species. The graphical representation of BLAST hits, significant sequence alignments, and sequence comparison have been illustrated in Figures 6, 7, and 8.

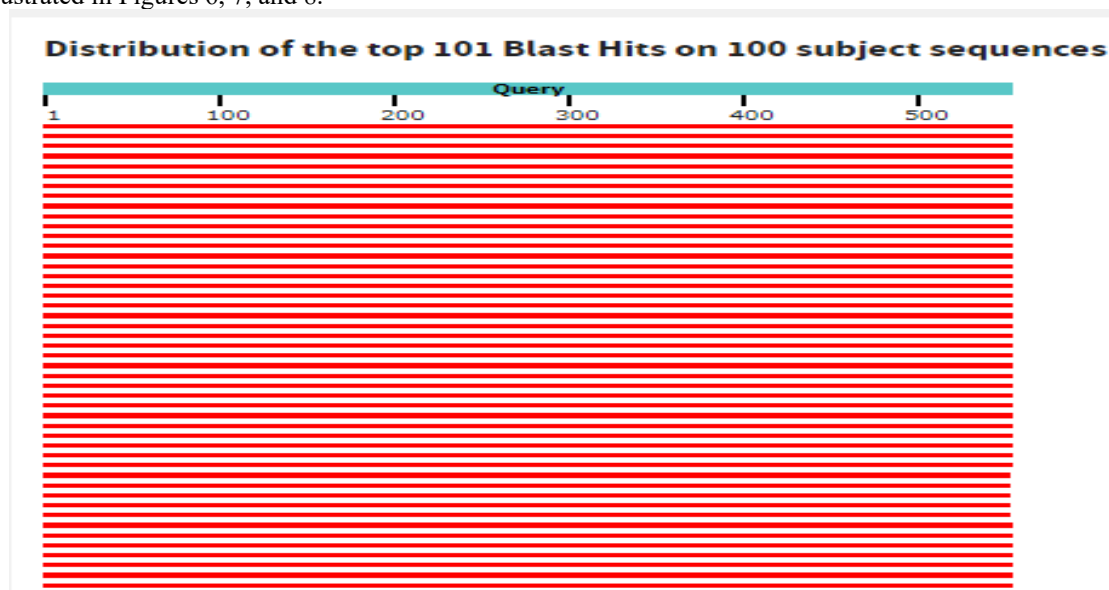


Figure 6. Graphic Representation of Top BLAST Hits Obtained for Trivandrum Isolate 1.

Descriptions	Graphic Summary	Alignments	Taxonomy						
Sequences producing significant alignments									
Download Select columns Show 100									
select all 100 sequences selected									
GenBank Graphics Distance tree of results MSA Viewer									
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
☒	Microbacterium sp. strain Atecer4N 16S ribosomal RNA gene, partial sequence	Microbacterium sp.	1003	1003	100%	0.0	99.28%	1380	MT386149.1
☒	Microbacterium sp. strain ZX196 16S ribosomal RNA gene, partial sequence	Microbacterium sp.	1003	1003	100%	0.0	99.28%	1489	MK680097.1
☒	Microbacterium indicum strain WT19 16S ribosomal RNA gene, partial sequence	Microbacterium indicum	1003	1003	100%	0.0	99.28%	1391	MN733234.1
☒	Microbacterium indicum strain B9H9 16S ribosomal RNA, partial sequence	Microbacterium indicum	1003	1003	100%	0.0	99.28%	1450	NR_047459.1
☒	Microbacterium indicum partial 16S rRNA gene, strain B9H9	Microbacterium indicum	1003	1003	100%	0.0	99.28%	1448	AM286267.2
☒	Microbacterium excoecariae strain CBSSP-1 16S ribosomal RNA gene, partial sequence	Microbacterium excoecariae	992	992	100%	0.0	98.92%	1488	MT138564.1
☒	Microbacterium amylolyticum strain DSM 24221 chromosome, complete genome	Microbacterium amylolyticum	992	1985	100%	0.0	98.92%	2542739	CP948253.1
☒	Microbacterium sp. strain X49 16S ribosomal RNA gene, partial sequence	Microbacterium sp.	992	992	100%	0.0	98.92%	1129	MH537065.1
☒	Microbacterium simlaoensis strain M12 16S ribosomal RNA gene, partial sequence	Microbacterium simlaoensis	992	992	100%	0.0	98.92%	1336	MH493794.1
☒	Microbacterium simlaoensis strain M12 16S ribosomal RNA gene, partial sequence	Microbacterium simlaoensis	992	992	100%	0.0	98.92%	1368	MH493793.1
☒	Microbacterium indicum strain NC091221 16S ribosomal RNA gene, partial sequence	Microbacterium indicum	992	992	100%	0.0	98.92%	840	OR015527.1
☒	Microbacterium excoecariae strain CBSSP-1 16S ribosomal RNA, partial sequence	Microbacterium excoecariae	992	992	100%	0.0	98.92%	1488	NR_181116.1
☒	Microbacterium sp. strain EQ1 P35524 16S ribosomal RNA gene, partial sequence	Microbacterium sp.	992	992	100%	0.0	98.92%	1372	CP969043.1
☒	Microbacterium sp. strain EQ1 P35509 16S ribosomal RNA gene, partial sequence	Microbacterium sp.	992	992	100%	0.0	98.92%	1338	CP969028.1

Figure 7. Significant sequence alignments obtained for Trivandrum isolate 1.

Microbacterium sp. strain Atecer4N 16S ribosomal RNA gene, partial sequence

Sequence ID: [MT386149.1](#) Length: 1380 Number of Matches: 1

Range 1: 171 to 726 [GenBank](#) [Graphics](#) [Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1003 bits(543)	0.0	552/556(99%)	1/556(0%)	Plus/Plus
Query 1	GCGGCCTATCAGCTTGTGGTGAGGTAATGGCTACCAAGGCGTCGACGGGTAGCCGGCC	60		
Sbjct 171	GCGGCCTATCAGCTTGTGGTGAGGTAATGGCTACCAAGGCGTCGACGGGTAGCCGGCC	230		
Query 61	TGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGC	120		
Sbjct 231	TGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGC	290		
Query 121	AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGATGAC	180		
Sbjct 291	AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGATGAC	350		
Query 181	GGCCTTCGGGTTGTAAACCTCTATTAGTAGGGAAGAAGCCTTCGCGTGACGGTACCTGCA	240		
Sbjct 351	GGCCTTCGGGTTGTAAACCTCTTTTAGTAGGGAAGAAGCCTTCGGGTGACGGTACCTGCA	410		
Query 241	GAAAAAGCGCCGGCTAACTACGTGCCAGCAGCGCGGTAATACGTAGGGCGCAAGCGTTA	300		
Sbjct 411	GAAAAAGCGCCGGCTAACTACGTGCCAGCAGCGCGGTAATACGTAGGGCGCAAGCGTTA	470		
Query 301	TCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTGCTGTGAAAAC TG	360		
Sbjct 471	TCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTGCGCTGCTGTGAAAAC TG	530		
Query 361	GAGGCTCAACCTCCAGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTG	420		
Sbjct 531	GAGGCTCAACCTCCAGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTG	590		
Query 421	GAAATCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCA	480		
Sbjct 591	GAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCA	650		
Query 481	GATCTCTGGGCCGTAAGTACGCTGAGGAGCGAAAGAGTGGGGAGCAAACAGGCTTAGAT	540		
Sbjct 651	GATCTCTGGGCCGTAAGTACGCTGAGGAGCGAAAGAGTGGGGAGCAAACAGGCTTAGAT	710		

Figure 8. Sequence alignment showing similarity between Trivandrum isolate 1 and the nearest bacterial match.

