International Journal of Agricultural Technology 2023 Vol. 19(5):2093-2100
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Isolation and screening of fish gut actinomycetes for antibacterial activity against Uropathogenic *Escherichia coli*

Jisha, K.[1], Gayathri, G.[1], Gopikrishnan, V.[2], Vareeket, R.[3] and Prabha, T. R.[4*]

[1]Department of Microbiology, Vels Institute of Science, Technology and Advanced Studies, Chennai- 600117, Tamil Nādu, India; [2]Centre for Drug Discovery and Development, Sathyabama Institute of Science and Technology, Chennai- 600119, Tamil Nādu, India; [3]Research Institute of Modern Organic Agriculture (RIMOA), Faculty of Agricultural Technology, King Mongkut's Institute of Technology, Ladkrabang (KMITL), Bangkok, Thailand; [4]Department of Biotechnology, Indian Institute of Technology Madras, Chennai-600036, Tamil Nādu, India.

Jisha, K., Gayathri, G., Gopikrishnan, V., Vareeket, R. and Prabha, T. R. (2023). Isolation and screening of fish gut actinomycetes for antibacterial activity against Uropathogenic *Escherichia coli*. International Journal of Agricultural Technology 19(5):2093-2100.

Abstract Actinobacteria are among the most studied prokaryotes due to their propensity to create microbial bioactive compounds. Actinobacterial members can be found in a wide variety of aquatic and terrestrial settings, incorporating marine habitats. Actinomycetes isolated from the marine environment are gaining popularity due to their diverse structures and unique biological properties of the secondary metabolic products. The research was investigated the antibacterial properties of actinobacteria associated with gut of marine fish against human pathogen. Approximately 20 morphologically distinct strains obtained from *Engraulis sp.* (anchovy fish) and *Trachurus trachurus* (horse mackerel) were proved for antibacterial efficacy against Extended spectrum beta-lactamase (ESBL) producing uropathogenic *Escherichia coli* (UPEC). The actinobacterial strain HM7 expressed antibacterial effect against UPEC.

Keywords: Actinobacteria, Fish gut, Antibacterial activity, Extended Spectrum Beta-Lactamase, Secondary metabolite

Introduction

In the mid-twentieth century, the development of antibiotics for the management of infectious maladies transformed the world of medicine. After using antibiotics for over seven decades, a recurring rend of resistance to antibiotics developed across some bacterial pathogens has been observed. Resistance manifests itself first among the most severely ill hospitalised patients, subsequently extends to other patients in the hospital, then ultimately affects the population. Such incidents are first geographically limited; however, when resistant microbial strains move from region to region, they eventually establish global endemicity. This trend has been seen in methicillin-resistant *Staphylococcus aureus* (MRSA), Extended-spectrum

* **Corresponding Author**: Prabha, T. R.; **Email:** drtrprabha@gmail.com

β-lactamase (ESBL)-producing Enterobacteriaceae, and penicillin-resistant *Staphylococci*. (ESBL)-producing *E. coli* are the most frequent cause of urinary tract infections (UTIs) that impart resistance to beta-lactam antibiotics like third- and fourth-generation cephalosporins and monobactams. Due to the failure of empirical therapy, which may cause major clinical consequences such sepsis, renal scarring, and extended hospitalization when compared to infection with non-ESBL strains, the increasing isolation of ESBL-producing E. coli is of concern on a global scale. Each time, the timely implementation of effective preventive measures to slow the spread of resistant infections was neglected (Schwaber and Carmeli, 2008; Vachvanichsanong *et al*., 2020). Therefore, there is an urgent need for the development and discovery of novel antibiotics to efficiently tackle the dangerous microorganisms that trigger disease (Sharma *et al*., 2016). Despite significant advancements in the disciplines of synthetic chemistry and engineered biosynthesis of antibacterial chemicals, nature continues to be the most abundant and flexible resource for novel antibiotics (Koehn and Carter, 2005; Baltz, 2006; Pelaez, 2006).

Because high-quality biological components from novel resources are investigated in persisting screens or when fresh evaluating technologies are deployed, novel structurally distinct natural products are uncovered, it is crucial to encourage these two characteristics of innovation in drug development programmes. Actinobacteria remains to be a prominent producer of unique natural compounds among prokaryotes (Goodfellow and Fiedler, 2010). Actinobacteria are Gram-positive bacteria that have the greatest percentage of guanine plus cytosine (G+C) in its DNA. These bacteria are essential to the biogeochemical recirculation of recalcitrant and organic material in the environment (Balagurunathan *et al*., 2010). Actinobacterial members, particularly those in the genus Streptomyces, produce around two-thirds of the antimicrobial drugs currently available on the market today (Barka *et al*., 2016).

