Research Article

Exploration and Profiling of Bacteria Symbionts in *Arothron stellatus* from the Thoothukudi Coast

P. Kanthimathi*, P. J. Joslin

Department of Zoology, St. Mary's College (Autonomous), Thoothukudi (Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli), Tamil Nadu, INDIA.

Submission Date: 22-05-2024; Revision Date: 17-07-2024; Accepted Date: 19-08-2024.

ABSTRACT

Marine organisms inhabit a distinct ecological niche compared to terrestrial counterparts, leading to significant variations in their secondary metabolite profiles. Symbiotic relationships between microorganisms and marine organisms are prevalent and diverse in the marine environment. In this study, pufferfish *Arothron stellatus* specimens were collected from the Thoothukudi coast and their associated liver bacteria were examined. Two symbiotic strains were isolated and characterized through cultural, morphological and molecular analyses. These isolates exhibited traits such as motility, rod-shaped morphology and both Gram-positive and Gram-negative features. Based on 16S rRNA gene analysis, the strains were identified as *Achromobacter xylosoxidans* (L_1) and *Bacillus cereus* (L_2). The respective sequences have been deposited in the Genebank under accession numbers OP420554 (L_1) and OP42055 (L_2). Tetrodotoxin (TTX) is a well-known toxin present in puffer fishes, and it is produced by various bacterial species associated with these fish. Our findings suggest that the isolated bacterial strains, *Achromobacter xylosoxidans* (L_1) and *Bacillus cereus* (L_2), may potentially contribute to TTX production. Further research is warranted to elucidate the synthesis mechanism of TTX and the role of bacteria in this process.

Keywords: *Arothron stellatus, Bacillus cereus, Achromobacter xylosoxidans*, GeneBank, 16S rRNA.

Correspondence:
Mrs. P. Kanthimathi
Ph.D Research Scholar (Reg.No-20112212192003)
Research Scholar,
PG and Research,
Department of Zoology,
St. Mary's College (Autonomous),
Thoothukudi-628001,
(Affiliated to Manonmaniam Sundaranar University,
Abishekapatti,
Tirunelveli), Tamil Nadu, INDIA.

Email: pkanthimathi77@gmail.com

INTRODUCTION

In the biosphere, the marine environment covers 70% of the earth and acts as the largest ecosystem for various living organisms, especially microbes. Marine microbiology has encouraged the interest of researchers to investigate unexplored marine environments from all over the world, with a focus on the discovery of novel bioactive compounds.[1] Fishes are a vital component of marine habitats. Pufferfish is an underutilized fish in any fishing. Pufferfish is commonly known as a fatal fish due to the presence of a neurotoxin called Tetrodotoxin (TTX) in its body organelles like the liver, gonad, skin, muscle and testis.[2] The symbiotic relationship between microorganisms and marine organisms is abundant and widespread in the sea. Diverse and widespread symbiotic microorganisms are hosted by different marine creatures.[3]

Bacterial symbiosis, that is, host-bacterial association for a prosperous relationship, is common in marine animals. These bacterial pathogens damage the host via invasive and toxic attributes. Invasion involves the ability of the bacterium to grow in the host, either in an extracellular environment or an intracellular environment. Invasive bacteria injure the host through the production of extracellular enzymes that damage the host tissue or through the modulation of the host response system, such as regulating cytokine expression. These bacteria sometimes produce secondary metabolites in the host.[4] There are studies related to the bacteria associated with pufferfish. TTX-bearing organisms and most of the reported strains belonged to the genera