Identification of *Microbacterium* species indicates the possible existence of environmental bacteria having survival and adaptation ability to marine host-associated environment. Environmental bacteria belonging to genus *Microbacterium* are common inhabitants of aquatic environments, involved in the cycling of nutrients (Zhou et al., 2022). In the same way, Trivandrum isolate 2 had maximum similarity with *Bacillus cereus*.

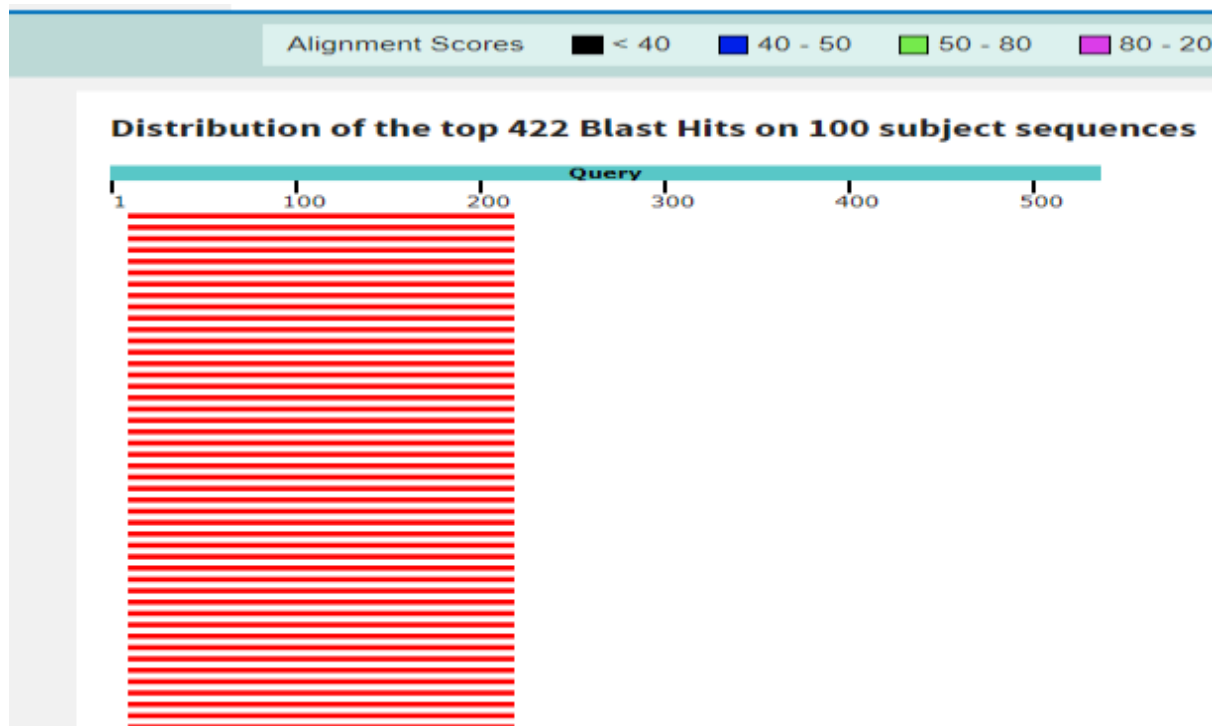


Figure 9. Graphic Representation of Top BLAST Hits Obtained for Trivandrum Isolate 2.

Descriptions									
Graphic Summary									
Alignments									
Taxonomy									
Sequences producing significant alignments									
Download									
Select columns									
Show 100									
select all 100 sequences selected									
GenBank									
Graphics									
Distance tree of results									
MSA Viewer									
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession	
☒ Bacillus cereus strain SGA0260 chromosome	Bacillus cereus	239	2711	38%	5e-58	87.16%	5045656	CP928009.1	
☒ Bacillus cereus strain SGA0262 chromosome, complete genome	Bacillus cereus	239	2711	38%	5e-58	87.16%	9947158	CP927820.1	
☒ Bacillus sp. JCHL-0493-1-1 16S ribosomal RNA gene, partial sequence	Bacillus sp. JCHL-0493-1-1	239	239	38%	5e-58	87.16%	1476	FJ785733.1	
☒ Bacillus pumilus strain S1 16S ribosomal RNA gene, partial sequence	Bacillus pumilus	237	237	38%	2e-57	87.04%	1482	ON026801.1	
☒ Bacillus thuringiensis strain PDAAR025_702 chromosome, complete genome	Bacillus thuringiensis	233	3145	38%	2e-56	86.70%	5263142	CP953965.1	
☒ Bacillus thuringiensis strain A0A-CP81 chromosome	Bacillus thuringiensis	233	3151	38%	2e-56	86.70%	5248960	CP949019.1	
☒ Bacillus sp. (in: Bacillus) strain MZUR14 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	233	233	38%	2e-56	86.70%	836	MT086208.1	
☒ Bacillus cereus strain DLOU-Vishal chromosome, complete genome	Bacillus cereus	233	3162	38%	2e-56	86.70%	5291741	CP940342.1	
☒ Bacillus sp. (in: Bacillus) strain rasta46 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	233	233	38%	2e-56	86.70%	1488	MH609688.1	
☒ Bacillus pumilus strain B011 gene for 16S ribosomal RNA, partial sequence	Bacillus pumilus	233	233	38%	2e-56	86.64%	1505	LC501436.1	
☒ Bacillus sp. JAS24-2 chromosome, complete genome	Bacillus sp. JAS24-2	233	2700	38%	2e-56	86.70%	5225480	CP931069.1	
☒ Bacillus cereus strain ASY1561 16S ribosomal RNA gene, partial sequence	Bacillus cereus	233	233	38%	2e-56	86.70%	732	MH309628.1	
☒ Bacillus thuringiensis strain SA23 16S ribosomal RNA gene, partial sequence	Bacillus thuringiensis	233	233	38%	2e-56	86.70%	1055	MK467567.1	
☒ Bacillus anthracis strain HD26-8YS87 chromosome, complete genome	Bacillus anthracis	233	3151	38%	2e-56	86.70%	5294779	CP950609.1	
☒ Uncultured bacterium clone Hidenov100 16S ribosomal RNA gene, partial sequence	uncultured bacterium	233	233	38%	2e-56	86.70%	277	MH102780.1	
☒ Bacillus cereus strain CC-1 chromosome, complete genome	Bacillus cereus	233	3166	38%	2e-56	86.70%	5279524	CP933179.1	
☒ Uncultured Bacillus sp. clone EQY2016 16S ribosomal RNA gene, partial sequence	uncultured Bacillus sp.	233	233	38%	2e-56	86.70%	1410	KY593164.1	
☒ Uncultured bacterium clone S2-90 16S ribosomal RNA gene, partial sequence	uncultured bacterium	233	233	38%	2e-56	86.70%	1040	KY563627.1	
☒ Bacillus sp. (in: firmicutes) 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in: firmicutes)	233	233	38%	2e-56	86.70%	632	KX062471.1	

Figure 10. Significant sequence alignments obtained for Trivandrum isolate 2.

