Microbial Pathogenesis 157 (2021) 105007

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Identification of Multi-Potent Protein Subtilisin A from halophilic bacterium *Bacillus firmus* VE2

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ARTICLE INFO

Keywords:
Saltpan soil
Halophiles
Antimicrobial protein
Bacillus firmus
Anticancer activity
Subtilisin A

ABSTRACT

Screening of halophiles with antimicrobial activity in saltpan soil samples from Nagapattinam district, Tamil Nadu, revealed isolate VE-2 as the most potent, identified as *Bacillus firmus* strain VE-2 through 16s rRNA gene sequencing. It had an optimum growth condition (OD 3.1) and antimicrobial protein (AMP) production (450 μg/mL) at 37 °C, pH 8, 25% NaCl, and 36 h incubation. SDS-PAGE analysis of the purified AMP showed the molecular weight of 36 kDa. HPLC analysis of the purified AMP showed different amino acids, such as asparagines, alanine, lysine, proline, threonine, glycine, cysteine, serine, aspartic acid leucine, and valine. Further characterization and identification using FT-IR, 2D-PAGE, MALDI-TOF, and *in-silico* analysis showed that the isolated AMP had the highest similarity to Subtilisin-A. It showed antibacterial activity against clinical bacterial pathogens like *S. aureus, S. pyogenes, C. diphtheria, E. coli, and P. aeruginosa* with the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration of 2.5 μg/mL and 20 μg/mL and also against various fungal pathogens such as *A. niger, A. flavus, C. albicans, C. tropicalis* and *C. parapsilosis* with the MIC and minimum fungicidal concentrations of 1.25–80 μg/mL. The purified AMP had excellent antioxidant potential, showed a scavenging effect against DPPH and Nitric oxide radicals, and displayed anticancer activity against HeLa cell lines with the IC_{50} values 53 μg/mL. Hence, the purified bioactive antimicrobial peptides (AMP) could also be used in anticancer therapies.

1. Introduction

Extremophilic microbes adapts to extreme abiotic stress. Halophiles are such a group of extremophiles adapted to saline environments. Halophiles are classified into slight (1–3% NaCl), moderate (3–15% NaCl), and extreme halophiles (>15% NaCl) [1,2]. The salinity in marine ecosystems generally ranges from 3 to 5%, which can be as high as 31.5% in the Dead Sea. Halotolerant are reliable sources of novel biomolecules and have pharmaceutical and industrial applications such as food colorant, antibacterial, antifungal, antiviral, antioxidant, and anticancer potentials [3–5]. Recent discoveries of novel enzymes from halophiles includes the serine alkaline protease and serine alkaline peptidase from *Gracilibacillus boraciitolerans* strain LO15 and *Virgibacillus natechei* sp. nov., strain FarD^T [6,7]. For the past two decades, in-depth researches have been conducted to isolates and characterize novel halophiles from different geographical locations such as in India [8], Korea [9], and China [10]. Antimicrobial compounds from marine halophiles have shown promising antimicrobials, although less than 1% of them have been explored so for [11]. For instance, *Bacillus pumilus,* a marine sediment isolate, exhibited antimicrobial activities against bacterial and fungal strains, including *E.coli, Staphylococcus aureus, Bacillus subtilis, Aspergillus niger,* and *Aspergillus flavo*us [12]. Since the discovery of antibiotics from soil actinomycetes (1940–1960s), the soil has been extensively mined in quest of novel antimicrobial compounds (Leisner, 2020). The rapid increase in antibiotic resistance incidences has necessitated the need for alternate niches for mining antimicrobials.

The present study aims to isolate, and characterize an antimicrobial compound from a halophilic bacterium isolated from the saltpan sediments collected from different marine environments within the Nagapattinam District, Tamil Nadu, India. The isolated antimicrobial

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https://doi.org/10.1016/j.micpath.2021.105007
Received 1 April 2021; Received in revised form 19 May 2021; Accepted 20 May 2021
Available online 25 May 2021
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