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2024.13.58

Plesiomonas shigelloides,[5] *Acinetobacter* sp., *Altromonas* sp.,[6] *Flavobacterium*,[7] *Vibrio* sp.,[8] *Actinomycetes*., *Bacillus* sp., *Microbacterium arabinogalactanolyticum*, *Serratia marcescens*,[9] *Nacardiopsis dassonvillei*.,[10] *Roaseobacter sp.*,[11] *Marinomonas* sp.,[12] *Bacillus horikoshii*,[13] *Vibrio Haveyi*,[14] *Cellulomonas fimi*., *Kytococcus sedentarius*,[15] *Aeromonas* sp.,[16] *Lysinibacillus fusiformis*,[17] *Raoutella terrigena,*[18] *Shewanella putrefaciens*,[19] *Providentia rettgeri*,[20] *Cyanobacteria* sp.,[21] *Bacillus cereus*.[22] Research focused on characterising and identifying bacteria associated with pufferfish in India is notably scarce. This scarcity of studies on Indian pufferfish underscores the need for comprehensive investigations. To address this gap, the present study isolates and characterises bacteria associated with the liver of *Arothron stellatus* specimens collected from the Thoothukudi coast.

MATERIALS AND METHODS

Collection of specimen

Arothron stellatus[23] pufferfish specimens were procured from the Thoothukudi fishing harbour. After collection, the fish were carefully rinsed with seawater, then promptly transported to the laboratory on dry ice and subsequently preserved in a deep freezer at -20ºC for future use. Following this, the fish underwent a thorough washing and precise dissection using sterilised instruments (a knife and scissors) to extract the liver tissue. These tissue samples were handled under aseptic conditions to facilitate the isolation of microbes.

Isolation and Cultivation of Symbiotic Bacteria

Zobell Marine Agar 2216 was employed for bacterial isolation. Sterilised dishes were used to prepare agar plates, with each plate containing 15 g of agar dissolved in 100 mL of Zobell 2216 medium, adjusted to a pH of 7.2. Subsequently, one gram of liver tissue was homogenised in a sterile blender and then suspended in 9 mL of sterile seawater. Serial dilutions ranging from 10^{-1} to 10^{-5} were performed, ultimately resulting in a final concentration of 0.09 g of tissue per mL^{-1}. Bacterial isolation was accomplished by spreading 0.5 mL of the diluted samples onto the marine agar plates. The plates were then incubated at 28ºC for a period of 2 to 3 days to allow for bacterial growth. Over time, colonies of mixed bacterial species developed on each plate. These colonies were subsequently purified using the streak plate method on fresh Zobell 2216 agar plates until discrete, individual colonies were obtained. Before bacterial identification through cultural, morphological and molecular characterization, the pure culture underwent multiple subcultures to ensure purity. Finally, the purified strains were preserved at -80ºC in glycerol for future reference.

Primary characterization and Identification of Isolates

The freshly obtained pure isolates were employed for initial characterization. This involved an examination of the cultural and morphological attributes of bacterial colonies, encompassing features such as shape, size, colour, margin, elevation, opacity, Gram staining and motility.[24]

Molecular characterization of bacteria

The bacterial strains (L_1 and L_2) isolated from the liver of puffer fish *Arothron stellatus* were subjected to molecular characterization.

Genomic DNA isolation

DNA isolation from the microbial sample was done using the EX pure Microbial DNA isolation kit developed by Bogar Biostores Pvt. Ltd.

Amplification of16S rDNA of Bacterial Isolate

Fragments of the 16S rDNA genes of the bacterial isolate were amplifiedusingtheprimers27 F(5'AGAGTTTGATCTGGCTCAG') and 1492R (5'TACGGTACCTTGTTACGACTT 3'). For amplification of 16S rDNA genes of bacterial isolate, PCR reaction mixture contained 5 µL of the extracted DNA, 1.5 uL of each of the primers 27F and 1492R, 5 µL of deionized water and 12 µL of TagMasterMix (Taq DNA polymerase is supplied in 2XT aq buffer.0.4 mM$dNTP_s$.3.2 m M$MgCl_2$ and 0.02% bromophenol blue) The temperature program and the cycle of reactions was as initial denaturation step at 95ºC for 2 min, followed by 25 cycles of denaturation at 95ºC for 30 sec, primer annealing at 50ºC for 30 sec and primer extension at 72ºC for 2 min with a final extension at 72ºC for 10 min.[25]

Sequencing of 16S rDNA

Removed unincorporated PCR primers and $dNTP_s$ from PCR products using the Montage PCR Clean-up kit (Millipore). The PCR product was sequenced using the primers. Sequencing reactions were performed using an ABI PRISM ®BigDyeTMTerminat or Cycle Sequencing Kits with AmpliTaqr DNA polymerase (FSenzyme) (Applied Biosystems). Single-pass sequencing was performed on each template using below16S rRNA universal primers. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples

were resuspended in distilled water and subjected to electrophoresis in an ABI3730×1 sequencer (Applied Biosystems).

