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Novel Anti-Streptococcal Peptide Produced by Mangrove Bacteria *Bacillus subtilis*

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A B S T R A C T

Streptococcus mutans is a primary dental pathogen causes dental caries, its multidrug resistance increases the complications in treating the diseases. Many bacteria in a microbially rich environment like mangrove ecosystem tend to produce a variety of antimicrobial compounds. In the present study, a bacterium was isolated which showed excellent anti-streptococcal activity against human oral pathogen *Streptococcus mutans*. Antibacterial activity not recorded for any other pathogens used. The active bacterium identified as *Bacillus subtilis* using phenotypic characterization. Further, the active compound from the culture supernatant of *B. subtilis* was partially purified by precipitation and solvent extraction. The active compound proved to be a peptide in nature. The minimal inhibitory concentration (MIC) of the partially purified anti-streptococcal peptide was 123±10 µg ml^{-1}, which is comparable to commercial antibiotics. The anti-streptococcal peptide found in the study can be evaluated for its use in treating dental diseases.

Introduction

Coastal ecosystem is a dynamic ecosystem, links terrestrial and marine ecosystems. Mangrove is a unique coastal ecosystem covered by many unique plant populations. Mangrove plants provide shelter for many living things (Ganesh Kumar *et al.,* 2010; Vasanthi *et al.,* 2014; Jha *et al.,* 2013; Balakrishnan *et al.,* 2014, 2017a; Chai *et al.,* 2017; Baskar and PrabaKaran, 2015, 2011a,b). Mangrove-associated bacteria tend to produce an enormous variety of antibacterial compounds for their survival in the highly competing niche (Balakrishnan *et al.,* 2014; Prakash *et al.,* 2015).

Bacteria can act as consortia to perform interactive and defensive mechanisms in a presenting environment or host and can operate as a sole living entity. This consortium called the microbiome; recently microbiological research focused on microbiome analysis to find the role of each bacteria to act as consortia (Balakrishnan *et al.,* 2017b; Balakrishnan and Mobley, 2017).

Among oral pathogens, *Streptococcus mutans* is an important pathogen causes dental caries (Ahrari *et al.,* 2015). Increasing multidrug resistance of *S. mutans* leads the way to search new antimicrobial compounds from the natural environment. Apart from multidrug resistance pathogenic bacteria like *E. coli*, *Streptococcus*, *Salmonella*, and *Shigella* tend to change their antigenic properties; this leads troubles in the strain level detection process (Barizuddin *et al.,* 2015; Balakrishnan *et al.,* 2016). In the present study, tried to isolate a potential bacterium with excellent antibacterial properties against the dental pathogen *S. mutans*. The isolated bacteria characterized up to species level using phenotypic characterization. Also, partially purified the active compound using precipitation and extraction techniques.

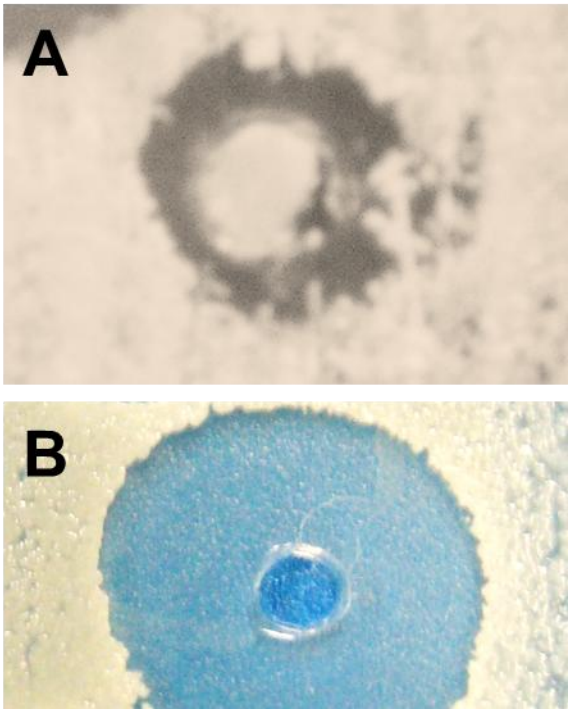
Mangrove soil samples collected from Mangrove ecosystem of Muthupet, Tamil Nadu, India (Lat: 10°20'20.5"N Lan: 79°32'36.8"E). Isolation was performed using marine agar plates. From the isolation procedure isolated 60 different bacteria and screened for antibacterial activity against *S. mutans*. Antimicrobial activity performed using disc diffusion assay using supernatant of the marine broth cultures of purified isolates. Among the isolates, one of them showed excellent anti-streptococcal activity. Phenotypic characterization of the active strain performed as per Bergey's Manual of Systematic Bacteriology (Logan and Vos, 2015). Based on the results the isolate identified as *Bacillus subtilis* (Table 1). There are several reports proved to isolate antibiotic-producing bacterial species from mangrove environment. Recently in our previous research project, we identified a *B. cereus* which showed a wide range of antimicrobial activity against many human pathogenic bacteria (Karthikeyan and Sahayarayan, 2017).

The present study also tested antibacterial activity of *B. subtilis* against other common bacteria such as *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Proteous mirabilis*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Shigella flexineri* and *Staphylococcus aureus*. However, the active bacteria did not show any antibacterial activity against the above-listed bacteria. The results confirm that the antibiotic produced by *B. subtilis* specific to *S. mutans* among the tested bacteria (Fig. 1).

Table.1 Phenotypic characters of *B. subtilis*

Test	*Result*	Test	*Result*	Test	*Result*
Colony colour	White	H_2S 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	+

Fig.1 Antimicrobial activity of *B. subtilis* against *S. mutans*. A; Initial antibacterial screening using culture supernatant, showing a zone of inhibition. B; Partially purified anti-streptococcal peptide showed higher antibacterial activity than supernatant



We suspected the antimicrobial compound is a proteinaceous substance (antimicrobial peptide). Partial purification of the antimicrobial compound carried out using culture supernatant of the bacterium in marine broth. The supernatant was precipitated using 60% ammonium sulphate saturation, followed by butanol extraction as described by Balakrishnan *et al.,* (2014). To prove proteinaceous nature; enzymatic treatments carried using lipase, pepsin, catalase, trypsin, lysozyme, and proteinase K. where the compound inactivated by proteolytic enzymes thus the active compound proved to be a protein-based compound.

MIC of the partially purified compound was tested using serial dilution method. The active compound showed MIC of 123±10 µg ml^{-1} against *S. mutans*. This level is nearly comparable to commercial antibiotics like penicillin (108±02 µg ml^{-1}) and erythromycin (98±01 µg ml^{-1}). If the compound is purified, it will show much lower MIC value, and that will make this compound more active than any other antibiotic used in treating dental caries. Balakrishna *et al.,* (2014) demonstrated the same thing for vibriocin, where the antibacterial efficiency of the active compound logarithmically increased after each step of purification. Many research studies proved on identify antimicrobial compounds from mangrove sediments. Only a few studies demonstrated the nature of the antimicrobial compound produced by associated mangrove bacteria (Hwanhlem *et al.,* 2014; Balakrishnan *et al.,* 2014). In the present study, the compound showed to be a protein in nature. Possibly the compound is an antimicrobial peptide. Similar kind of antimicrobial peptide reported from *Vibrio parahaemolyticus* and the peptide active against only pathogenic *Vibrios* (Balakrishnan *et al.,* 2014). Based on the present study the

anti-streptococcal peptide identified can be used in treating dental caries by controlling the growth of *S. mutans* in parts of the oral cavity.

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