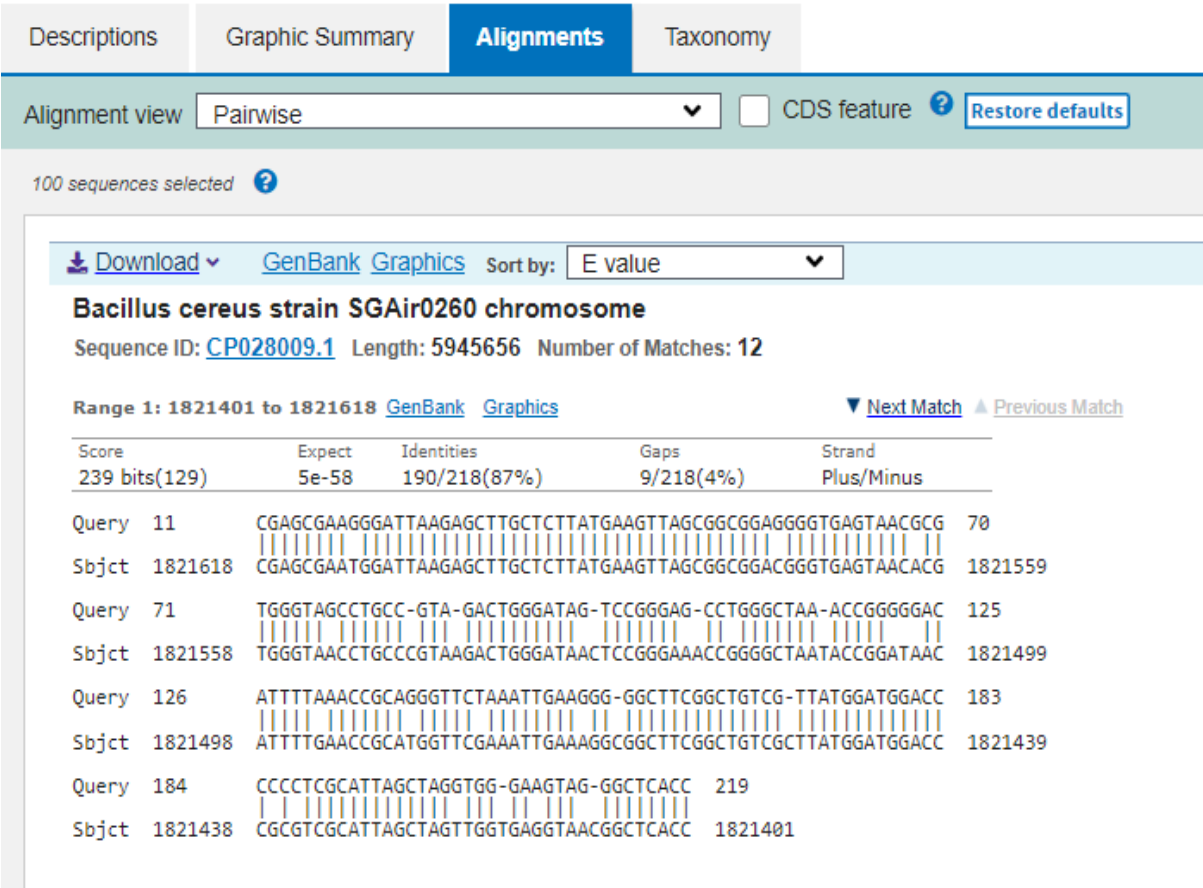


Figure 11. Sequence alignment showing similarity between Trivandrum isolate 2 and *Bacillus cereus*.

The bacterial species within the genus *Bacillus* have been isolated from fish gut tracts and are characterized by their ability to secrete extracellular enzymes responsible for the breakdown and digestion of nutrients (Ray et al., 2012). Furthermore, Thoothoor isolate 1 was found to be *Bacillus cereus* through BLAST search results.

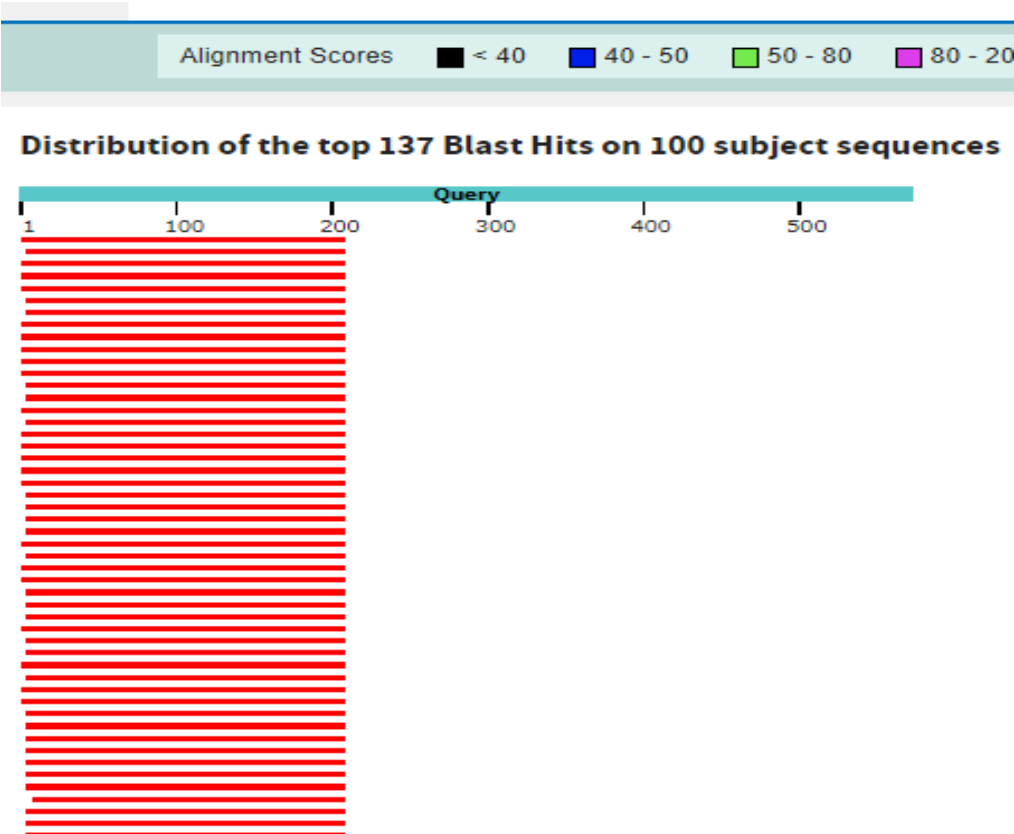


Figure 12. Top BLAST matches for Thoothoor isolate 1.