Phylogenetic Analysis

The 16S rDNA sequence was aligned using the NCBI blast similarity search tool. The phylogeny analysis of the query sequence with the closely related sequence of blast results was performed, followed by multiple sequence alignment. The program MUSCLE 3.7 was used for multiple alignments of sequence.[26] The resulting aligned sequences were cured using the program Gblocks0.91b. These Gblocks eliminate poorly aligned positions and divergent regions[27] (removes alignment noise). Finally, the program PhyML 3.0 Al RT was used for phylogeny analysis, and HKY85 was used as a Substitution model. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data while being one order of magnitude faster. The program Tree Dyn198.3 was used for tree rendering.[28]

RESULTS

Cultural and morphological characteristics of isolated bacteria

Two distinct isolates exhibiting unique characteristics were chosen from the Zobell Marine Agar 2216 plates. These selected isolates underwent streaking to attain a pure culture. Subsequently, two bacterial strains from the liver were designated and labelled as L_1 and L_2. The colony morphology of the isolates was thoroughly examined and documented in Table 1, which included observations on their shape, colour, margin and elevation. Notably, the form of the bacterial isolate colonies appeared circular and irregular. The isolates exhibited colours ranging from dull white to white. Margins of the bacterial isolates were identified as both undulate and entire. Regarding elevation, some isolates were raised, while others were flat. Gram-positive and Gram-negative characteristics were observed in the isolated strains (L_1 and L_2); these strains presented as both short and long rods, displaying motility (Table 1).

Molecular identification of the strains

A total of two bacterial isolates from the liver of puffer fish *Arothron stellatus* are subjected to molecular characterization. The DNA isolates from the two cultured bacterial isolates were subjected to 27F and 1492R primer and the amplification of the 16S rRNA gene for isolates is shown in Figure 1. The 16S rDNA sequence of strains was aligned using NCBI BLAST Similarity Search Tool and the newly isolated strains were identified as *Achromobacter xylosxidans* (L_1) (Figures 1, 2), *Bacillus cereus* (L_2) and as it shared the highest similarity 99% with this species (Figures 3, 4). Those bacterial strains deposited were done by the program phyML.3.0 aLRT and the program tree Dyn 198.3 was used for tree rendering.

The amplicons were requested in the applied Biosystems platform and identified as *Achromobacte rxylosxidans* (L_1), *Bacillus cereus* (L_2). The sequence was submitted in the DNA gene bank of NCBI with an accession number of L_1 -OP420554, L_2 -OP420555.

DISCUSSION AND CONCLUSION

In the current investigation, two distinct strains of bacteria, namely *Achromobacter xylosoxidans* (L_1) and Bacillus cereus (L_2), were isolated from the liver of the pufferfish *Arothron stellatus*. This finding builds upon the work of Myoung-Ja Lee *et al.* (2000),[29] who previously identified three Vibrio strains in the intestine of the pufferfish *Fugu vermicularis* radialus. This study revealed that these isolated strains could be further categorised into three distinct groups based on various characteristics, including colour, surface morphology, gram stain reaction, dimensions (width and length), spore formation, and motility. Further, our research extended to the isolation and identification of *Bacillus* spp. and *Achromobacter* spp. in the liver of the pufferfish *Arothron stellatus*. This was achieved through a combination of cultural and morphological techniques. In the study conducted by Tu Hoang Nguyen *et al.* (2015),[30] bacteria were isolated and identified through a combination of biochemical tests and 16S rRNA sequence analysis. The isolated bacterium was determined to be facultatively

Table 1: Cultural and Morphological characteristics of isolated bacteria.