However, while screening old strains that is in use results in the expensive rediscovery of known chemicals, it is getting harder to detect new metabolites from common actinomycetes. The bioprospecting approach is works on the idea that novel secondary metabolites can be discovered by analyzing a limited number of dereplicated, unusual actinomycetes that have been collected from marine habitats (Goodfellow and Fiedler, 2010). Due to the capacity to synthesize a wide range of natural substances and their specialized systems for adjusting to harsh settings, microorganisms found in marine habitats have garnered a lot of interest (Solingen *et al.,* 2001). It has also been observed that actinobacteria are present in marine organisms like fish (Karthiga Rani *et al*., 2016). However, there are very few data on the actinobacteria associated with fish guts that have antimicrobial properties (Sanchez *et al*., 2012).

The goal of the current work was to isolate actinobacteria from the fish guts of two marine fish species, *Engraulis* sp. (anchovy fish) and *Trachurus trachurus* (horse mackerel), in order to better understand their antibacterial potential.

Materials and Methods

Fish procurement and pretreatment

Two species of marine fishes, *Engraulis* spp. (Anchovy fish) and *Trachurus trachurus* (Horse mackerel) were caught off the coast of Chennai, Tamil Nadu, India, in Kovalam (Lat: 12.78700N, Long: 80.25040E). To prevent external microbial contamination, the samples were brought to the lab as quickly as feasible. After being transported to the lab, the surface sterilization was done by immersing in a 70% ethanol solution for 30 seconds, and the fish gut was extracted (Figure 1). 0.85% saline is used to homogenise the fish gut material. In a 250 ml flask, 1g of homogenates pooled intestinal segment was combined with 100 ml of 0.85% saline, and the flask was then placed in a shaker incubator at 55 °C for 30 minutes. By eliminating the majority of undesirable spore-forming and Gram-negative bacteria, this environment facilitated the isolation of actinobacteria (Vijayabaskar and Somasundaram, 2008).

Figure1. Gut portion of a. *Engraulis* spp., b. *Trachurus trachurus*

Isolation of actinobacteria

By using the spread plate technique, actinobacteria were isolated using Kuster's Agar and Starch Casein Agar that was diluted with 50% sea water and added with nystatin (100 µg/ ml) and nalidixic acid (20 µg/ ml) to prevent the development of both fungi and Gram-negative bacteria (Radhakrishnan *et al*., 2006). The solution was serially diluted up to 10^5 dilutions using sterile distilled water blank. 100 µl of aliquots from dilutions 10^3, 10^4, and 10^5 were transferred over SCA plates and spread using sterile L-rod. Actinobacterial colonies with distinct morphologies were chosen, purified, and subcultured using ISP2 (yeast extract malt extract) agar medium and 30% glycerol stocks were maintained at -20 °C.

Actinobacterial cultural characterization

All of the chosen actinobacterial colonies were inoculated onto ISP2 agar medium to perform the cultural characterization. At 28 °C, each plate was incubated for 10 days. Growth, consistency, aerial mass colour, reverse side pigment, and the formation of soluble pigment showed some of the cultural traits that were noted (Radhakrishnan *et al*., 2014).

Antibacterial activity testing of actinobacteria

The ESBL-positive Uropathogenic *Escherichia coli* was used to assess the antibacterial activity of actinobacterial cultures by applying the agar plug method. For the purpose of producing secondary metabolites, actinobacterial cultures were cultivated on ISP2 agar plates for 10 days at 28 °C. Extracellular secondary metabolites are released into the culture media during incubation. Using a well cutter, agar plugs of 5 mm diameter were cut from the ISP2 agar plates after the period of incubation and laid over the Mueller Hinton agar plate swabbed with test pathogens. For 24 hours, plates were incubated at 37 ± 2.0°C. To evaluate and keep track of antibacterial activity, the zone of inhibition's diameter was assessed. The mean value of this trial was computed after being conducted in triplicates.

Results

Isolation and characterization of actinobacteria

Totally 20 actinobacterial strains with various morphologies (Figure 2) were selected from two different marine fishes (*Engraulis sp.*-11, *Trachurus trachurus*- 9) maintained at 4°C on ISP2 agar slants. All of the actinobacterial cultures grew well during recovery and storage. Cultural characteristics of the actinobacteria are represented in Table 1.

