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Original Research Article

Screening for Antibacterial activity of Bacteria isolated from Marine sediment

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A B S T R A C T

The marine environment has revealed to be a promising source of biologically active compounds as several unique molecules are derived from microorganisms. They produce a variety of metabolites with many therapeutic applications. Bacteria hold a prominent position because they have a wide diversity and proven ability to produce new compounds. Looking at the wide importance of Bacteria in various fields, the present study was undertaken to isolate Bacteria from sediment samples from coastal regions of Suryalanka, Bapatla, Andhra Pradesh, India for identification of the most potent isolate to assess antibacterial activity. Four bacterial Isolates were selected for antibacterial screening by agar well method using the cell free supernatant. Out of four isolates, isolate AVCS-4 is proven to be more potent and tentatively identified as *Bacillus* genus by phenotypic, physiological and biochemical analysis.

Keywords

Marine Bacteria, Antibacterial activity, *Bacillus*

Introduction

Though ocean comprises 70% of the earth surface area, marine bacteria of this habitat are not fully explored for their bioactive compounds. The notable abilities of marine bacteria to produce chemically unique bioactive molecules are supported by characterization of a wealth of intriguing new structures through the application of classical screening and isolation techniques (Gulder *et al.*, 2009). Discovery of antibiotic lead molecules through screening of microbial secondary metabolites is becoming progressively significant. Microorganisms have been reported to produce around 23,000 bioactive secondary metabolites (Shinn *et al.*, 2013) with a wide range of industrially valuable activities, such as antibacterial, antifungal, antimalarial, cytotoxic, anticancer etc.,

Bacillus genus is a key antibiotic resource and being observed as a promising starting point in the hunt for new inhibitory substances as it is proficient of producing enormous antibacterial products. Over 800 antibiotic metabolites, such as gramicidin, bacitracin and polymyxin B have been reported from different species of *Bacillus* sp (Xiaoyong Zhang *et al.*, 2010). Several compounds of clinical value are being assayed in vitro to control the growth of pathogenic microbes. *S*everal non-ribosomal compounds, cyclic lipopeptidides of iturin group and macrolactones such as surfactins, fengycins and plipastatins have also identified from *Bacillus* through multistep mechanisms including the selection and condensation of amino acid residues (Stein *et al.*, 2005; Urdaci *et al.*, 2004). Subtilosin

A, an anionic macrocyclic peptide reported from *B. subtilis* and *B. amyloliquefaciens* showed strong bactericidal activity against *L. monocytogenes* (Babasaki *et al.,* 1985; Sutyak *et al.,* 2008). (Nagao *et al.,* 2001) described 18 macrolactins produced by *Bacillus* sp. Out of which seven compounds with a molecular mass of 402 Da identified as potent antibacterial, antiviral and cytotoxic agents (Romero-Tabarez *et al.,* 2006).

A large number antibiotics including bacteriocins, glycopeptides, lipopeptides and cyclic peptides showed difference in their basic chemical arrangements (Cladera-Olivera *et al.,* 2004; Baindara *et al.,* 2013). Two potent antibiotics cyclic lipopeptides, maribasins A and B with broad range of antimicrobial activity were isolated from the fermentation broth of the marine *B. marinus* (Zhang *et al.,* 2010). *B. marinus* also produced difficidin with broad spectrum of antibiotic activity like clinical antibiotics such as bacitracin, pumulin, laterosporin, gramicidin, colistin and polymyxin against different pathogenic bacteria.

Bacillus also reported to produce antifungal antibiotics such as mycobacillin and zwittermicin. Nearly 10,000 potential compounds from marine bacteria is being discovered every year (Sarma *et al.,* 2009). However the potentiality and exploitation of *Bacillus* sp of marine origin for production of potential antibiotics is yet to be worked out. The purpose of our study is to explore the hidden prospects of bacteria from marine environment in relation to the study of new potential drugs.

Materials and Methods

All the media ingredients and chemicals required for the present work were obtained from Hi media Lab (Mumbai) Ltd.

Pathogenic strains

The bacterial pathogens used as target strains in the current research work were *Escherichia coli* (MTCC1696), *Staphylococcus aureus*(MTCC3160), *Salmonella typhi* (MTCC8587), *Pseudomonas aeruginosa*, S*erretia marcescens, Proteus vulgaris* (brought from clinical laboratory) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India.

Isolation of marine bacteria

Marine soil sediment was collected from Surya Lanka coast of Bapatla, Andhra Pradesh and the samples were stored in polythene covers under chilled condition for further experiments. Marine bacteria were isolated by using serial dilution technique. The soil samples were serially diluted (10^{-1} to 10^{-9}) and the dilutions were inoculated on Beef extract- peptone agar media (Lanhong Zheng *et al.,* 2014). The inoculated plates were incubated at 37ºC, for 24-48 hours. Morphologically different bacterial colonies were selected from10^{-5} to 10^{-7} dilutions. The experiment was done in triplicate.