Bacterial Strain	Size	Pigmentation	Form	Margin	Elevation	Gram staining and Motility
L1	Small	Dull white	Circular	Filiform	Flat	+ ive Motile
L2	Small	white	Circular	Entire	Raised	-ive Motile

CONTIG L_1

GTGCCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGC-GTAAAGCGTGCGCAGGCGGTTCGGAAAGAAAGATGTGAAATCCCAGAGCTTA-ACTTTGGGAACTGCATTTTTAACTACCGGGCTAGAGTGTGTCAGAGGGAGGTG-GAATTCCGCGTGTAGCAGTGAAATGCGTAGATATGCGGAGGAACACCGATGGC-GAAGGCAGCCTCCTGGGATAACACTGACGCTCATGCACGAAAGCGTGGGGAG-CAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGCT-GTTGGGGCCTTCGGGCCTTGGTAGCGCAGCTAACGCGTGAAGTTGACCGC-GTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCA-CAAGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTT-GACATGTCTGGAATGCCGAAGAGATTTGGCAGTGCTCGCAAGAGAACCGGAACA-CAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCC-GCAACGAGCGCAACCCTTGTCATTAGTTGCTACGAAAGGGCACTCTAATGAGA-CTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCT-TATGGGTAGGGCTTCACACGTCATACAATGGTCGGGACAGAGGGTCGCCAACCC-GCGAGGGGGAGCCAATCCCAGAAACCCGATCGTAGTCCGGATCGCAGTCTGCAACTC-GACTGCGTGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGTCGCGGTGAATAC-GTTCCCGGGTCTTGTACACACCG

RESULT: *Achromobacter xylosoxidans*

Figure 1: Partial Sequence of 16S rDNA of *Achromobacter xylosoxidans.*

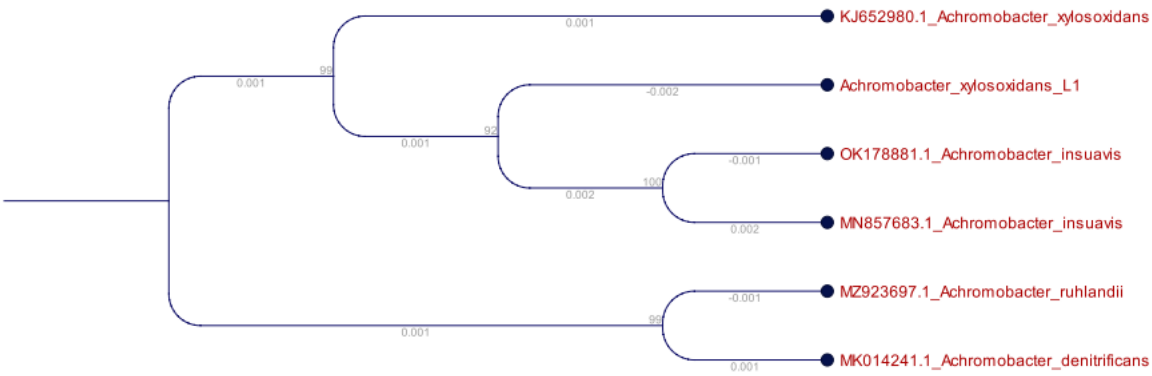

Figure 2: Phylogenetic Tree showing the phylogenetic position of L_1 based on 16S rDNA sequence analysis.

anaerobic, exhibiting the ability to utilize and produce acid through the fermentation of d-glucose. A partial 16S rRNA sequence, consisting of 540 base pairs, was deposited in the DDBJ database under Accession Number AB828395. Upon analysis using the NCBI BLAST tool, it demonstrated 99% homology to *Enterococcus faecium* AUS004 and 97% homology to *Enterococcus faecium* DSM 20477. This discovery marked the first instance of TTX-producing *E. faecium* being isolated from puffer fish in Vietnam. In our current investigation, bacterial strains L_1 and L_2, associated with the liver of pufferfish, were subjected to PCR analysis using the 16S rRNA spacer gene. They were identified as *Achromobacter xylosoxidans* (L_1) and *Bacillus cereus* (L_2). The obtained sequences were compared against entries in the database using BLAST. The resulting sequences were deposited in the GenBank database with accession numbers OP420554 and OP420555, respectively, showing high similarity, with 99% and 100% similarity to the 16S rRNA gene sequences. Lu and Yi (2009)[13]

