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Phenotypic characteristics, phylogenetic analysis and characterization of alkaline proteases of marine bacteria *Geomicrobium halophilum, Oceanobacillus oncorhynchi,* and *Oceanobacillus khimchii*

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Abstract

In this report, protease-producing haloalkaliphilic bacteria from sea water have been investigated. Seven bacterial strains belonging to *Geomicrobium halophilum, Oceanobacillus oncorhynchi*, and *Oceanobacillus khimchii* were isolated and characterized based on their colony characteristics, cell morphology, biochemical properties, antibiotic sensitivity, and 16S rRNA gene sequence analysis. The analysis based on 53 phenotypic characters (cluster analysis) generated a phenogram establishing correlation with the phylogenetic placement. A maximal level of phenotypic similarity among the protease producing bacteria was evident

at only 46% in the phenogram. An overall coherence was evident between the patterns reflected in phenogram and phylogram. Four isolates of different geographical locations belonging to *Geomicrobium halophilum* were scattered in the phenogram, displaying significant variations in their phenotypes. Further, stability and catalysis of the extracellular alkaline proteases of these isolates were studied. The bacteria were able to grow in the range of pH 7-10 and temperatures between 37-55 °C. The proteases were active and stable at pH 7-13 with the optima between 9.5 and 10.5, although a rapid loss of activity was observed at pH 6.5 and below. Most of the proteases had temperature optima at around 60 °C, while they were stable in the range of 37-60 °C, with the retention of 100-80% activities after 1h of incubation at 60-90 °C. As a unique feature, the enzymes were resistant to urea denaturation (2-8 M) for a period of 2-24 h. The addition of NaCl had profound effect on the resistance against chemical denaturation.

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Contributions

All authors contributed to study conception and design. RHJ performed experiments related to enzyme stability and catalysis. HBB contributed to analysis of numerical taxonomy and phylogeny. RHJ and VHR compiled and interpreted the data and wrote the manuscript. HBB also analyzed the data and edited the manuscript. SPS supervised the work, helped in analyzing the data and edited the manuscript.

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Ethics declarations

The submitted manuscript is original, unpublished and not under simultaneous consideration by another journal.

Consent for publication

All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they have no indirect or direct conflict of interest.

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Highlights

• Marine bacteria characterized on the basis of morphology, biochemical characters, antibiotic sensitivity and enzyme secretion

• Bacterial diversity assessed by phylogenetics and numerical taxonomy

• Stability of proteases at high temperatures, wide pH range and in Urea indicates their potential applications in detergent, leather, food, dairy, and cosmetics industries

• Salt dependent resistance against denaturants was revealed

• Study significantly adds to the diversity and enzymatic potential of the haloalkaliphilic bacteria of sea water

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