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PHARMACEUTICAL POTENTIALS OF BACTERIA FROM SALTPANS OF GOA, INDIA

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ABSTRACT:

Solar salt pans consist of a series of inter linked pans where gradients of salinity occur due to evaporation of seawater. Salinity in these ponds reaches as high as 400 psu during the peak salt-producing season and as low as 5 psu during the monsoons. Salt pans are extreme environments which inhabit organisms that thrive high salinities, temperatures and withstand severe solar radiations. Such organisms are capable of producing interesting metabolites which may benefit mankind. Salt pan water, salt and sediment samples were collected from nine saltpans from North and South Goa to isolate bacteria. The bacterial extracts have been screened for various biological activities to ascertain their biomedical importance. A total of 63 out of 1178 cultures were found to be active showing antioxidant, anti gastric ulcer, antifungal, memory enhancing activity and activity against neurological disorders, anticancer, amylase, amylase inhibitory, protease and protease inhibitory activity. This study highlights the biomedical potential of bacteria from salt pans and encourages further exploration of such bacteria for therapeutic activities.

Keywords: anticancer, bioactive compounds, salt pans, therapeutic agents.

[I] INTRODUCTION

Marine microbial metabolites have gained tremendous importance in past few decades due to their potential to be 'molecules of the future'. With the increasing problem of drug resistance there is a dire need to isolate, identify and utilize newer molecules of biomedical importance. Newer niches are constantly being sought to identify potential producers of bioactive molecules. Saltpans are an extreme environment, which inhabit organisms that survive very high salinities, high temperatures and withstand severe solar radiations. Hence these organisms could serve sources of novel secondary metabolites. Various halophilic and halotolerant microbes inhabiting the salt pans are yet to be fully explored as potential producers of pharmaceutically significant molecules. Few reports are available on their antimicrobial potential in India [1,2,3].

Goa's traditional salt industry is said to have been a major supplier of salt to the country and an exporter to some foreign countries since the 10th century. But since 2002, only about 16 salt pans are in use for the commercial production of natural salt. Salinity in these ponds ranges from 10 to 400 psu during the peak salt-manufacturing season i.e. between November to May. We have isolated 1178 bacteria from water, salt and sediment samples from nine saltpans and screened the lyophilised aqueous extracts of these bacteria for various biological activities.

[II] MATERIALS AND METHODS

2.1. Sample collection:

Nine saltpans from North and South Goa were selected for sampling. Sampling was restricted to the crystallizer ponds from the salt pans of viz. Ambeli, Arpora, Cavellosim, Curca, Morjim, Nerul, Ribandar, Shiroda and Siridao. Water samples from overlying saltpans, were collected in sterile disposable bottles and chilled on ice. Sediment samples were collected using a 10 -cm corer and salt crystals were collected using a sterile spatula. Core samples and salt crystals

were sealed in sterile plastic bags and transported at 4°C in an ice box and processed within a 24 h period.

2.2. Isolation of bacteria:

In brief, serial dilutions were carried out with sterile salt pan water and 0.2ml of the sample was spread plated, in triplicates, on Nutrient Agar (NA) supplemented with 5% NaCl. The plates were incubated at 37°C until the appearance of colonies. The strains isolated were purified, sub cultured and stored at 4°C. All the media and media components used for the experiments were procured from HiMedia, Mumbai, India unless otherwise specified.

All the isolates growing on these plates were further tested for growth on media C agar [composition in g/L Soluble starch 25, Glucose 10, Yeast Extract 2, Calcium carbonate 3, Trace salt solution 1ml; pH 7.5 (Trace salt solution g/100mL FeSO4.7H2O 0.5, CuSO4.5H2O 0.5, ZnSO4.7H2O 0.5 & MnCl2.4H2O 0.5] and media D agar [composition in g/L Tryptone 15, Soyatone 5, Sodium chloride 5, agar 15; pH 7.3] plates and were selected for further processing.

2.3. Preparation of extracts:

Isolated colonies were inoculated into 40ml of media C or media D broth and kept on shaker for 7 days. The broth was sonicated using an ultra sonicator Vibra cell™ sonicator [Sonics & Materials, Inc. Danbury, CT, USA] with a cycle of 40 seconds with pulse and 40 seconds without pulse, twice. The contents were centrifuged at 10,000 rpm for 20 minutes. The tubes were always kept immersed in ice during the process. The supernatant was decanted and dispensed as 1ml aliquots, frozen and lyophilized using [CoolSafe™ 110 Freeze Dryer, Scanvac, DK]. The freeze dried samples were dispatched to various Council of Scientific and Industrial Research (CSIR) laboratories to evaluate their antioxidant (Indian Institute of Chemical Technology, Hyderabad), anti gastric ulcer against Helicobacter pylori (Indian Institute of Chemical Biology, Calcutta), antifungal (Central Institute of Medicinal and Aromatic Plants, Lucknow), memory enhancing activity & activity against neurological disorders such as anti anxiety, anti dementia, anti depressant & anti parkinsons (Central Drug Research Institute, Lucknow) and anticancer (Indian Institute of Integrative Medicine, Jammu) activity.

