

Original Research Article

Screening of Marine Actinomycetes as Probiotics for Production of Bacteriocin

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ABSTRACT

Keywords

Actinomycetes,
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Antibacterial
activity,
Pathogens
and probiotic

Total 59 actinomycetes colonies were isolated from six different coastal areas of India. These 59 isolates were morphologically distinct on the basis of colony morphology and colour of pigments. Probiotic properties viz., resistance to low pH and tolerance against bile salt 0.3% were tested. Seventeen isolates had grown at low pH 3 and 0.3% bile salt. Out of 17 isolates, 12 isolates had shown antibacterial activity against identified human pathogens such as *K. pneumoniae ssp pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus casseliflavus*, *Staphylococcus sciuri* and MTCC pathogens viz., *Escherichia coli* (1687) *Pseudomonas aeruginosa* (1688) *Salmonella typhi* (531) *Bacillus subtilis* (8960) *Proteus vulgaris* (742) *Klebsiella pneumoniae* (535) *Staphylococcus aureus* (96). 5 Actinomycetes isolates showing broad spectrum antibacterial activity.

Introduction

Marine environment are opening an unexpected new horizon for finding novel organisms for valuable products. Oceans found nearly 70% of earth surface and microorganisms has grown in marine environments are different from terrestrial organisms (Takizawa *et al.*, 1993). So, Marine derived antibiotics are more efficient at fighting microbial infections than terrestrial bacteria have not developed any resistance them (Donia and Humann, 2003).

Actinomycetes are the dominant group of soil population together with bacteria and fungi. They have shown Gram-positive bacteria having high G+C (>55%) content in

their DNA and they are originally considered as an intermediate group between bacteria and fungi. They are free living, saprophytic bacteria, and a major source for production of antibiotics. They play a major role in recycling of organic matter (Lacey *et al.*, 1978), production of novel pharmaceuticals, nutritional materials, cosmetics, enzymes, antitumour agents, enzyme inhibitors, immune-modifiers and vitamins. Streptomyces are especially prolific and can produce a great many antibiotics (around 80% of the total antibiotic production) and active secondary metabolites (Williams, 1978). A little is known about the actinomycetes diversity of

marine sediments, which is an inexhaustible resource that has not been properly exploited. For the past half century, majority of antibiotics and antitumor drugs have come from terrestrial actinomycetes; however, discoveries of new pharmaceuticals from terrestrial actinomycetes have trailed off dramatically in recent years. There with, actinomycetes from marine environments have aroused the keen interest of researchers (Maldonado *et al.*, 2005; Ward and Bora, 2006; Kozue *et al.*, 2008). The marine environment has several peculiar characteristics that are not seen in terrestrial areas. Microorganisms living in the marine environment are obviously expected to be different from terrestrial ones.

It is reported that marine actinomycetes not only have several new species, but also have plenty novel structures with potent bioactivities (Lin and Liu, 2010). According to incomplete statistics, the number of novel compounds isolated from marine actinomycetes in the 21th century is more than twice of the last century. Research results showed that marine actinomycetes would become another important microorganism resource for pharmaceutical industries, like terrestrial actinomycetes. From certain marine *Actinomycete* isolates, many bioactive molecules with useful properties such as antimicrobial agents (Mohan RR. *et al.* 2005) and antitumor compounds (Maskey *et al.*, 2004) have been discovered. *Streptomyces scopuliridies* and *Streptomyces pluripotents* are novel a bacteriocin producing *Streptomyces* (Faris *et al.*, 2011). These shown to produce a broad spectrum bacteriocin. The strains of *Actinobacteria* belonging to the genus *Streptomyces* might be promising probiotics in aquaculture because they produce compounds with potential bioactivity against pathogens of fish and shellfish.

Materials and Methods

Collection of marine samples: Marine water and sediment samples were collected from six different coastal areas of India, name as Calangute beach Goa, Dadar chaupathy Mumbai, Gopalpur Orissa, Thirumullavaram beach Kerala, Elliot's beach Chennai, Angellar beach. Samples were collected from 5 to 15 cm depth and stored in dark during transport to laboratory, stored for further study.

Isolation of *Actinomycetes*: Starch casein agar was used for isolation and enumeration of *Actinomycetes*. The medium supplemented with 25 ug/ml streptomycin to inhibit bacterial contamination. In conventional dilution technique, 1ml of marine water sample were suspended in 10 ml sterile saline and 0.1 ml of suspension from this spread over starch casein agar contain 3% salt and incubated for 7–9 days at 30°C. 1gm of marine sediments were suspended in 10 ml of sterile saline and homogenized by vortex mixer. Dilution technique 10^{-4} – 10^{-6} had done and 0.1 ml of suspension from this spread over starch casein agar contains 3% salt and incubated for 7-9 days at 30°C. After incubation the obtained colonies were purified and sub-cultured on SCN agar plates and stored for further studies.