Screening for antibacterial Activity of the isolate

Spot Inoculation method

Test organisms of bacterial pathogens *E.coli* (MTCC1696), S.*marcescens, S.aureus* (MTCC3160), *S.typhi* (MTCC8587), *P.aeruginosa, P.vulgaris* were cultured on Nutrient Agar Medium by pour plate method. Marine bacterial colonies were spotted onto the agar plates inoculated with test organisms and incubated at 37°C for 24–48 h. Antibacterial activity was recorded as clear zone in the turbid growth of pathogens (Gram *et al.,* 2010).

Preparation of culture filtrate

Nutrient broth of 100 ml was prepared and one loop full of bacterial culture was transferred for antibiotic production. The flasks were incubated in at 30°C. When the optical density at 600 nm was approximately 1.5, the bacterial cultures were transferred under aseptic conditions into 1 L flasks, containing 400 ml sterile media. The flasks were then incubated in the dark at 30°C. Following fermentation, the cultures were centrifuged at 10000 rpm for 15 minutes at 4°C to obtain the cell free culture broth.

Agar Well Diffusion Method

Antibacterial activity was determined by agar well diffusion assay with slight modifications (Tagg and McGiven, 1971). Nutrient agar media plates were swab-inoculated with the test organisms grown in nutrient broth for 12 h and wells were made with cork borer. 50 µl of cell free supernatant of marine bacteria was added into the well and plates were incubated for 24 h at 37°C. Antibacterial activity was measured in terms of diameter (mm) of the inhibition zone produced around the well.

Phenotypic and Biochemical molecular identification of the organism

Phenotypic, biochemical and molecular characterization were carried out to identify the organism according to Bergey's manual of systematic bacteriology**.**

Proteolytic activity of the organism

The proteolytic activity of the strain was assayed using skim milk agar medium. Skim milk agar medium plates were prepared and the bacterial isolate was spotted on the medium. The plates were incubated at 37ºC for 24-48 hours. Formation of clear zone around the spot indicates the proteolytic activity of the bacteria.

Lecithinase Activity of the organism

Egg yolk agar was prepared and the organism was spotted on to the agar. Agar plates were incubated for 24–48 h at 37ºC to detect pattern of lecithinase production (Ben *et al.*, 2006).

Results and Discussion

21 morphologically distinct colonies were selected and screened for antibacterial activity by spot inoculation and well diffusion methods. Out of 21 isolates four isolates (AVCS1, AVCS2, AVCS3 & AVCS4) have shown a prominent antibacterial activity against six different test organisms (Table 1). Cell free supernatant of four selected AVSC isolates were tested for antibacterial activity by well diffusion method. Isolate AVCS-4 has shown highest zone of inhibition against *E. coli* (21 mm) followed by *S. typhi* (18 mm), *S. aureus* (16 mm), *P. mirabilis* (12 mm), *P. aeruginosa* (19 mm) and *S. marcescens* (15 mm) as shown in Table:1 and Fig:1

According to Bergey's manual of Systematic Bacteriology, Phenotypic and biochemical tests predicted that the organism belongs to *Bacillus* genus (Table 2 and Table 3).

AVCS4 has shown a clear zone around the colony in Casein hydrolysis and no lecithinase activity indicated that it is positive for proteolytic enzymes and negative for lecithinase (Fig 2)

The discovery of new antibiotics is required due to the increasing prevalence of multiple resistance pathogenic microorganisms to the drugs that are presently in clinical use

(Burgess *et al.*, 1999). *Bacillus subtilis* JM4 isolated from soil produce Subpeptin JM4-A and subpeptin JM4-B which are active against a broad spectrum of bacteria, including *Salmonella*, *Bacillus cereus*, *Staphylococcus aureus* (Wu *et al.*, 2005). *B. sonorensis* MT39 isolated from marine soil produce Sonorensin which has Antimicrobial activity against *L. monocytogenes* and *S. aureus* (Chopra *et al.*, 2014). Antibacterial screening of 21 isolates showed that 4 isolates exhibited zone of inhibition against test microorganisms by spot inoculation method, further confirmed with culture broth of AVCS1, AVCS2, AVCS3, AVCS4 isolates by agar well diffusion method, isolate AVCS4 has shown zone of inhibition from 12 mm to 21 mm against test organisms. It is identified as *Bacillus* genus, with the typical phenotypic characteristics such as gram positive, rod shape and ability to sporulate. The isolate was also screened for protease, lipase and lecithinase in order to screen the anticancer potentiality. The isolate has shown positive activity against protease. Proteases are produced by large number of microorganisms, but *Bacillus* strains are documented as important sources of commercial proteases because of their capability to secrete large quantities of enzymes with high activity (Beg and Gupta, 2003; Joo *et al.*, 2004). A marine *Bacillus* strain identified as *Bacillus* sp. MIG has shown maximum activity of proteases which are commercially used (Mona, 2006). Continuous emergence of disease, the development of drug resistant bacteria and increasing prices of medicines call for the discovery of less expensive microbial based medicine. The antibacterial potential can be attributed to the secondary metabolites that are presenting varying degrees. The most effective strain (AVCS-4) isolated is a Gram-positive bacterium tentatively predicted as *Bacillus* sp by physiological and biochemical characterization, this information will benefit in future for the optimization studies and physical factors to achieve maximum antibiotic production. Results from the present study can provide base line information about the antibacterial potential of isolate AVCS4.