The isolated colonies were directly tested for amylase, amylase inhibitory, protease and protease inhibitory activity using plate assay method. MD (Media D) plate was prepared with 1% Starch (soluble starch). Cultures were inoculated on MD Starch agar plates and kept for 48 hours minimum and 3-4 days maximum incubation. Amylase producers were detected with iodine. The colony producing amylases exhibited a clear halo on a blue background which was recorded. Iodine vapours from the plate were allowed to evaporate and subsequently the plate was flooded with commercial amylase Diastase (Alpha amylase) (s d fine-chem) 1% solution (100 micro liter per plate) and incubated for 15 minutes. The amylase was discarded and again the plate was flooded with iodine. Amylase inhibitors exhibited a dark blue halo on a clear plate. The halo size was recorded. To detect protease producers and inhibitors Media D plate was prepared with 1% purified skimmed milk (HiMedia, India), cultures were inoculated and incubated for 48 hours (minimum) and 3-4 days (maximum). The protease producers showed a halo around the colony. The plates were flooded with commercial protease 1% Typsin (HiMedia, India) solution and allowed to react for 15 minutes. The Trypsin was decanted and a white halo was observed around the colonies showing protease inhibitory activity.

[III] RESULTS AND DISCUSSION

Microbes produce secondary metabolites as a response to the effects of the surrounding biotic and abiotic environment. The presently known secondary microbial metabolites exhibit a wide number of varied and versatile bioactivities. The marine microbes, especially those from extreme environments like salt pans and other hypersaline environments represent a massive under developed resource. In this regard, screening and isolation of large number of potential isolates from diverse environments, facilitates the availability of large number of cultures for the depository and in turn would enable us to obtain better isolates for the production of drugs against specific varied diseases. Such potential and novel marine microbes could then be exploited for large scale production of vital drugs. For achieving the above objectives screening for potential bioactive compounds from these hypersaline marine bacteria; water, sediment and salt crystals were explored.

In all 1178 bacteria were isolated from water, sediment and salt crystals from nine different salt pans of Goa. Colonies appeared on their respective solid media within 24 h of incubation. All isolates except BGUM 59 MC showed luxurious growth on Media D whereas BGUM 59 MC showed better growth on Media C. The culture extracts were sent to various laboratories for testing their respective bioactivities. Isolates were tested, in house, for amylase producing & inhibitory activity as well as protease producing & inhibitory activity. Sixty three cultures were found to produce metabolites of pharmaceutical significance (Refer Figure 1). Details of the culture extracts showing various activities are given in Table 1.

