Production of antimicrobial agents by marine *Bacillus subtilis*: Application of Plackett-Burman and Box Behnken experimental designs

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Background

Bacterial resistance is a rapidly escalating threat to public health as our arsenal of effective antibiotics dwindles. Bacterial resistance to antibiotics refers to the insensitivity of bacteria to the antimicrobial actions of a given antibiotic. The organism in question may develop the ability to destroy the antibiotic or to grow in its presence. Therefore, there is an urgent need for new antibiotics. (Penesyan *et al.*, 2015).

Bioactive compounds are traditionally screened from terrestrial microorganisms. However, the opportunity of finding novel antibiotics from terrestrial microorganisms has diminished. The environments between oceanic and terrestrial systems are very different. As such, marine microorganisms may produce bioactive compounds not found in terrestrial habitats. It has been reported that marine invertebrates harbor a higher population of bacteria able to produce novel bioactive compounds. Previous studies on microbes associated with sponges have revealed that some bacteria have a great potential for producing useful natural products. The search of new microorganisms, having unique physiological and metabolic capabilities, aids to better comprehend the ecosystem and provides opportunities to discover new compounds of commercial importance (Silvi *et al*., 2013).

Different previous studies concerned the production of antimicrobial agents by *Bacillus* sp. (Anjum *et al*., 2016; *Samanta et al*., 2017).

Objectives

The main objective of the present work is to select and identify a competent marine isolate capable of producing antimicrobial agents and to optimize its production by applying different experimental designs and other techniques.

Materials & Methods

- Screening of different marine isolates for production of antimicrobial agents against *Salmonella typhimurium* (ATCC 14028), Methicillin resistant *Staphylococcus aureus* (ATCC 43300), *Listeria monocytogenes* (ATCC 19111), *Aeromonas hydrophila* (ATCC 35654), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12228), *Escheria coli* (ATCC 8739), *Candida albicans* (ATCC 10231), *Pseudomonas aeruginosa* (ATCC 9027) and *Aspergillus niger* (ATCC 16404).
- Biochemical, MALDI-TOF MS based proteomic identification in addition to molecular identification of the promising isolate.
- Time course production of the antimicrobial agents.
- Effect of different factors (fermentation medium, carbon sources, nitrogen sources) on production of the antimicrobial agents.
- Optimization of fermentation conditions using Plackett-Burman and Box-Behnken designs
- Effect of physical and chemical mutations on production of the antimicrobial agents.
- Effect of immobilization by adsorption on different support materials in addition to entrapment technique on production of antimicrobial agents.

RESULTS

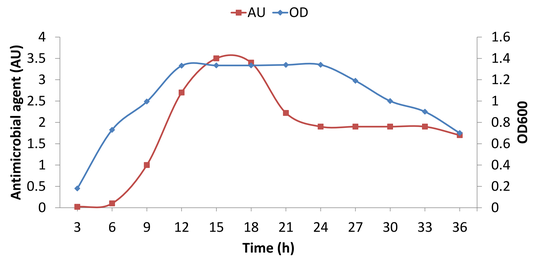
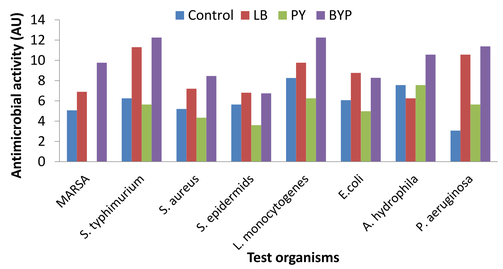


Figure 1: Growth curve and antimicrobial agent production by *B. subtilis* AD35



LB: Luria Bertani medium; PY: Peptone yeast medium; BYP: Beef yeast peptone medium

Figure 2: Effect of medium type on production of antimicrobial agent by *B. subtilis* AD35

