

Enumeration of halophilic forms in parangipettai saltpan and its antagonistic activities against *Vibrio* sp.

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ABSTRACT

Totally 27 halobacterial strains were isolated from parangipettai saltpan environment, based on the antagonistic activities 7 halobacterial strains were identified as halophilic bacterial strains were related to *Bacillus* sp., *Halobacterium* sp., *Halobacillus* sp., *Halobacterium* sp., *Staphylococcus aureus*, *Halobacterium salinarum*, *Halobacillus salinus*. The isolate *H. salinus* showed maximum antibacterial activities against the entire pathogenic *Vibrio* sp. and showed highest zone of inhibition against *V. paraheamolyticus* (13mm), *V. anguillarum* (12mm), *V. alginolyticus* (11mm). The halobacterium specie showed maximum zone inhibition against *V. paraheamolyticus* (10mm) and minimum antibacterial activities were observed in the *H. salinarum* which showed against *V. harveyi* (3mm). The results indicated that halophilic bacterial strain *H. salinus* is a good candidate for the production of novel bioactive compounds.

1. INTRODUCTION

In aquaculture industries the bacterial forms such as *Vibrio* spp. cause severe economic loss in worldwide [1]. The prevention and control of such diseases many remedies has been proposed use of antibiotics, vaccines and immunostimulants etc. The use antibiotics will lead the formation of drug resistant forms, it is more difficult to control and eradication of these kind of bacterial forms [2]. Salt pond organisms are defined as halophilic microbial forms which usually inhabit salt rich environments. Halophilic microbial forms are economically important because it produce several bioactive compounds which are useful for many pharmaceutical industries [3]. Among the halophilic microbial forms, the halophilic bacterial forms are mostly known for its secondary metabolites such as proteins, amino acids, etc [4].

In recent years many reports are available for the application of halophilic bacterial forms as a probiotic in shrimp aquaculture [5] [6] [7] [8]. On this above observation present study has been carried out to evaluate the antimicrobial potent halobacterial strains from parangipettai salt pan environments.

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2. MATERIALS AND METHOD

2.1. Collection of sample

Sediment samples were collected from parangipettai saltpan environment at six intervals of one year. The samples were collected from sterile plastic bags and kept 4°C until transported to the laboratory for further processing.

2.2. Enumeration of halophilic bacterial forms

Halphilic bacterial forms were isolated using selective halophilic agar medium. Sediment sample were serially diluted, an aliquot 0.1ml of each dilution from 10⁻³ to 10⁻⁶ was taken and spread on the surface of halophilic agar medium. Plates were incubated at 37 °C for 3-4 weeks. After incubation different colonies were selected and purified for further investigation.

2.3. Morphological and biochemical identification

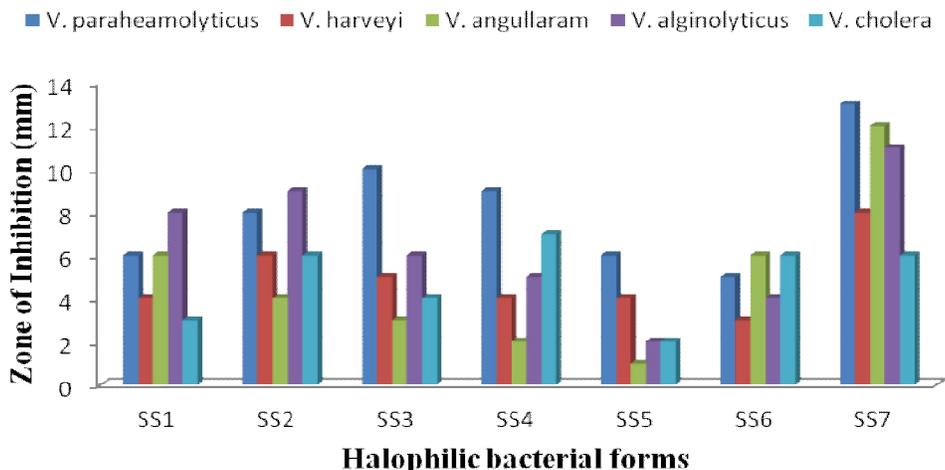
The isolated bacterial forms were subjected into morphological and biochemical identification using standard Bergey's manual [9].

2.4. Antimicrobial screening

The isolated halophilic bacterial forms were screened for its antimicrobial potentials against shrimp pathogens (*Vibrio paraheamolyticus*, *V. harveyi*, *V. anguillarum*, *V. alginolyticus*, *V. cholera*) were obtained from Marakanam hatchery.

Table. 1: Biochemical identification of halophilic forms.