Screening for probiotic properties

Resistance to low pH and low bile salts:

Tolerance to acidic pH was examined by growing the strains in SCN at a pH of 1, 2, 3. The isolates were seeded on agar and broth medium, incubated at 30°C for 7 days, and the presence or absence of growth was recorded on the 7th day and onward. Prepared SCN with different conc. of bile salt viz., 0.1%, 0.2%, 0.3% and observed the

growth of isolates on that bile salt (Milagro *et al.*, 2015).

Screening for bacteriocin assay:

(A) Cross-streak method: Antibacterial activities of isolates were streaked across diameter on Muller Hinton agar plates. After incubation at 30°C for 6 days, 24 hour of culture of pathogens streaked perpendicular to the central strip of actinomycetes culture. All plates were again incubated at 37°C for 24 hrs and zone of diameter was measured (Mohan Remya and Ramasamy Vijayakumar, 2008)

(B) Agar well diffusion method: Amongst 59 isolated marine bacteria further screening has been carried out for their probiotic nature on the basis of antibacterial activity towards distinct human pathogens and showing multiple antibiotic resistance by disk diffusion method.

A lawn of indicator strains including *Escherichia coli*, *Klebsiella pneumoniae sp.*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus sp.*, and MTCC pathogens was made over the surface of Muller Hinton Agar. The plates were allowed to dry and wells were prepared on surface of agar plates.

Cell free extract of isolates was prepared by growing them overnight in SCN broth, cells were separated by centrifugation at 10,000xg for 15 minute and supernatant was collected and neutralized up to pH 7 using 0.1 N NaOH and then used for bacteriocin assay. The crude extract at 100ul quantity was incorporated in respective well and plates were incubated at 37°C for 24 hours. The results were recorded by observing and measuring zone of inhibition (Kanagaraj *et al.*, 2012). Amongst isolates those were selected showing broad antibacterial activity.

Results and Discussion

Isolation of actinomycetes: Total 59 actinomycetes were isolated from different marine water and sediment samples as shown in table 1.

Morphology of Actinomycetes on SCN: In our study, 59 isolates were isolated from different coastal area of India. Thirty one strains characteristics of actinomycetes from the rest of same sample site based on their morphology, colour of pigments and arrangement of mycelia (Milagro *et al.*, 2015), for a long time, it was believed that actinomycetes strains isolated from marine environments were runoffs of their terrestrial counterparts (Table 2).

Screening of probiotic:

Resistance to low pH and bile salt: Out of 59 actinomycetes isolates, 17 isolates were grown at low pH 3 and 0.3% bile salts, as shown in figure 1. The marine actinomycete strain *Streptomyces sp* LCJ94 can be used as a probiotic in aquaculture as the marine actinomycetes have advantages like the production of antibiotic agents, bioactive compound, the degradation of macromolecules like protein and starch in the aquaculture ponds (Gunasekaran Mohanraj and Thangavel Sekar, 2013). Thus the marine isolate *Streptomyces sp.* LCJ94 used as a whole probiotic or purify the active compounds for combating the diseases caused in aquaculture.

All actinomycetes strains had a similar behaviour to different pH exhibiting no growth at pH 1–3, but growing at pH higher than 3 (Milagro *et al.*, 2015). It has been estimated that the survival rate of traditional probiotics, hosts gut is 20–40% being acidity one of the main obstacles (Bezkorovainy, 2001).

Bacteriocin assay

Cross streak method: Five actinomycetes isolates viz. A4, A5, A7, A17, A38 had shown broad spectrum antagonistic activity against human pathogens and MTCC pathogens (Fig. 2). The 51.1% of actinomycetes strains isolated from shrimp farms had antibacterial activity against *Vibrios* (You *et al.*, 2005). A similar antimicrobial effect just for 12–08% of actinobacteria tested against *Vibrio anguillarum* (Zheng *et al.*, 2008).