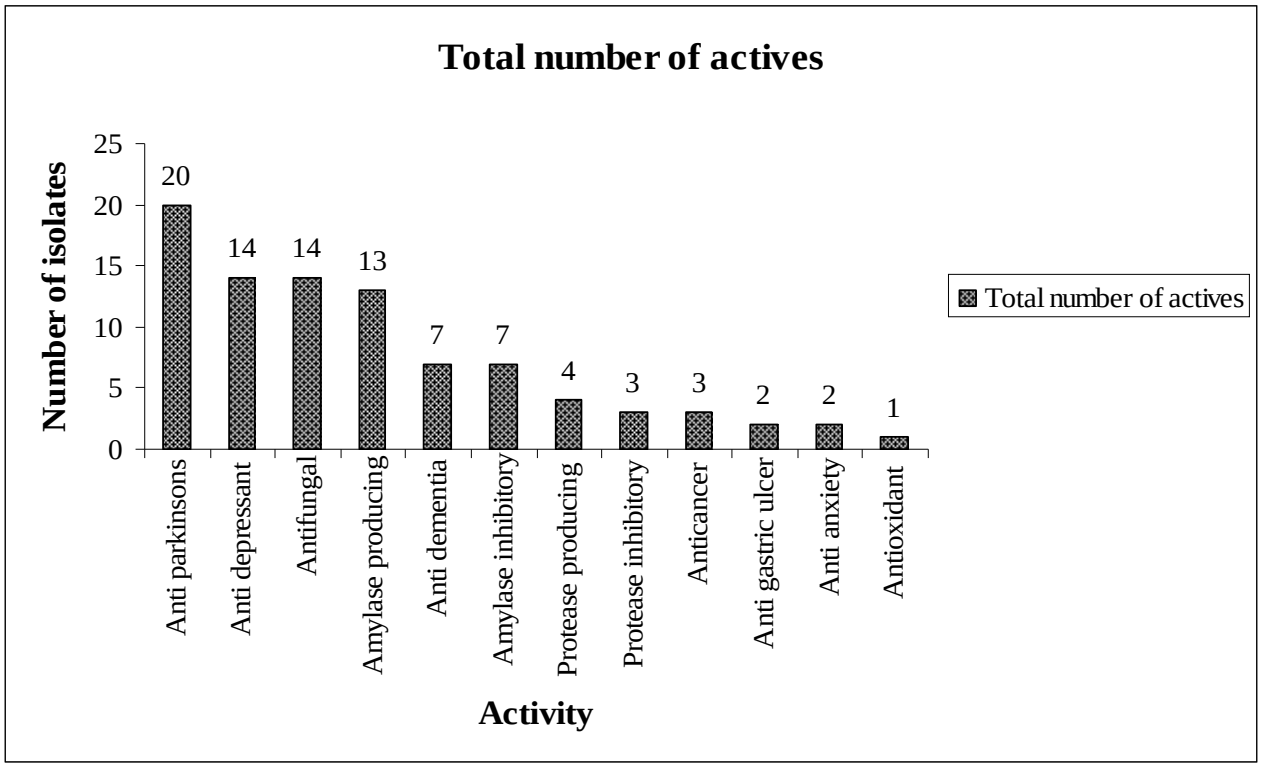

Figure 1: Activity profile of sixty three bacterial isolates.

From the active cultures, a maximum of 32% showed anti Parkinson's activity followed by 22% which showed anti depressant & antifungal

activity, each. Seven cultures (11%) showed anti dementia activity. Three isolates showing anti cancer activity (against colon and uterine cancers) were consistent in their in vitro activity, however in vivo trials are in progress. Two isolates showed anti gastric ulcer activity (against *Helicobacter pylori*) & anti anxiety activity and only 1 culture showed antioxidant activity. When the cultures were tested for enzyme production, 21% i.e. 13 isolates produced amylases while only 6% exhibited protease activity. When tested for enzyme inhibitors 11% produced amylase inhibitors while nearly 5% produced protease inhibitors.

Novel metabolites produced by marine actinomycetes have shown promising antibacterial, anticancer, antifungal, anti inflammatory, anti malarial and neuritogenic activities [4]. Debbab et al. (2010) [5] have reviewed some of the recent new bioactive compounds from marine bacteria and fungi. Bacteria from Weihai solar saltern, China have been screened for antimicrobial and cytotoxic activities [6]. Though there are reports on diversity of microbes from salt pans in India [7,8,9,10] not much is found in literature specifically with respect to bioactive compounds from salt pan isolates. Dhanasekaran et al.(2005) [1] have reported salt pan actinomycetes producing antibacterial activity. *Saccharopolyspora salina* VITSDK4, isolated from a saltpan marine soil sample collected at the Marakkanam coast of the Bay of Bengal, India, was profoundly antagonistic with fungal and Gram positive pathogens [11]. Kamat and Kerkar (2004, 2007) [2,3] have reported a halotolerant *Acinetobacter* sp. from saltpans of Ribandar, Goa producing antibacterial compound.

Activity	Laboratory	Culture No. BGUM_ _ _
Antioxidant	IICT, Hyderabad	009MD.
Anti gastric ulcer	IICB, Calcutta	158MD,159MD.
Antifungal	CIMAP, Lucknow	005MD,006MD,007MD,038MD,047MD,058MD,066MD,072MD,073MD,078MD ,133MD,136MD,165MD,186 MD.
Memory enhancing activity & activity against neurological disorders (i) to (iv)		As detailed below
(i) Anti anxiety	CDRI, Lucknow	837MD,1091MD.
(ii) Anti dementia	CDRI, Lucknow	059MC,262MD,346MD,348MD,370MD,740MD,741MD.
(iii) Anti depressant	CDRI, Lucknow	100MD,107MD,256MD,257MD,264MD,265MD,298MD,327MD,461MD,465MD ,471MD,472MD,473MD,474MD.
(iv) Anti Parkinson's	CDRI, Lucknow	102MD,103MD,109MD,236MD,299MD,305MD,307MD,312MD,313MD,314MD ,315MD,359MD,373MD,375MD,376MD,440MD,457MD,458MD,799MD,806M D.
Anticancer	IIIM, Jammu	014MD,016MD,017MD.
Amylase producing	In house testing	005MD,014MD,016MD,017MD, 059MC,072MD, 078MD,102MD, 236MD,315MD,440MD,465MD,837MD.
Amylase inhibitory	In house testing	109MD,262MD,307MD,314MD,373MD,375MD,799MD.
Protease producing	In house testing	016MD,236MD,264MD,806MD.
Protease inhibitory	In house testing	014MD,465MD,799MD.

Table 1: Details of culture extracts showing various activities

Vidyasagar et al. (2007) [12] have reported *Chromohalobacter* sp. TVSP101 from solar

salterns of Tuticorin, Tamilnadu, India producing halothermophilic protease.

After our preliminary findings, efforts are now in progress to partially purify the active ingredient and confirm the activity. To our knowledge, for the first time in India, such large numbers of halobacteria have been screened for various biological activities. There is a need to continue exploring saltpan ecosystem for more potential cultures, some of which could hold promising solutions to the evolving drug resistance in human pathogens.

[V] CONCLUSION

The present study highlights the biotechnological significance of bacteria from saltpans and emphasizes that these microbes are probably a source for the discovery of novel secondary metabolites.

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