Strain N.O	Pigments	Gram stain	Motility	catalase	oxidase	Indole	MR	VP	citrate	Urease	Hydrolysis		
											Starch	Gelatin	casein
SS1	+	+	-	+	+	-	-	-	-	-	+	-	+
SS2	+	-	+	+	+	+	+	-	-	-	-	+	-
SS3	+	+	+	+	+	+	+	-	-	-	-	+	+
SS4	+	-	+	+	+	+	+	-	-	-	-	+	-
SS5	+	+	-	+	-	-	+	+	-	-	+	+	+
SS6	+	-	-	+	+	+	-	-	-	-	-	+	-
SS7	+	+	-	-	-	-	-	-	-	-	-	+	+

**Fig. 1:** antibacterial activities of halophilic bacterial isolates.

Antimicrobial activities were quantified by using well diffusion method [10]. For evaluation, tetracycline (100µg/ml) was used as standard antibiotic and DMSO as negative control. Muller Hinton agar was used for this study. Plates were incubated at 37 °C, after incubation zone of inhibition was measured.

3. RESULT

Totally 27 halophilic bacterial forms are isolated from the sediment sample of parangipettai salt pan environment. Out of that 16 strains acceptable antagonistic activities among them 7 halophilic bacterial strains showed high amount of antibacterial activities towards the shrimp pathogens.

3.1. Identification of halophilic bacterial isolates

The phenotypic characterization indicated that 7 (SS1-SS7) halophilic bacterial strains were related to *Bacillus* sp. (SS1), *Halobacterium* sp (SS2), *Halobacillus* sp. (SS3), *Halobacterium* sp (SS4), *Staphylococcus aureus* (SS5), *Halobacterium salinarum* (SS6), *Halobacillus salinus* (SS7)(Table.1).

3.2. Antimicrobial activities

All the seven halophilic bacterial isolates showed high amount of antimicrobial activities towards the shrimp pathogens (*Vibrio paraheamolyticus*, *V. harveyi*, *V. anguillarum*, *V. alginolyticus*, *V. cholera*). The isolate *H. salinus* (SS7) showed maximum antibacterial activities against the entire *Vibrio* sp. isolate *H. salinus* SS7 showed highest zone of inhibition against

V. paraheamolyticus (13mm), *V. anguillarum* (12mm), *V. alginolyticus* (11mm). Followed by isolate *Halobacillus* sp. (SS3) showed maximum zone inhibition against *V. paraheamolyticus* (10mm) and minimum antibacterial activities were observed in the isolate *H. salinarum* (SS6), which showed against *V. harveyi* (3mm). Rest of the isolates showed acceptable antibacterial activities against dreadful shrimp pathogens (Fig. 1).

4. DISCUSSION

In recent past, the use of halophilic forms in industrial application has been increased. It produces wide range of bioactive compounds such as enzymes (protease, amylase, cellulases, etc.), extracellular polysaccharides (EPS), proteins, etc [11]. On this aspect, in the present study was carried out to identify novel halophilic forms from parangipettai salt pan environment. Totally 27 halophilic bacterial forms are isolated out of those 7 strains were screened based on its antagonistic activities against shrimp pathogens. Ganesan *et al.* [12] isolated 4 halophilic bacterial strains from Pichavaram mangrove environment. Similarly Sanju *et al.* [13] isolated and identified 9 bacterial strains from Kovalam salt pan environment. In the present study, all the 7 halophilic bacterial strains were identified by using their morphological and biochemical identifications. The strains are belongs to the species of *Halobacterium* spp., *Halobacillus* sp., *Halobacillus salinus*, *Staphylococcus aureus*, *Halobacterium salinarum*, *Halobacillus salinus*. The four halophilic bacterial

forms such as *V. harveyi*, *Halomonas* sp., *V. fluvialis* and *Halobacterium* sp were isolated from pichavaram mangrove forest, India [12]. Likewise in Samphar salt lake 108 halobacterial isolates were isolated based on the enzyme production 21 strains were identified using morphological and molecular level identification, the strains belongs to the genera of *Marineobacter*, *Virgibacillus*, *Halobacillus*, *Geomicrobium*, *Chromohalobacter*, *Oceanobacillus*, *Bacillus*, *Halomonas* and *Staphylococcus* [15]. Among seven halophilic bacterial isolates *Halobacillus salinus* (SS7) showed maximum antibacterial activities against the entire *Vibrio* sp. isolate SS7 showed highest zone of inhibition against *V. parahaemolyticus* (13mm), *V. anguillarum* (12mm), *V. alginolyticus* (11mm). Kavitha *et al.* [14] isolated *Haloferax Volcanii* KPS1 from Kovalam salt pan and it inhibit the growth of both gram positive and gram negative pathogenic bacterial forms. Similarly Ashok kumar and Mayavu, (2014) screened 25 halobacterial strains from Marakanam salt pan environment, based on the antagonistic activities against shrimp pathogens among them only one strain *Bacillus* Mk22 has been showed high amount of antagonistic activities.

5. CONCLUSION

In parangipettai salt pan environment 27 halobacterial strains isolated, in that 7 halobacterial strains were identified by using both morphological and biochemical methods. The isolate *H. salinus* inhibited all the shrimp pathogens. The strain *H. salinus* has produced several interesting compounds and thus may be good candidate for the pharmaceutical industry.

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