Agar well diffusion method: These five isolates A4, A5, A7, A17 and A38 had shown antibacterial activity against identified human pathogens and MTCC pathogens. A17 had shown Highest zone of inhibition and broad spectrum antibacterial

activity (Fig. 3). The strain MUSC 135(T) exhibited a broad spectrum bacteriocin against the pathogen MRSA, ATCC BAA - 44, *Salmonella typhi* ATCC 19430(T) and *Aeromonas hydrophilla* ATCC 7966(T). (Mohan Remya and Ramasamy Vijayakumar, 2008) The isolation and characterization of bacteriocin producing strains from extreme environment would provide lead to approaches like biopreservative.

In conclusion, the present study shows that the marine actinomycetes isolated from different coastal areas of India may play a good role as a biocontrol agent as probiotics and bacteriocin producers. The actinomycetes isolates from marine environment showed broad spectrum antibacterial activity against pathogens.

Table.1 Isolation of actinomycetes

Sr. No.	Name of coastal area	No. of Isolates
1	Elliot’s beach, Chennai (water)	-
2	Elliot’s beach, Chennai (sediments)	-
3	Calangute beach, Goa (water)	05
4	Calangute beach, Goa (sediments)	12
5	Thirumullavaram beach, Kerala(water)	03
6	Thirumullavaram beach, Kerala(sediments)	18
7	Gopalpur, Orissa(water)	-
8	Gopalpur, Orissa(sediments)	07
9	Dadar chaupati, Mumbai(water)	-
10	Dadar chaupati, Mumbai(sediments)	09
11	Angellar beach(water)	-
12	Angellar beach(sediments)	05
	Total	59

Table.2 Morphology of actinomycetes on SCN

Sr. No.	Colony	Colour of pigments
A5	Powdery	Yellow
A7	Slight sticky	Gray
A17	Sticky	White
A31	Sticky	Red
A37	Powdery	Yellow