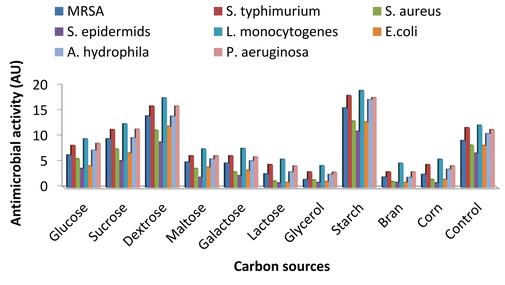


Figure 3: The effect of different carbon sources on the production of antimicrobial agent by *B. subtilis* AD35

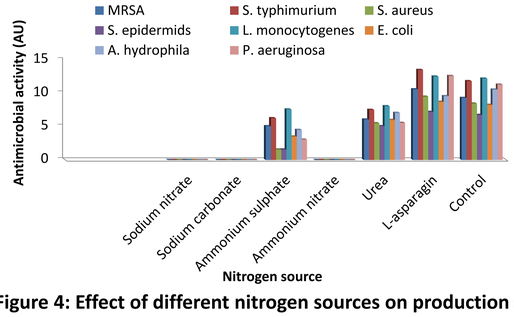


Figure 4: Effect of different nitrogen sources on production of antimicrobial agent by *B. subtilis* AD35

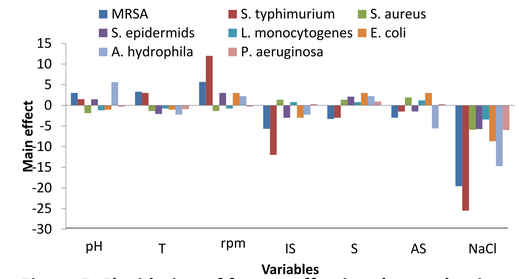


Figure 5: Elucidation of factors affecting the production of antimicrobial agents

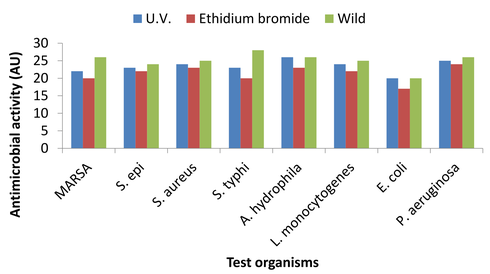


Figure 6: Effect of physically and chemically induced mutations on antimicrobial agent production by *B. subtilis* AD35.

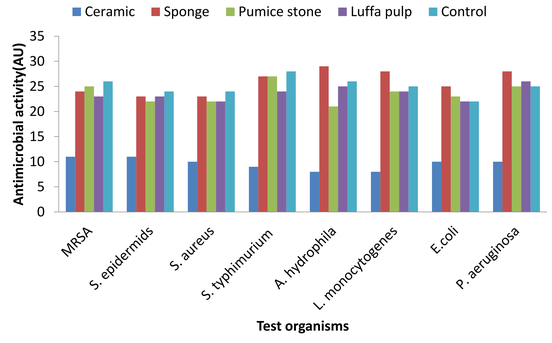


Figure 7: Effect of various adsorption materials on the production of antimicrobial agent by *B. subtilis* AD35.

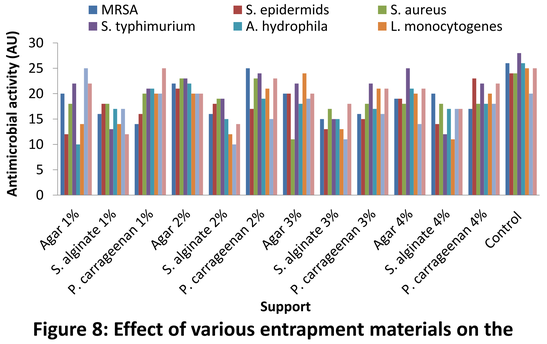


Figure 8: Effect of various entrapment materials on the production of antimicrobial agent by *B. subtillis* AD35.

CONCLUSIONS

Results of the current study confirmed the potential of marine natural microbial communities for production of antimicrobial agents which enables the development of a technology for pharmaceutical and aquaculture applications.

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