

Original Research Article

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## Antibacterial Activity of a Novel *Bacillus cereus* isolated from Mangrove Ecosystem

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### ABSTRACT

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Mangrove forests are highly nutrient rich environment paves the way to the microbially vibrant ecosystem. Bacteria survive in this ecological niche tend to produce symbiotic or antagonistic metabolites, which is vital for many biotechnological applications. Muthupet mangrove is one of the important mangrove forests in India. In the present study, isolated some bacterial strains and the isolated strains were subjected to screen its antibacterial potential against human pathogenic bacteria. The antimicrobial assay showed one potential bacterium could able to produce antibacterial compound against enterobacteria members. The highest activity was recorded against *E. coli*. Phenotypic characterization of the active isolate proved that the isolate is *Bacillus cereus*. The antimicrobial compound produced by *B. cereus* showed its promising biotechnological application.

### Introduction

Coastal region is a vital marine ecosystem which connects land and ocean, this can be categorized as estuaries, coral reefs, intertidal zones, lagoons, mangroves and salt marshes (Chai *et al.*, 2017; Balakrishnan *et al.*, 2017; Vasanthi *et al.*, 2014; Jha *et al.*, 2013; Ganesh Kumar *et al.*, 2010). Mangrove forests are multifaceted and vibrant ecosystem acting as a shelter for many living things adjacent to the coastal ecosystem (Chai *et al.*, 2017; Balakrishnan *et al.*, 2017). Mangroves also act as fencing between the coastal and estuarine ecosystem. Plant species present in this environment tend to tolerate high amount of salt concentration, thus providing shelter

and food for many associated plants and animals in the ecosystem (Baskar and PrabaKaran 2011a, 2011b and 2015). Mangrove ecosystem are rich in nutrients allows high diversity of microbial community compared to terrestrial ecosystem (Balakrishnan *et al.*, 2017). This vast diversity of the microbes increases its metabolic and genetic capability of showing unique phenotypic characters, and the characters are beneficial in many ways to the human community (Balakrishnan *et al.*, 2017; Baskar and PrabaKaran 2015). There are several research work reported on antimicrobial compound production, plant growth promoter

producing, bioremediation and many more biotechnological activities reported from mangrove associated microorganisms (Balakrishnan *et al.*, 2017; Baskar and PrabaKaran 2015; Prakash *et al.*, 2015). Producing antimicrobial compounds is one of the major survival properties of bacterial population present in mangrove (Prakash *et al.*, 2015; Balakrishnan *et al.*, 2014). Thus it can compete with other microbial community present in the highly competitive niche. In the present study, isolated a potential antibacterial compound producing bacterium from Muthupet mangrove ecosystem, located on the southeast coast of India. The isolated bacteria identified using phenotypic characteristics and evaluated its antimicrobial potential against pathogenic bacteria.

## Materials and Methods

### Isolation of bacteria

Soil samples were collected from dense mangrove forest area of Muthupet mangrove ecosystem (10°20'20.5"N 79°32'36.8"E). In a sterile container, 10 g of soil sample collected and transported immediately to the laboratory. Collected samples subjected to extraction by adding 10 ml of Phosphate Buffer Saline (PBS) and vortexing for 2 min.

After extraction 1 ml of the extracted content transferred into a tube containing 9 ml of sterile PBS. The content was mixed well by vortexing, and subjected for serial dilution. From dilution 5, 6 and 7 one milliliter of the sample taken and plated onto a Zobel marine agar. The plates were Incubated for 24 hrs at 37 °C. After incubation; individual colonies were selected and streaked on a new ZMA plates.

### Antibacterial assay

Each isolated colonies were subjected to antimicrobial production test, for that the

bacteria was grown into the ZMB for 18hrs at 37 °C. After incubation supernatant was collected using centrifugation. 20 µL collected supernatant transferred to an agar plate containing 5 mm wells and streaked with desired pathogen (Table 1). The plate was incubated for 18 hrs at 37 °C. After incubation the plates were observed for the presence of a zone of inhibition (Balakrishnan *et al.*, 2014). The size of inhibition zone was recorded.

### Characterization of potential bacteria

For characterizing the active isolate of the study, experiments were performed as described in Bergey's Manual of Systematic Bacteriology (Logan and Vos, 2015).

## Results and Discussion

Among 83 isolates isolated 4 of them showed antimicrobial activity. Among the four active isolates, one of the isolates showed excellent activity. Phenotypic characterization results were compared with *Bergey's Manual of Systematic Bacteriology* and revealed that isolate is *Bacillus cereus* (Logan and Vos, 2015) (Table 1).

Among the ten pathogenic bacteria used in the study, *B. cereus* showed its antibacterial activity against five bacteria. All the five sensitive bacteria are gram negative; this means the bacteria *B. cereus* isolated in the study produces antibacterial substances against a wide range of Gram-negative bacteria.

Like this many gram-positive bacteria were reported for production of the antimicrobial compound against Gram-negative bacteria (Hu *et al.*, 2017; Sumi *et al.*, 2014). Also, the all sensitive bacteria in the study come from the Enterobacteriaceae family; these are considered to be highly pathogenic to the human.

**Table.1** Phenotypic characters of *B. cereus*

Test	Result	Test	Result	Test	Result
Colony colour	White	H <sub>2</sub> S Production	-	Glucose	+
Grams staining	+	Catalase	+	Glycerol	+
Motility	+	Oxidase	+	Inositol	-
Spore production	+	Urease	+	Lactose	+
pH range	4 – 10	Gelatinase	+	Maltose	+
Optimum pH	7±0.2	Nitrate Reductase	+	Mannitol	+
Temperature °C	10-45	Arginine decarboxylase	+	Mannose	-
Optimum °C	37±0.2	Lysine decarboxylase	-	Raffinose	-
Range of NaCl (%)	0.5-10	Ornithin decarboxylase	-	Rhamnose	-
Optimum NaCl (%)	0.5	Arabinose	+	Sorbitol	+
Indole	-	Cellobiose	+	Sucrose	+
Methyl Red	+	Dextrose	-	Starch	+
Voges Proskauer	+	Fructose	+	Trehalose	+
Citrate	+	Galactose	-	Xylose	-

**Table.2** Antibacterial activity of *B. cereus*

Type strains	Zone of Inhibition (mm)
<i>Bacillus subtilis</i>	-
<i>Enterococcus faecalis</i>	-
<i>Escherichia coli</i>	13.1
<i>Klebsiella pneumonia</i>	10.7
<i>Micrococcus luteus</i>	-
<i>Proteous mirabilis</i>	12.2
<i>Pseudomonas aeruginosa</i>	-
<i>Salmonella enteric ser typhi</i>	7.2
<i>Shigella flexineri</i>	8.9
<i>Staphylococcus aureus</i>	-

In recent years many new pathogenic strains from the family of Enterobacteriaceae threatening human life by changing its pathogenicity and resisting many commercially used antimicrobial compounds (Balakrishnan *et al.*, 2016; Barizuddin *et al.*, 2015).

The highest activity was recorded against *E. coli* (Table 2); this is the dominant species of bacteria present in the human gastrointestinal tract tent to change its genotype and cause many life threatening diseases (Balakrishnan and Mobley 2017; Balakrishnan *et al.*, 2016).

The antibacterial compound produced by *B. cereus* can be used as a drug against Gram-negative pathogens. However, before the application, there are a lot of work needs to be done to proves its specificity and toxicity to the humans. Nowadays it is also important to consider its effect on inducing antimicrobial resistance in bacteria.

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