Descriptions	Graphic Summary	Alignments	Taxonomy					
Sequences producing significant alignments								
Download Select columns Show 100								
select all 100 sequences selected								
GenBank Graphics Distance tree of results MSA Viewer								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒ Bacillus cereus strain AAF3 16S ribosomal RNA gene, partial sequence	Bacillus cereus	309	309	36%	4e-79	93.72%	816	MF191709.1
☒ Bacillus cereus strain ASWISA1 16S ribosomal RNA gene, partial sequence	Bacillus cereus	305	305	35%	5e-78	93.66%	732	MN309828.1
☒ Bacterium strain BS1172 16S ribosomal RNA gene, partial sequence	bacterium	305	305	36%	5e-78	93.27%	1463	MK924360.1
☒ Bacillus tropicus strain SA39 16S ribosomal RNA gene, partial sequence	Bacillus tropicus	305	305	36%	5e-78	93.27%	1057	MK467594.1
☒ Bacillus tropicus strain SA23 16S ribosomal RNA gene, partial sequence	Bacillus tropicus	305	305	36%	5e-78	93.27%	1055	MK467552.1
☒ Bacillus tropicus strain SS9 16S ribosomal RNA gene, partial sequence	Bacillus tropicus	305	305	35%	5e-78	93.66%	1085	MK467544.1
☒ Bacillus sp. (in firmicutes) strain WPT 16S ribosomal RNA gene, partial sequence	Bacillus sp. (in firmicutes)	305	305	35%	5e-78	93.66%	1395	KY347914.1
☒ Bacillus sp. BTAI chromosome, complete genome	Bacillus sp. BTAI	305	4135	36%	5e-78	93.27%	5219042	CP125950.1
☒ Bacillus sp. EJB8 16S ribosomal RNA gene, partial sequence	Bacillus sp. EJB8	305	305	36%	5e-78	93.27%	1518	DQ451098.2
☒ Bacillus paranthracis isolate 1702 chromosome, complete genome	Bacillus paranthracis	305	3886	36%	5e-78	93.27%	5407047	CP113375.1
☒ Bacillus paranthracis isolate 4M chromosome, complete genome	Bacillus paranthracis	305	3759	36%	5e-78	93.27%	5408718	CP113365.1
☒ Bacillus tropicus strain K10 16S ribosomal RNA gene, partial sequence	Bacillus tropicus	305	305	36%	5e-78	93.27%	1440	QPT721103.1
☒ Bacillus cereus strain WGT1 16S ribosomal RNA gene, partial sequence	Bacillus cereus	305	305	35%	5e-78	93.66%	1453	QP388443.1
☒ Bacillus sp. BT MASC 1 16S ribosomal RNA gene, partial sequence	Bacillus sp. BT MASC 1	305	305	35%	5e-78	93.66%	890	KJ438150.1

Figure 13. BLAST matches with significant alignments for Thoothoor isolate 1.

Descriptions

Graphic Summary

Alignments

Taxonomy

Alignment view

Pairwise

CDS feature

Restore defaults

100 sequences selected

Download

GenBank

Graphics

Bacillus cereus strain AAF3 16S ribosomal RNA gene, partial sequence

Sequence ID: MF191709.1

Length: 816

Number of Matches: 1

Range 1: 3 to 209

GenBank

Graphics

Next Match

Previous Match

Score	Expect	Identities	Gaps	Strand
309 bits(167)	4e-79	194/207(94%)	1/207(0%)	Plus/Plus
Query 2	ACTTGCAAGTCGAGCGATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTG	61		
Sbjct 3	ACTTGCAAGTCGAGCGATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTG	62		
Query 62	AGTAACACGTGGGTAACCTGCCATAA-ACTGGGATAACTCCGGGAAACCGGGGCTAATA	120		
Sbjct 63	AGTAACACGTGGGTAACCTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATA	122		
Query 121	CGGGATAACCTTTTGAACCGCAGGGTTCTAAATTTAAAGGCGGCTTCGGCTGTCTTTAT	180		
Sbjct 123	CCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTAT	182		
Query 181	GGATGGAACCGCGTACCAATAGCTAGT	207		
Sbjct 183	GGATGGACCCGCGTCGCATTAGCTAGT	209		

Figure 14. Sequence alignments for Thoothoor isolate 1 and Bacillus cereus.

In view of the findings obtained, it is likely that Bacillus cereus is a common culturable member of the tuna gut microbiome population. Second Thoothoor isolate was found to show highest identity at the sequence level with Lacrimispora amygdalina.

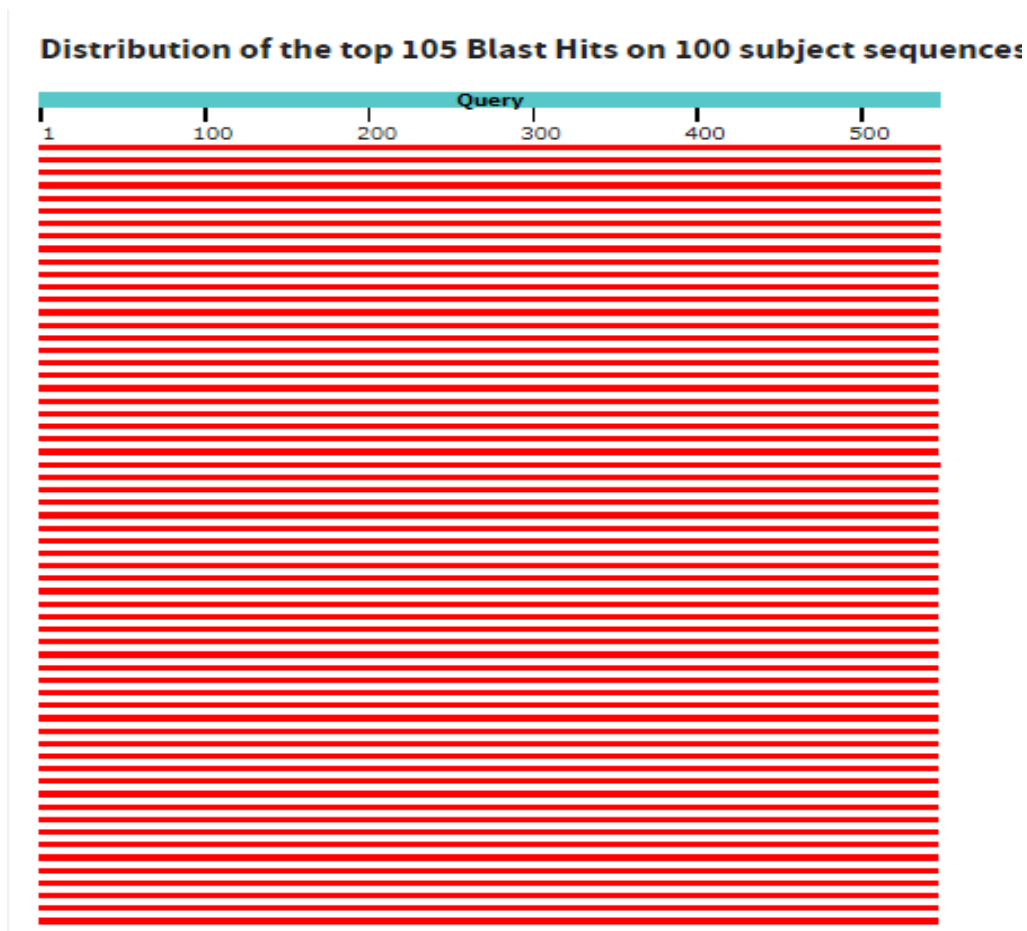


Figure 15. Graphical representation of top BLAST hits for Thoothoor isolate 2.