CONTIG L_2

TAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG-GCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCT-GACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTT-GTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAAC-CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC-GTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGAT-GTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTG-CAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAG-GAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGC-GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGC-TAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAGCACTCC-GCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCA-CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT-GACATCCTTTGACAACCCTAGAGATAGGGCTTTTCCTTTCGGGAGCAGAGTGACAG-GTGGTGCATGGTTGTTGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAAC-GAGCGCCACCCTTGATTTTAGTTGCCATCATTTAGTTGGGCCCTTTAAGGTGACT-GCCGGTGACCAACCGGAGGAAGGTGGGGAAGACGTCAAATCATCATGCCCCTTAT-GACCTGGGCTACCCCCGTGGTACAATGGACGGTACAAAGAGCTGCAAGGCCCCGAG-GTGGAACTAATTTCATAAAACCGTTTTCAGTTCGGATTGTAGGCTGCAACTTGCCTA-CAAGAAGCTGGAATCGCTAGTAATCGCGGATCAGCCTGCCGCGGTG

RESULT: *Bacillus cereus*

Figure 3: Partial Sequence of 16S rDNA of *Bacillus cereus.*

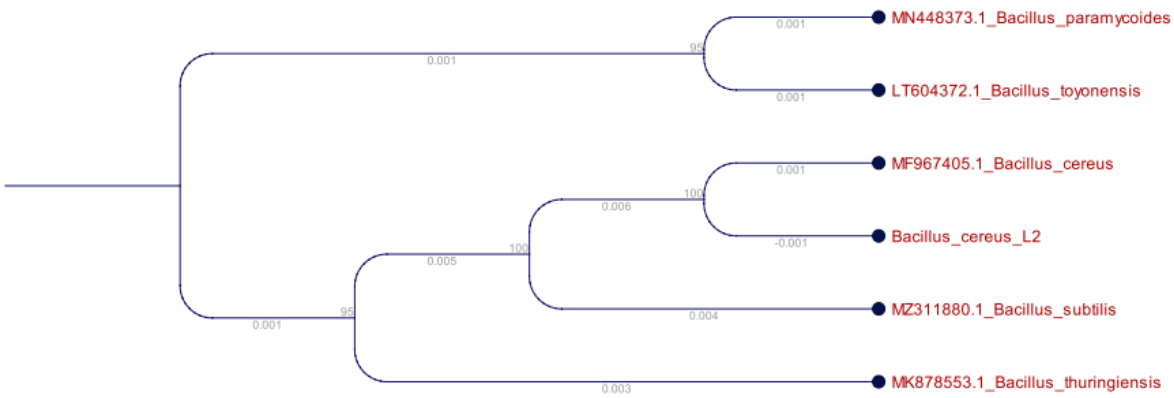

Figure 4: Phylogenetic Tree showing the phylogenetic position of L_2 based on 16S rDNA sequence analysis.

successfully isolated a strain of *Bacillus horikoshii* from the liver of a pufferfish. Additionally, Wang and Fan (2010)[17] provided insights into the characteristics of a Bacillus strain isolated from the pufferfish *Fugu obscurus*. Building upon these earlier discoveries, our present study further supports the notion that Bacillus species could potentially serve as parasitic or symbiotic bacteria within puffer fish, potentially contributing to the production of TTX during their growth phases. This collective body of research emphasises the significance of understanding the intricate relationships between these bacteria and their puffer fish hosts, shedding light on the mechanisms behind TTX production.