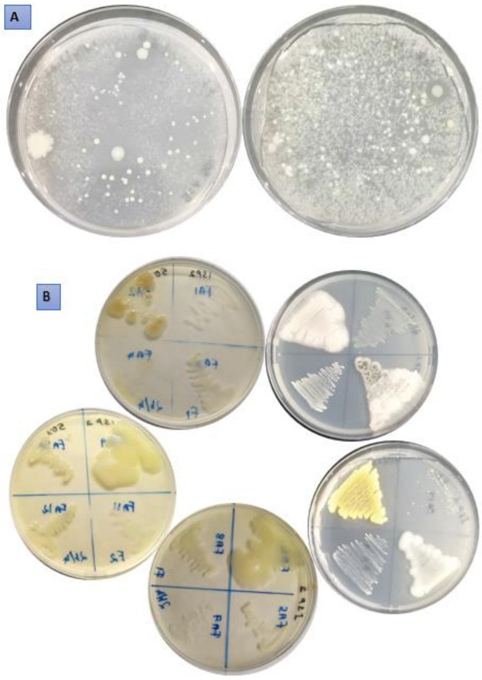

Figure 2. Isolation of actinobacteria on A-SCA plate, B- ISP2 agar plate

Table 1. Cultural characteristics of actinobacterial strains

Sl no.	Strain	Cultural characteristics			
		Consistency	AMC	RSP	SP
1	EA1	Powdery	White	-	-
2	EA2	Opaque	Pale yellow	-	-
3	EA3	Rough	White	-	-
4	EA4	Powdery	White	-	-
5	EA5	Rough	White	-	-
6	EA6	Leathery	Creamy White	-	-
7	EA7	Powdery	White	-	-
8	EA8	Powdery	White	-	-
9	EA9	Leathery	Yellow	Yellow	-
10	EA10	Powdery	White	-	-
11	EA11	Leathery	White	-	-
12	HM1	Leathery	White	-	-
13	HM2	Powdery	White	-	-
14	HM3	Powdery	White	-	-
15	HM4	Powdery	White	-	-
16	HM5	Leathery	Creamy white	-	-
17	HM6	Powdery	White	-	-
18	HM7	Leathery	White	-	-
19	HM8	Powdery	White	-	-
20	HM9	Powdery	White	-	-

AMC- Aerial mass colour, RSP- Reverse side pigment, SP- soluble pigment

Antibacterial activity testing

During the primary screening, only one actinobacterial strain (HM7) retrieved from *Trachurus trachurus* had significant antibacterial activity (Zone of inhibition- 10mm) against ESBL-positive Uropathogenic *E. coli*.

Discussion

The need to battle the rise in infections caused by microorganisms resistant to antibiotics, together with the hunt for new anticancer and antiviral chemicals, is what spurs the pharmaceutical industry's search for innovative therapeutic agents (Basik *et al*., 2003; Gontang *et al*., 2007). Over fifty percent of the medications currently utilizing across various therapeutic divisions were created using therapeutic ingredients mostly derived from natural materials. Taxonomically diverse bacterial groups found in marine environments have distinctive physiological and morphological traits allowing them to endure conditions of high pressure, high salinity, and high temperature, as well as the potential to produce various secondary metabolites not found in terrestrial microbes (Eccleston *et al.* 2008). because of the structural variety and distinctive biological activity of the secondary metabolites, actinomycetes isolated from the marine ecosystems are currently of great interest (Manivasagan *et al*., 2014). In our study, we explored the antibacterial activity of marine actinomycetes associated with gut of two fishes namely *Engraulis* sp. (Anchovy fish) and *Trachurus trachurus* (Horse mackerel). One of the fish gut isolates showed significant antibacterial activity. Actinomycetes isolated from marine fishes have been shown to be antibacterial action, according to reports. Vignesh *et al*. 2019 reported the antibacterial activity of actinobacterial strains from the marine fish gut against *S. aureus*, *E. coli, and S. enterica*. The *Streptomyces* sp. and *Micromonospora* sp. were procured by Jami *et al*. (2015) from the guts of *Schizothorax zarudnyi* and *Schizocypris altidorsalis* and demonstrated antibacterial efficacy against various fish diseases. The secondary metabolites from *Streptomyces* sp. isolated from marine fish, *Carcharhinus amblyrhynchos* showed antibacterial activity against *Mycobacterium tuberculosis and ESBL Klebsiella pneumonia ATCC 13882* (Vaishali *et al*., 2021).

Fighting the threat is posed by multidrug-resistant bacteria necessitates the search for new bioactive agents. Actinobacteria associated with marine fish guts is concerned the main focus of this investigation, and it is determined that they may be capable of producing compounds with antibacterial activity. In our research, strain HM7 showed a minimal antibacterial activity. The fish gut is a valuable and promising natural resource for the isolation of bioactive actinobacteria.

Acknowledgments

The authors would like to acknowledge Centre for Drug Discovery and Development (CDDD), Sathyabama Institute of Science and Technology, Chennai- 600119, Tamil Nādu, India for their generous support in conducting the laboratory work.

References

Balagurunathan, R., Radhakrishnan, M. and Somasundaram, S. T. (2010). L-Glutaminase producing actinomycetes from marine sediments–selective isolation, semi quantitative assay and characterization of potential strain. Australian Journal of Basic and Applied Sciences, 4:698-705.