Descriptions	Graphic Summary	Alignments	Taxonomy					
Sequences producing significant alignments								
Download Select columns Show 100								
select all 100 sequences selected								
GenBank Graphics Distance tree of results MSA Viewer								
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lacrimispora amygdalina strain JB20 16S ribosomal RNA gene, partial sequence	Lacrimispora amygdalina	985	985	100%	0.0	99.27%	1437	MT264911.1
Lacrimispora amygdalina strain JB12 16S ribosomal RNA gene, partial sequence	Lacrimispora amygdalina	985	985	100%	0.0	99.27%	1394	MT264792.1
Lacrimispora amygdalina strain JB4 16S ribosomal RNA gene, partial sequence	Lacrimispora amygdalina	985	985	100%	0.0	99.27%	1414	MT264780.1
Clostridium indicum strain PI-S10-A1B 16S ribosomal RNA gene, partial sequence	Clostridium indicum	985	985	100%	0.0	99.27%	1509	MH193004.3
Bacterium enrichment culture clone OTU1 16S ribosomal RNA gene, partial sequence	bacterium enrichment culture	985	985	100%	0.0	99.27%	1169	KP715407.1
Clostridium indicum strain PI-S10-A1B 16S ribosomal RNA, partial sequence	Clostridium indicum	985	985	100%	0.0	99.27%	1509	NR_180648.1
Lacrimispora amygdalina strain BR-10 16S ribosomal RNA gene, partial sequence	Lacrimispora amygdalina	985	985	100%	0.0	99.27%	1485	ON754040.1
Clostridium sp. AIP 321.05 16S ribosomal RNA gene, partial sequence	Clostridium sp. AIP 321.05	985	985	100%	0.0	99.27%	1362	DQ507245.1
Lacrimispora amygdalina strain BR-10 16S ribosomal RNA, partial sequence	Lacrimispora amygdalina	985	985	100%	0.0	99.27%	1485	NR_115211.1
Uncultured bacterium clone RMA169 16S ribosomal RNA gene, partial sequence	uncultured bacterium	983	983	99%	0.0	99.26%	1274	JF681680.1
Uncultured bacterium clone RMA168 16S ribosomal RNA gene, partial sequence	uncultured bacterium	983	983	99%	0.0	99.26%	1270	JF681679.1
Uncultured bacterium clone RMA161 16S ribosomal RNA gene, partial sequence	uncultured bacterium	983	983	99%	0.0	99.26%	1274	JF681672.1
Uncultured bacterium clone RMA145 16S ribosomal RNA gene, partial sequence	uncultured bacterium	983	983	99%	0.0	99.26%	1284	JF681656.1
Uncultured bacterium clone RMA143 16S ribosomal RNA gene, partial sequence	uncultured bacterium	983	983	99%	0.0	99.26%	1274	JF681654.1

Figure 16. Important sequence alignments for Thoothoor isolate 2.

Lacrimispora amygdalina strain JB20 16S ribosomal RNA gene, partial sequence

Sequence ID: [MT264911.1](#) Length: 1437 Number of Matches: 1

Range 1: 272 to 816 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
985 bits(533)	0.0	541/545(99%)	0/545(0%)	Plus/Plus
Query 1	CCGACCTGAGAGGGTGACCGGCCACATTGCGACTGAGACACGGCCCAAACCTCTACGGGA	60		
Sbjct 272	CCGACCTGAGAGGGTGACCGGCCACATTGGGACTGAGACACGGCCCAAACCTCTACGGGA	331		
Query 61	GGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCTGATCCAGCGACGCCGCGTGACT	120		
Sbjct 332	GGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCTGATCCAGCGACGCCGCGTGACT	391		
Query 121	GAAGAAGTATTTTCGGTATGTATAGGTCTATCAGCAGGGAAGAATATGACGGTACCTGACT	180		
Sbjct 392	GAAGAAGTATTTTCGGTATGTAAAGGTCTATCAGCAGGGAAGAAAATGACGGTACCTGACT	451		
Query 181	AAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTAT	240		
Sbjct 452	AAGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGTTAT	511		
Query 241	CCGGATTACTGGGTGTAAAGGGAGCGTAGACGGCGATGCAAGTCTGGAGTGAAAGCCCG	300		
Sbjct 512	CCGGATTACTGGGTGTAAAGGGAGCGTAGACGGCGATGCAAGTCTGGAGTGAAAGCCCG	571		
Query 301	GGGCTCAACCCCGGACTGCTTTGGAAACTGTGTTGCTAGAGTGCAGGAGAGGTAAGTGG	360		
Sbjct 572	GGGCTCAACCCCGGACTGCTTTGGAAACTGTGTTGCTAGAGTGCAGGAGAGGTAAGTGG	631		
Query 361	AATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAAGTCGCGAAGGCCG	420		
Sbjct 632	AATTCCTAGTGTAGCGGTGAAATGCGTAGATATTAGGAGGAACACCAAGTCGCGAAGGCCG	691		
Query 421	CTTACTGGACTGTAAGTACGCTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATA	480		
Sbjct 692	CTTACTGGACTGTAAGTACGCTTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATA	751		
Query 481	CCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTTGGGGAGCAAAGCTCTTCGGT	540		
Sbjct 752	CCCTGGTAGTCCACGCCGTAAACGATGAATACTAGGTGTTGGGGAGCAAAGCTCTTCGGT	811		
Query 541	GCCGC	545		

Figure 17. Sequence alignment depicting identity between Thoothoor isolate 2 and *Lacrimispora amygdalina*.

Lacrimispora genera consist of anaerobes which undergo fermentation metabolism and can break down organic compounds. Presence of such isolates suggests that anaerobic niches are present in fish intestines (Sivasubramanian et al., 2012). Sequence analysis for Kanyakumari isolate 1 showed identification of *Escherichia coli*.