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Fig.1 Antibacterial activity of AVCS-4

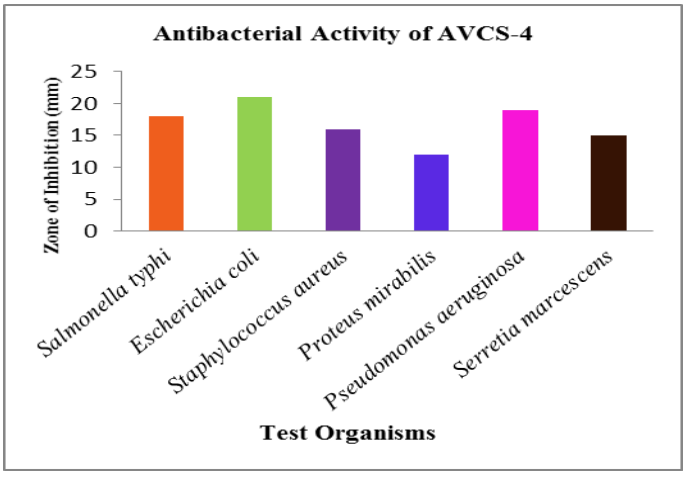

Fig.2 Proteolytic and Lecithinase activity of AVCS-4

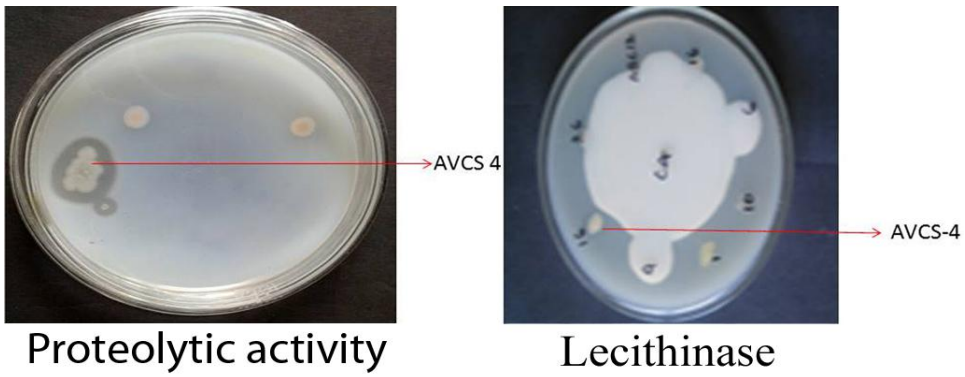

Table.1 Zone of Inhibition against test organisms

S.No	Test Organisms	Zone of Inhibition (mm)
1.	*Salmonella typhi*	18 mm
2.	*Escherichia coli*	21 mm
3.	*Staphylococcus aureus*	16 mm
4.	*Proteus mirabilis*	12 mm
5.	*Pseudomonas aeruginosa*	19 mm
6.	*Serretia marcescens*	15 mm

Table.2 Phenotypic Characteristics of AVCS4

S.No	**Tests Performed**	**Results**
1.	Morphology	Smooth, circular
2.	Gram Staining	Positive
3.	Spore formation	Positive
4.	Shape	Rods

Table.3 Biochemical characteristics of AVSC4

S.No	Biochemical tests	Results
1.	Indole	Negative
2.	Methyl red	Negative
3.	Voges proskauer	Negative
4.	Citrate utilization	Positive
5.	Starch hydrolysis	Positive
6.	Gelatin	Positive
7.	Casein hydrolysis	Positive
8.	Urease	Positive
9.	Oxidase	Negative
10.	Catalase	Positive
11.	Nitrate utilization	Negative
12.	Protease activity	Positive
13.	Lecithinase	Negative
14.	Lipase	Positive
15.	Motility	Positive

Table.4 Proteolytic and Lecithinase activities of AVCS-4

Proteolytic activity	Lecithinase Activity
Positive	Negative

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