Baltz, R. H. (2006). Marcel Faber Roundtable: is our antibiotic pipeline unproductive because of starvation, constipation or lack of inspiration?. Journal of Industrial Microbiology and Biotechnology, 33:507-513.

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H. P. and van Wezel, G. P. (2016). Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and molecular biology reviews, 80:1-43.

Basik, M., Mousses, S. and Trent, J. (2003). Integration of genomic technologies for accelerated cancer drug development. Biotechniques, 35:580-593.

Eccleston, G. P., Brooks, P. R. and Kurtböke, D. I. (2008). The occurrence of bioactive micromonosporae in aquatic habitats of the Sunshine Coast in Australia. Marine drugs, 6:243-261.

Gontang, E. A., Fenical, W. and Jensen, P. R. (2007). Phylogenetic diversity of gram-positive bacteria cultured from marine sediments. Applied and environmental microbiology, 73:3272-3282.

Goodfellow, M. and Fiedler, H. P. (2010). A guide to successful bioprospecting: informed by actinobacterial systematics. Antonie Van Leeuwenhoek, 98:119-142.

Jami, M., Ghanbari, M., Kneifel, W. and Domig, K. J. (2015). Phylogenetic diversity and biological activity of culturable Actinobacteria isolated from freshwater fish gut microbiota. Microbiological research, 175:6-15.

Karthiga Rani, M., Chelladurai, G. and Jayanthi, G. (2016). Isolation and identification of bacteria from marine market fish Scomberomorus guttatus (Bloch and Schneider, 1801) from Madurai district, Tamil Nadu, India. Journal of Parasitic Diseases, 40:1062-1065.

Koehn, F. E. and Carter, G. T. (2005). The evolving role of natural products in drug discovery. Nature reviews Drug discovery, 4:206-220.

Manivasagan, P., Venkatesan, J., Sivakumar, K. and Kim, S. K. (2014). Pharmaceutically active secondary metabolites of marine actinobacteria. Microbiological research, 169:262-278.

Pelaez, F. (2006). The historical delivery of antibiotics from microbial natural products—can history repeat?. Biochemical pharmacology, 71:981-990.

Radhakrishnan, M., Deeparani, K. and Balagurunathan, R. (2006). Production of bioactive compounds by solid state fermentation from actinomycetes of Andaman sediments. Indian Journal of Applied Microbiology, 6:92-98.

Radhakrishnan, M., Pazhanimurugan, R., Gopikrishnan, V., Balagurunathan, R. and Kumar, V. (2014). Streptomyces sp D25 isolated from Thar Desert soil, Rajasthan producing pigmented antituberculosis compound only in solid culture. Journal of Pure and Applied Microbiology, 8:333-337.

Sanchez, L. M., Wong, W. R., Riener, R. M., Schulze, C. J. and Linington, R. G. (2012). Examining the fish microbiome: vertebrate-derived bacteria as an environmental niche for the discovery of unique marine natural products. PloS one, 7:e35398.

Schwaber, M. J. and Carmeli, Y. (2008). Carbapenem-resistant Enterobacteriaceae: a potential threat. Jama, 300:2911-2913.
Sharma, P., Kalita, M. C. and Thakur, D. (2016). Broad spectrum antimicrobial activity of forest-derived soil actinomycete, Nocardia sp. PB-52. Frontiers in microbiology, 7:347.
Solingen, P., Meijer, D., Kleij, W. A., Barnett, C., Bolle, R., Power, S. D. and Jones, B. E. (2001). Cloning and expression of an endocellulase gene from a novel streptomycete isolated from an East African soda lake. Extremophiles, 5:333-341.
Vachvanichsanong, P., McNeil, E. B. and Dissaneewate, P. (2020). Extended-spectrum beta-lactamase *Escherichia coli* and *Klebsiella pneumoniae* urinary tract infections. Epidemiology and infection, 149:e12. https://doi.org/10.1017/S0950268820003015
Vaishali, R., Ruchi, P., Subhashini, D., Vignesh, A., Manigundan, K., Gopikrishnan, V., Revathy, K., Anbarasu, S., Radhakrishnan, M. and Joseph, J. (2021). Antibacterial, antitubercular and anticancer activity of gut-associated Streptomyces enissoceasilis SFA isolated from marine fish Carcharhinus amblyrhynchos. Research Journal of Biotechnology, 16(1).
Vignesh, A., Ayswarya, S., Gopikrishnan, V. and Radhakrishnan, M. (2019). Bioactive potential of actinobacteria isolated from the gut of marine fishes. Indian Journal of Geo Marine Sciences, 48:1280-1285.
Vijayabaskar, P. and Somasundaram, S. T. (2008). Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen *Aeromonas hydrophila*. Biotechnology, 7:124-128.

(Received: 14 June 2023, Revised: 15 August 2023, Accepted: 27 September 2023)