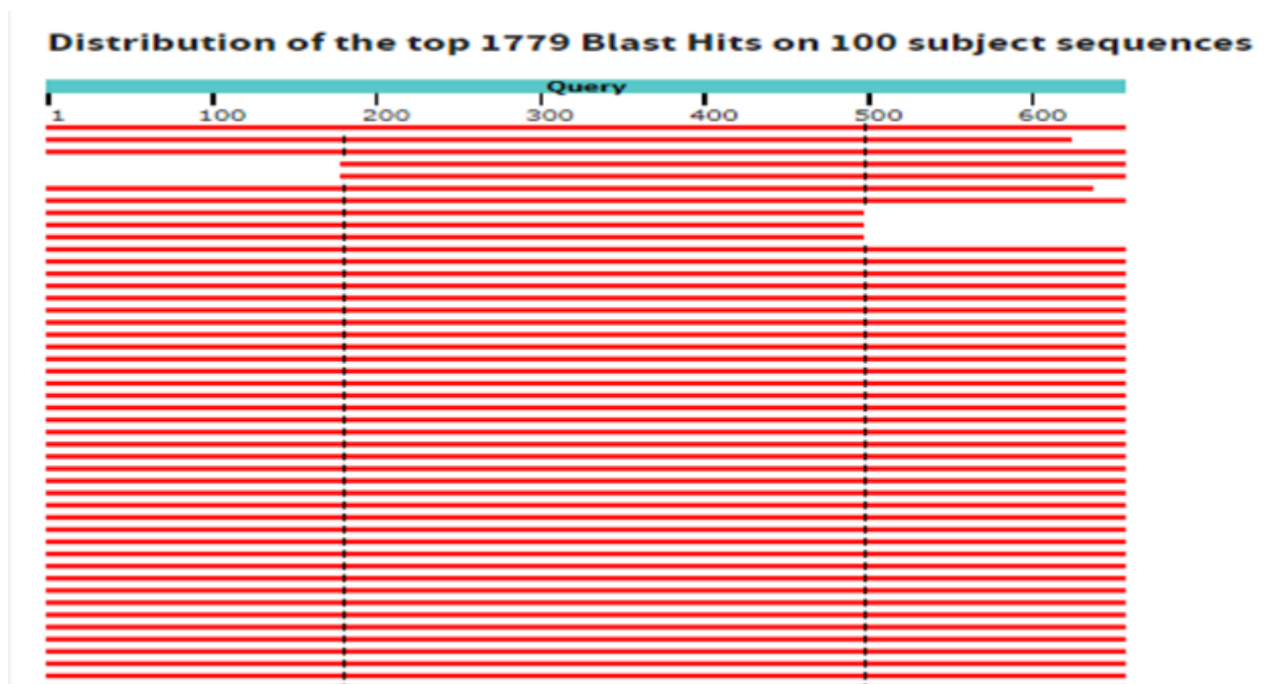


Figure 18. Graphical representation of top BLAST hits for Kanyakumari isolate 1.

Descriptions

Graphic Summary

Alignments

Taxonomy

Sequences producing significant alignments

Download

Select columns

Show

100

select all

100 sequences selected

GenBank

Graphics

Distance tree of results

MSA Viewer

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
☒	Escherichia coli partial 16S rRNA gene, strain RFR-3	Escherichia coli	891	1191	100%	0.0	99.19%	862	LT599761.1
☒	Unidentified marine bacterioplankton clone P2-4B 118 16S ribosomal RNA gene, partial sequence	unidentified marine bacte...	569	1124	95%	2e-157	99.05%	1371	KC001002.1
☒	Uncultured bacterium clone ELU005-T44-S-NIPCRAMp2Na 000616 16S ribosomal RNA gene, part...	uncultured bacterium	569	1174	100%	2e-157	99.05%	1444	HQ739009.1
☒	Uncultured bacterium clone 19bd21treat2 16S ribosomal RNA gene, partial sequence	uncultured bacterium	569	859	72%	2e-157	99.05%	877	GU171257.1
☒	Uncultured bacterium clone 9bd21treat2 16S ribosomal RNA gene, partial sequence	uncultured bacterium	569	859	72%	2e-157	99.05%	877	GU171253.1
☒	Kosakonia sp. strain Clone10 16S ribosomal RNA gene, partial sequence	Kosakonia sp.	564	1070	96%	9e-156	98.74%	1245	OQ928108.1
☒	Escherichia coli O157:H7 str EDL933 chromosome, complete genome	Escherichia coli O157:H7...	564	8335	100%	9e-156	98.74%	5138920	CP124740.1
☒	Escherichia sp. strain KOS 1021-5/24-TC-P5 16S ribosomal RNA gene, partial sequence	Escherichia sp.	564	896	75%	9e-156	98.74%	1078	OQ918368.1
☒	Escherichia sp. strain KOS 1021-5/21-TC-P3 16S ribosomal RNA gene, partial sequence	Escherichia sp.	564	896	75%	9e-156	98.74%	1024	OQ918366.1
☒	Escherichia sp. strain KOS 1021-3/15-T-P1 16S ribosomal RNA gene, partial sequence	Escherichia sp.	564	896	75%	9e-156	98.74%	1073	OQ918338.1
☒	Escherichia coli strain ECS07 16S ribosomal RNA gene, partial sequence	Escherichia coli	564	1196	100%	9e-156	98.74%	1442	OQ891229.1

Figure 19. Important sequence alignments for Kanyakumari isolate 1.

Escherichia coli partial 16S rRNA gene, strain RFR-3									
Sequence ID: LT599761.1 Length: 862 Number of Matches: 2									
Range 1: 1 to 494 GenBank Graphics Next Match Previous Match									
Score	Expect	Identities		Gaps		Strand			
891 bits (482)	0.0	490/494 (99%)		0/494 (0%)		Plus/Plus			
Query 1		GGGCGCAAGCCTGATGCAAGCCATGCCGCGTGTATGAAAGAGGCCTTCGGGTTGTAAAGTA				60			
Sbjct 1		GGGCGCAAGCCTGATGCAAGCCATGCCGCGTGTATGAAAGAGGCCTTCGGGTTGTAAAGTA				60			
Query 61		CTTTCAGCGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGA				120			
Sbjct 61		CTTTCAGCGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGA				120			
Query 121		AGAAAGCACCAGGCTAACTCCGTGCCAGCAGCCGCGTAATACGGAGGGTGCAAGCGTTAAG				180			
Sbjct 121		AGAAAGCACCAGGCTAACTCCGTGCCAGCAGCCGCGTAATACGGAGGGTGCAAGCGTTAAG				180			
Query 181		GCGGCCCTTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCATAACAGGATTA				240			
Sbjct 181		GCGGCCCTTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCATAACAGGATTA				240			
Query 241		GATACCTTGGTAGTCCACGCCGTAAACGATGTGCGACTTGGAGGTTGTGCCCTTGAGGCGT				300			
Sbjct 241		GATACCTTGGTAGTCCACGCCGTAAACGATGTGCGACTTGGAGGTTGTGCCCTTGAGGCGT				300			
Query 301		GGCTTCGGAGCTAACGCGTTAAGTCGACCGCTTGGGAGTACGGCCGCAAGGTTAATAC				360			
Sbjct 301		GGCTTCGGAGCTAACGCGTTAAGTCGACCGCTTGGGAGTACGGCCGCAAGGTTAATAC				360			
Query 361		TCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTAATTTCGATGCAAC				420			
Sbjct 361		TCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTAATTTCGATGCAAC				420			
Query 421		GCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTATCCAGAGATGGATTGGTGCCTT				480			
Sbjct 421		GCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTCAGAGATGGATTGGTGCCTT				480			
Query 481		CGGGAACTGTGAAGA 494							
Sbjct 481		CGGGAACTGTGAAGA 494							

Figure 20. Sequence alignment indicating identity between Kanyakumari isolate 1 and Escherichia coli.

Presence of *E. coli* could either be due to environmental contact or transient colonization via food consumption, which has been shown earlier in marine fishes (Luna et al., 2012). Maximum similarity of second isolate from Kanyakumari is observed with *Oceanisphaera ostreae*.