SUMMARY

This study primarily centered on the isolation and identification of bacteria associated with the liver of puffer fish *Arothron stellatus*. The colony morphology of the isolates was thoroughly examined and the 16S rDNA sequence of strains was aligned using NCBI BLAST Similarity Search Tool and the newly isolated strains were identified as *Achromobacter xylosxidans* (L_1), *Bacillus cereus* (L_2) and as it shared the 99% of highest similarity and the sequence was submitted in the DNA gene bank of NCBI with an accession number of L_1 -OP420554, L_2-OP420555. Finally, two distinct bacterial strains were successfully isolated and identified as *Achromobacter xylosoxidans* (L_1) and *Bacillus cereus* (L_2). This finding strongly implies a potential link between these bacteria and the toxin production found in puffer fish. However, it is essential to note that further in-depth research is imperative to understand their involvement in TTX synthesis and toxin production comprehensively. A more detailed investigation is warranted to unravel the intricate mechanisms underlying this phenomenon.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Manivasagn P, Venkatesan J, Sivakumar K, Kim S K. Pharmaceutically active Secondary metabolites of marine action bacteria. Microbiol Res 2014;169:262-78. http//doi.org/10.1016/jmicers.2013.07.014.
2. Arakawa Q, Hwang DF, Taniyama, S and Takatani T. Toxins of Puffer fish that cause human intoxications. Coastal Environmental and Ecosystem issues of the East China sea, 2010:227-44.
3. Nithyand p, Manju S, Pandian S K. Phylogenetic characterization culturable Actinomycetes associated with the mucus of the coral *Acropora digitifera* from Gulf of Mannar. FEMS Microbiol lell. 2011;31F:112-8.,
4. Petti R K. Culturability and secondary metabolite diversity of extreme microbes: expanding contribution of deep sea and deep-sea vent microbes to natural product discovery. MarBiotechnol. 2011;13:1-11.
5. Smith M M and Heemstra PC. Tetraodontidae. In: MM Smith and PC Hemmstra, Editors, Smith's sea fishe, Springer-Verlag, Berlin. 1987:894-903.
6. Do HK, Kongure K, Simidu U. Identification of deep-sea sediment bacteria which produce tetrodotoxin. Appl. Environ Microbiol. 1990;56:1162-3.
7. Do HK, Hamasaki K, Dhwada K, Simidu U, Noguchi T, Shida Y, *et al*.: Presence of tetrodotoxin and tetrodotoxin producing bacteria which in freshwater sediments. Appl, Environ, Microbial. 1993;59:3934-7.
8. Lee M J, Jeong D Y, Kim W S, Kim H D, Kim C H, Park W W, *et al*. A tetrodotoxin producing *Vibrio* strain LM-1, from the puffer fish *Fugu Veremicularis radiates*. Appl Environ, Microbiol. 2000;66:1698-701.
9. Yu C F, Yu P H, Chan P L, Yan Q, Wang P K.Two novel species of tetroidotoxin producing bacteria isolated from toxic marine puffer fishes. Toxicon. 2004;44:641-7.
10. Wu Z, Xie L, Xia G, Zhang J, Nie Y, Hu J, *et al*, Toxicity and distribution of tetrodotoxin producing bacteria in puffer fish *Fugu rubripes* collected from the Bohai sea of china. Toxicon. 2000;46:471-6.
11. Maran B A, Iwamoto E, Okuda J, Matsuda S Taniyama S, Shida Y, *et al*. Isolation and characterization of bacteria from the copepod *Pseudocaligus fugu* ectoparasitic on the panther puffer *Takifugu paradalis* with the emphasis on TTX. 2007. Toxicon 50:779-790.
12. Wang XJ, Yu RC, Luo X, Zhou MJ, Lin XT. Toxin screening and identification of bacteria isolated from highly toxic marine gastropod *Nassarius semiplicatus*, Toxicon. 2008;52:55-61.
13. Lu Y, Yi R. *Bacillus horikoshii*, a tetrodotoxin producing bacterium isolated from the liver of puffer fish. 2009. Ann, Microbioal. 2009;59:453-8.