Fig.1 Screening of probiotic

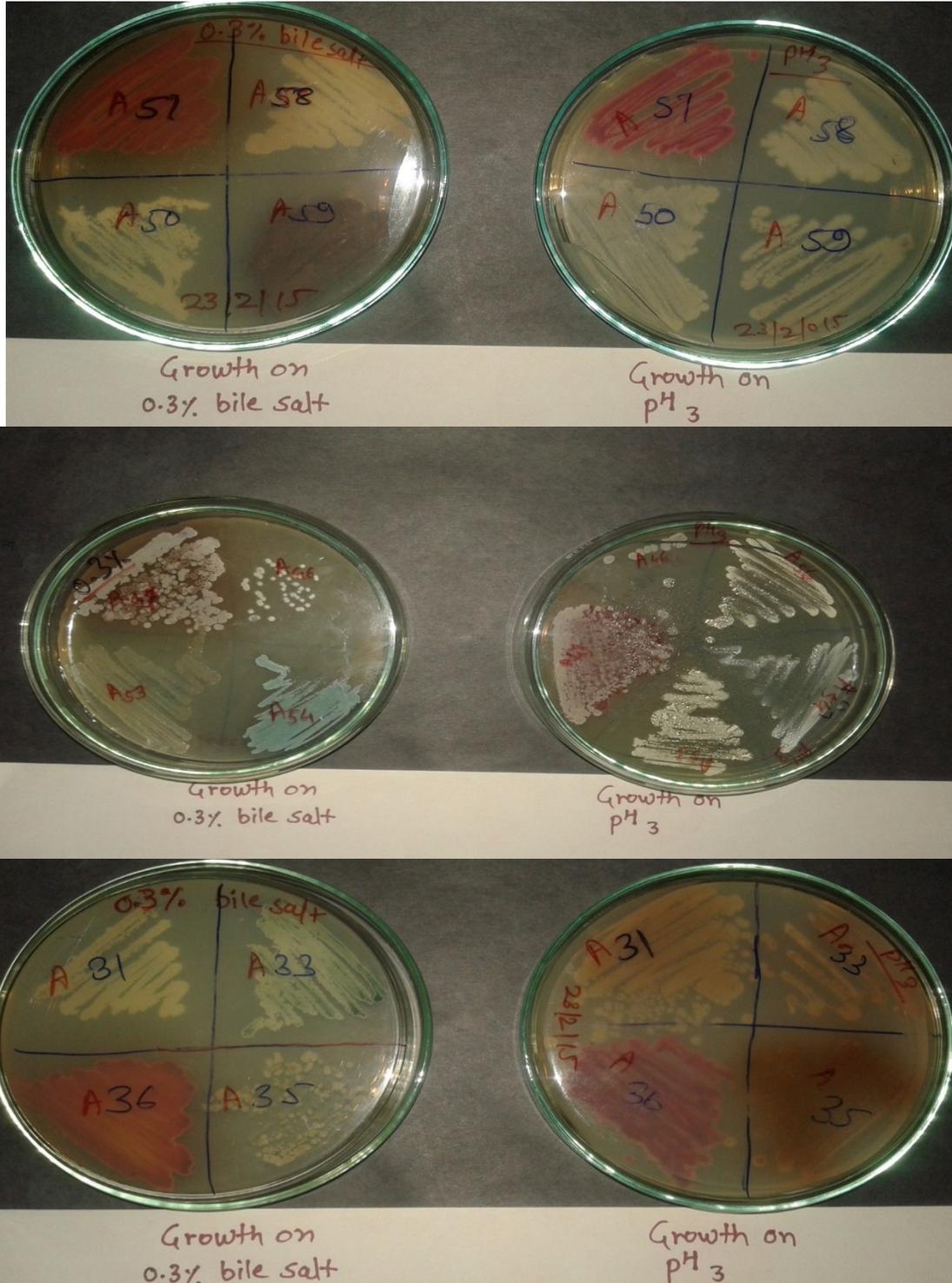
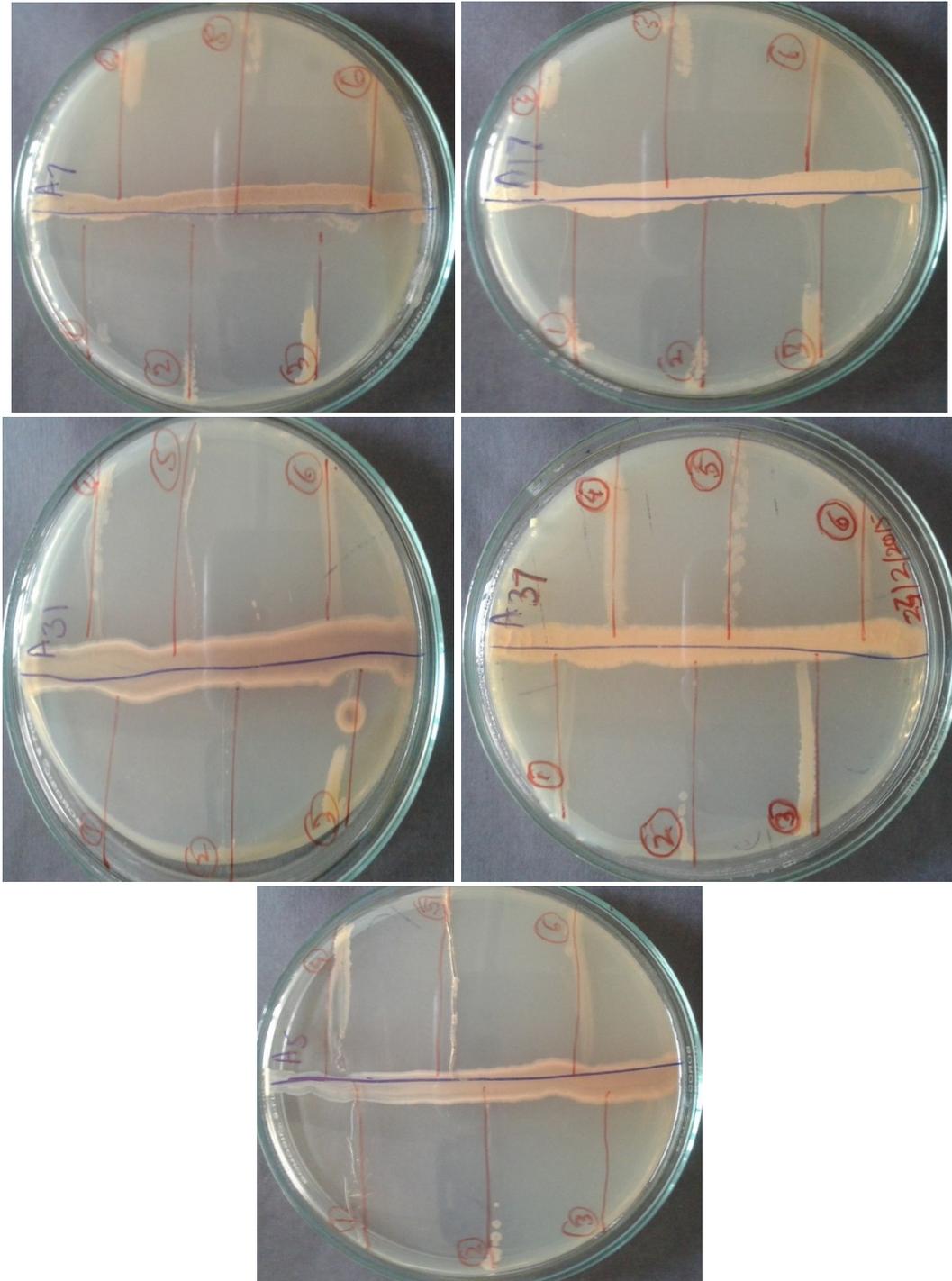
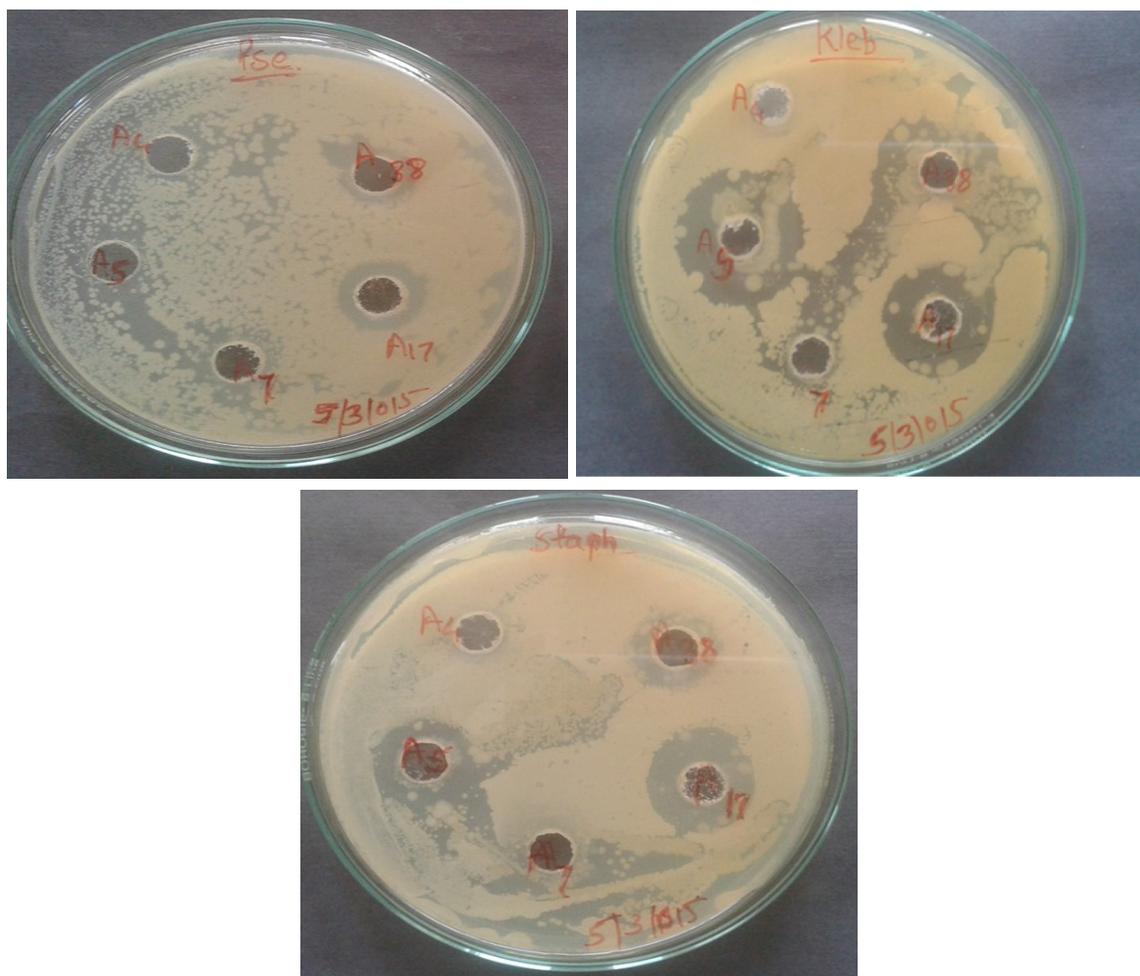


Fig.2 Cross streak method



Legend: A5, A7, A17, A38, A31- *Actinomycetes* and 1- *Escherichia coli* (1687), 2- *Pseudomonas aeruginosa* (1688), 3- *Salmonella typhi* (531), 4- *Bacillus subtilis* (8960) 5- *Klebsiella pneumoniae* (535), 6- *Staphylococcus aureus* (96).

Fig.3 Agar well diffusion method



Legends: A4,A5,A7,A17,A38- Actinomycetes

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