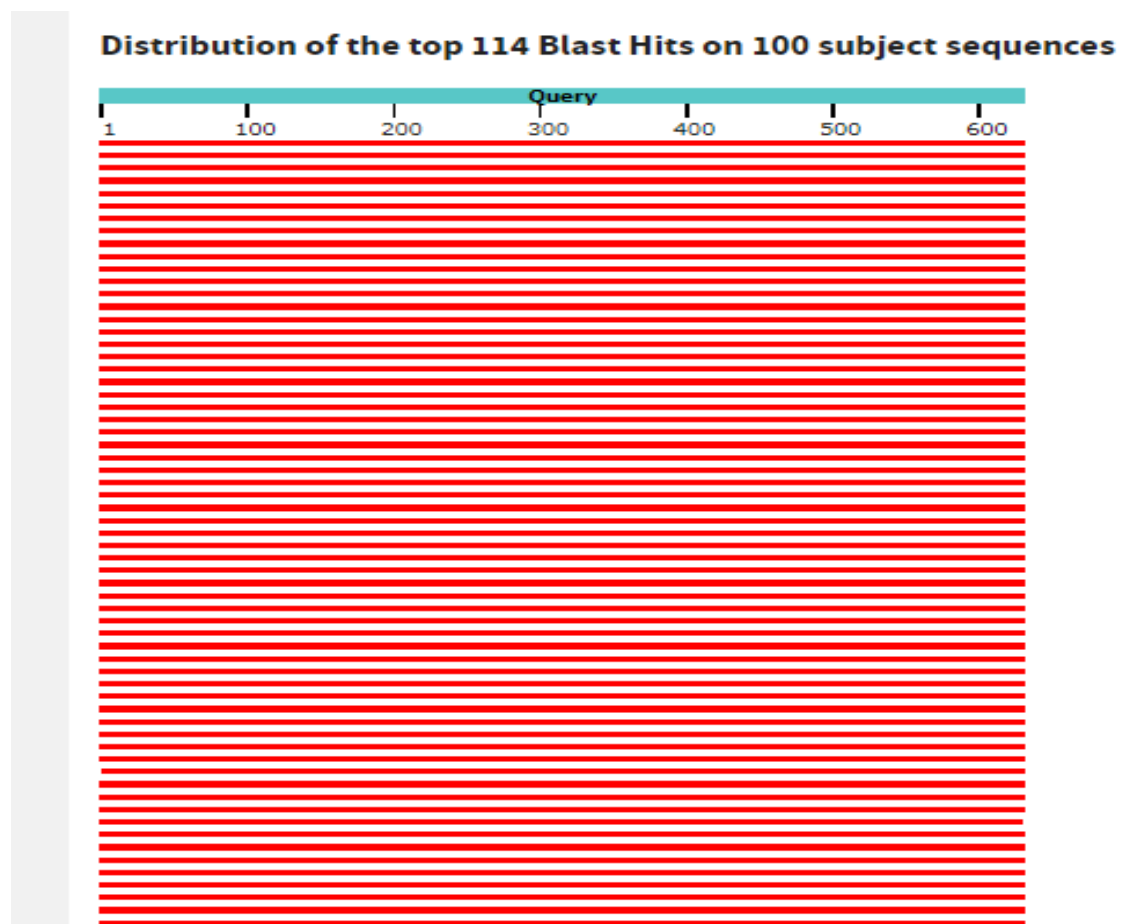


Figure 21. Graphical representation of top BLAST hits for Kanyakumari isolate 2.

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
✓	Oceanisphaera ostreae strain 20_KNS1_Sed_R1 16S ribosomal RNA gene, partial sequence	Oceanisphaera ostreae	1120	1120	100%	0.0	98.88%	1408	MN080177.1
✓	Oceanisphaera ostreae strain 4_KNS1_Sed_R4 16S ribosomal RNA gene, partial sequence	Oceanisphaera ostreae	1112	1112	100%	0.0	98.72%	1402	MN080153.1
✓	Oceanisphaera ostreae strain T-w6 16S ribosomal RNA, partial sequence	Oceanisphaera ostreae	1112	1112	100%	0.0	98.72%	1485	NR_109099.1
✓	Oceanisphaera sp. 48/2010/ 16S ribosomal RNA gene, partial sequence	Oceanisphaera sp. 48/2010/	1112	1112	100%	0.0	98.72%	1490	GQ891117.1
✓	Oceanisphaera sp. SS1.15 16S ribosomal RNA gene, partial sequence	Oceanisphaera sp. SS1.15	1107	1107	100%	0.0	98.56%	1504	KC160849.1
✓	Oceanisphaera sp. SS1.16 16S ribosomal RNA gene, partial sequence	Oceanisphaera sp. SS1.16	1103	1103	100%	0.0	98.41%	1505	KC160850.1
✓	Oceanimonas sp. SS1.31 16S ribosomal RNA gene, partial sequence	Oceanimonas sp. SS1.31	1079	1079	100%	0.0	97.77%	1503	KC160864.1
✓	Oceanimonas sp. SS1.27 16S ribosomal RNA gene, partial sequence	Oceanimonas sp. SS1.27	1079	1079	100%	0.0	97.77%	1503	KC160861.1
✓	Oceanimonas sp. SS1.18 16S ribosomal RNA gene, partial sequence	Oceanimonas sp. SS1.18	1079	1079	100%	0.0	97.77%	1503	KC160852.1
✓	Oceanisphaera sp. SS1.13 16S ribosomal RNA gene, partial sequence	Oceanisphaera sp. SS1.13	1079	1079	100%	0.0	97.77%	1503	KC160847.1
✓	Uncultured bacterium clone XJDN-868 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1074	1074	100%	0.0	97.61%	1485	KJ454331.1
✓	Uncultured bacterium clone XJDN-350 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1074	1074	100%	0.0	97.61%	1516	KJ454073.1
✓	Oceanimonas sp. SS1.23 16S ribosomal RNA gene, partial sequence	Oceanimonas sp. SS1.23	1074	1074	100%	0.0	97.61%	1503	KC160857.1
✓	Oceanisphaera profunda strain SM1222 chromosome, complete genome	Oceanisphaera profunda	1068	8348	100%	0.0	97.45%	3508860	CP021377.1
✓	Uncultured bacterium clone XJDN-562 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1068	1068	100%	0.0	97.45%	1485	KJ454178.1
✓	Uncultured bacterium clone XJDN-146 16S ribosomal RNA gene, partial sequence	uncultured bacterium	1068	1068	100%	0.0	97.45%	1483	KJ453971.1
✓	Oceanimonas sp. SS1.26 16S ribosomal RNA gene, partial sequence	Oceanimonas sp. SS1.26	1068	1068	100%	0.0	97.45%	1504	KC160860.1

Figure 22. Significant sequence alignments identified for Kanyakumari isolate 2.

Oceanisphaera ostreae strain 20_KNS1_Sed_R1 16S ribosomal RNA gene, partial sequence