14. Campbell S, Harda R M, Defelice S V, Bienfang P K, Li Q X. Bacterial production of tetrodotoxin in the pufferfish *Arothron hispidus*. Nat, Prod, Res. 2009;23:1630-40.
15. Bragadeeswaran S, Therasa D, Prabhu K, Kathiresan K. Biomedical and pharmacological potential of tetrodotoxin producing bacteria isolated from marine pufferfish *Arothron hispidus,* Muller, 1841. 2010. J, Venom Anim, Toxins Trop, Dis. 16:421-76.
16. Yang G, Xu J, Liang S, Ren D, Yan X, Bao B. A novel TTX producing *Aeromonas* isolated from the ovary of *Takifugu obscures*. Texicon, 2010;56:324-9.
17. Wang J, Fan Y, Yao Z. Isolation of a *Lysinibacillus fusiformis* strain with tetrodotoxin producing ability from pufferfish *Fugu obscures* and the characterization of this strain. Toxicon. 2010;56:640-643.
18. Yu V C, Yu P H, Ho K C, Lee F WIsolation and identification of a new tetrodotoxin-producing bacterial species, *Raoultella terrigena* from Hong Kong marine puffer fish *Takifugu niphobles*. Drug. 2011;9:2384-96.
19. Auawithoothij W, Noomhorm A. *Shewanella putrefaciens* a major microbial species related to tetrodotoxin TTX accumulation of pufferfish *Lagocephalus lunaris*. J, Appl, Microbiol, 2012;113:459-65.
20. Tu N, Tu Q, Tung H, Hieu D, Remero jovel S. Detection of tetrodotoxin producing *Providencia rettgeri* T892 in *Lagocephalus* pufferfish World. Microbiol, Biotechnol. 2014;30:1829-35.
21. Zhenchi Li Jinglin Tian, Yukun Lai, Chiu HongLee Zongweicai and Chun Fai Yu Pufferfish Gut Microbita Studies Revealved Unique Bacterial Co-Occurrence Patterns and New Insights on Tetrodotoxin Producers. Drug.18, 2022;5:278, doi:10-339/md18D50278.
22. Yang L, Zhang L, Yin P, Ding H, Xiao Y, Zeng JIsolation and toxigebic characteristics of a tetrodotoxin producing bacteria. 2022. Turkish journal of fiesheris and Aquatic Science. 22: 1, TRJFAS19531.
23. Laecepede B G E. Histoire naturelle des poisons. Paris PLASSAN IMPRIMEURLIBRIRE, 1958;4:728.
24. Breed R S, E G D Murray and N R Smith. Bergey's Mannual of Determinative Bacteriology, seventh edition, The Willams and Wikins company, Baltimore, (i)-xviii 1-1094, (Internatinal code of Bacteriological Nomenculture) P X excl, Schizophycea 9.30.
25. Relman D A Universal bacterial 16S rDNA amplification and sequencing In Persing, D H, Simth T H, Tenover F C, White T J(Eds). Diagnostic Molecular Microbiology Principles and Application, InASM, Washington DC, pp: 489-495.
26. Edgar R C, MUSCLE. Multiple sequence aligment with high accuracy and high throughput. Nucleic Acids Res. 2004;32(5):1792-97.
27. Talvera G and Castresana J. Improvement of high phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignment. Systematic Biology. 2007;56:564-77.
28. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevent F, *et al*. Phylogeny ferrous Phylogenetic analysis for the non-Specialist, Nucleic Acids.2008. Res1:36.
29. Myoung J A, Lee Dong Jeong, Wooseong Kim, HYUN Dae Kim, Cheorl HO Kim, Won Whan Park, *et al*. A tetrodotoxin-Producing vibrio strain, LM-1, from the Pufferfish *Fugu vermicularis* radiates. 2000. Applied and environmental Microbiology. Apr P-1698-1701.
30. Tu Hoang Nguyen, Huu Ngoc NGUYEN, Dat Van Nghe and Kim Hoang Nguyen Biological Activities of Tetrodotoxin-Producing *Enterococcus faecium* ADI Isolated from Puffer Fishes.2015. Biomed research international. Vol Article ID-973235: 8 pages.

Cite this article: Kanthimathi P, Joslin PJ. Exploration and Profiling of Bacteria Symbionts in Arothron stellatus from the Thoothukudi Coast. Asian J Biol Life Sci. 2024;13(2):466-71.