Sequence ID: [MN080177.1](#) Length: 1408 Number of Matches: 1

Range 1: 214 to 840 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1120 bits(606)	0.0	620/627(99%)	0/627(0%)	Plus/Plus
Query 1	CCAAGGCGACGATCTCTAACTGGTCTGAGAGGATGACCACTGAGGACTGAGACAC	60		
Sbjct 214	CCAAGGCGACGATCTCTAACTGGTCTGAGAGGATGACCACTGAGGACTGAGACAC	273		
Query 61	GGCCAGACTCCTACGGGAGGAGCAGTGGGAATATTGCACAATGCCGGAACCTGAT	120		
Sbjct 274	GGCCAGACTCCTACGGGAGGAGCAGTGGGAATATTGCACAATGCCGGAACCTGAT	333		
Query 121	GCAGCCATGCCGCGTGTGTGAAGTAGGCCTTCGGGTTGTAAGCACTTTCAGTGGTGAGG	180		
Sbjct 334	GCAGCCATGCCGCGTGTGTGAAGTAGGCCTTCGGGTTGTAAGCACTTTCAGTGGTGAGG	393		
Query 181	AAAGGTAAAGGGTTAATAACTCCTTGTGTGACGTTAACCACAGAAGAAGCACCGGCTAA	240		
Sbjct 394	AAAGGTAAAGGGTTAATAACTCCTTGTGTGACGTTAACCACAGAAGAAGCACCGGCTAA	453		
Query 241	CTCCGTGCCAGCAGCCGCGGTAAACGAGGGTGCAAGCGTTAATCGGAATAACTGGGCG	300		
Sbjct 454	CTCCGTGCCAGCAGCCGCGGTAAACGAGGGTGCAAGCGTTAATCGGAATAACTGGGCG	513		
Query 301	TAAAGCGCACGAGGCGGTTTGTAAAGCCAGATGTGAAAGCCCCGAGCTCAACTCGGGAA	360		
Sbjct 514	TAAAGCGCACGAGGCGGTTTGTAAAGCCAGATGTGAAAGCCCCGAGCTCAACTCGGGAA	573		
Query 361	CTGCATTGTGAACTCGCAACTAGAGTCTTGTAGAGGGGGTAGAATTTCCAGTGTAGCG	420		
Sbjct 574	CTGCATTGTGAACTCGCAACTAGAGTCTTGTAGAGGGGGTAGAATTTCCAGTGTAGCG	633		
Query 421	GTGAAATGCGTAGAGATTGGAAGGAATACCACTGGCGAAGGCGGCGGGTGGACAAAGAC	480		
Sbjct 634	GTGAAATGCGTAGAGATTGGAAGGAATACCACTGGCGAAGGCGGCGGGTGGACAAAGAC	693		

Figure 23. Alignment of sequence demonstrating similarity to Kanyakumari isolate 2 and Oceanisphaera ostreae.

Oceanisphaera ostreae is a bacterium associated with the marine environment that can be found in seawater and marine life forms. It emphasizes yet again the impact of the surrounding marine environment on the bacteria community within the gut of tunas.

Table 2. Bacterial isolates identified through 16S rRNA gene sequencing

Location	Isolate	Identified Species
Trivandrum	Colony 1	Microbacterium sp.
Trivandrum	Colony 2	Bacillus cereus
Thoothoor	Colony 1	Bacillus cereus
Thoothoor	Colony 2	Lacrimispora amygdalina
Kanyakumari	Colony 1	Escherichia coli
Kanyakumari	Colony 2	Oceanisphaera ostreae

The bacterial taxa identified in the present study are consistent with existing literature describing diverse gut microbial communities in marine fishes. Members of the genus *Bacillus* have frequently been observed within the intestinal tracts of fish and are recognized for their involvement in nutrient breakdown and the production of enzymes (Ray et al., 2012). Similarly, *Microbacterium* species are commonly found in aquatic environments while *Oceanisphaera* bacteria are linked to the existence in the marine environment. The detection of different groups of bacteria in samples taken from Thoothoor, Kanyakumari, and Trivandrum suggests the potential impact of environmental factors on gut microbial diversity. Such findings are also supported by previous studies where habitat, food preference, and environment parameters played significant roles in determining the gut microbiome of fish species (Lokesh & Kiron, 2016; Egerton et al., 2018; Cao, 2025).

4. CONCLUSION

Gut microorganisms associated with *E. affinis* and *T. albacares* were isolated and identified from specimens obtained from three geographic regions, which included Thoothoor, Kanyakumari, and Trivandrum. The gut tissues were homogenized, serially diluted, and then inoculated in Nutrient Agar medium to isolate pure colonies of bacteria. The selected colonies were purified through subcultures, preserved as stocks, and grown in liquid media for further molecular studies. Genomic DNA was extracted from bacterial cultures and subjected to molecular characterization by 16S rRNA gene amplification and sequencing. Sequences were compared with the NCBI BLAST database to identify bacteria. Sequence analyses showed the presence of *Bacillus cereus* and *Microbacterium* species in samples from Trivandrum, while those from

Thoothoor had *Bacillus cereus* and *Lacrimispora amygdalina*. On the other hand, Kanyakumari samples had isolates such as *Escherichia coli* and *Oceanisphaera ostreae*. These results show that there are diverse culturable bacterial communities found inside the intestines of tuna fishes, with differences in bacterial community composition from different geographic locations. The study provides baseline information of culturable bacteria in the gut microbiome of *E. affinis* and *T. albacares*, and helps us gain more knowledge regarding microbe diversity in tuna fish populations.

REFERENCES

1. Alikunhi, N. M., Batang, Z. B., AlJahdali, H. A., Aziz, M. A., & Al-Suwailem, A. M. (2017). Culture-dependent bacteria in commercial fishes: Qualitative assessment and molecular identification using 16S rRNA gene sequencing. *Saudi Journal of Biological Sciences*, 24(6), 1105-1116.
2. Cao, S. (2025). Shaping the salmonid gut microbiota: meta-analytical insights and dietary approaches to enhance fish health and performance.
3. Egerton, S., Culloty, S., Whooley, J., Stanton, C., & Ross, R. P. (2018). The gut microbiota of marine fish. *Frontiers in microbiology*, 9, 873.
4. Govindaraj, K., Samayanpaulraj, V., Narayanadoss, V., & Uthandakalaipandian, R. (2021). Isolation of lactic acid bacteria from intestine of freshwater fishes and elucidation of probiotic potential for aquaculture application. *Probiotics and antimicrobial proteins*, 13(6), 1598-1610.
5. Lokesh, J., & Kiron, V. (2016). Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. *Scientific reports*, 6(1), 19707.
6. Loughran, T. (2021). Chasing catch: climate-driven distribution and abundance of Pacific bluefin tuna and Japanese anchovy.
7. Luna, G. M., Vignaroli, C., Rinaldi, C., Pusceddu, A., Nicoletti, L., Gabellini, M., ... & Biavasco, F. (2010). Extraintestinal *Escherichia coli* carrying virulence genes in coastal marine sediments. *Applied and environmental microbiology*, 76(17), 5659-5668.
8. Ray, A. K., Ghosh, K., & Ringø, E. J. A. N. (2012). Enzyme-producing bacteria isolated from fish gut: a review. *Aquaculture nutrition*, 18(5), 465-492.
9. Sivasubramanian, K., Ravichandran, S., & Kavitha, R. (2012). Isolation and characterization of gut micro biota from some estuarine fishes. *Marine Science*, 2(2), 1-6.
10. Sylvain, F. É., Holland, A., Bouslama, S., Audet-Gilbert, É., Lavoie, C., Val, A. L., & Derome, N. (2020). Fish skin and gut microbiomes show contrasting signatures of host species and habitat. *Applied and environmental microbiology*, 86(16), e00789-20.
11. Tarnecki, A. M., Burgos, F. A., Ray, C. L., & Arias, C. R. (2017). Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. *Journal of applied microbiology*, 123(1), 2-17.
12. Teletchea, F. (2009). Molecular identification methods of fish species: reassessment and possible applications. *Reviews in Fish Biology and Fisheries*, 19(3), 265-293.
13. Zhou, J. S., Cheng, J. F., Li, X. D., & Li, Y. H. (2022). Unique bacterial communities associated with components of an artificial aquarium ecosystem and their possible contributions to nutrient cycling in this microecosystem. *World Journal of Microbiology and Biotechnology*, 38(4), 72