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EXTREMELY HALOPHILIC ARCHAEA AND EUBACTERIA ARE RESPONSIBLE FOR FREE RADICAL SCAVENGING ACTIVITY OF SOLAR SALTS OF GOA – INDIA

Jyothi J Alvares[1] and Irene J Furtado[2]*
[1,2] Department of Microbiology, Goa University, Taleigao Plateau, Goa- India. 403206.
*Corresponding author - ijfurtado@unigoa.ac.in

ABSTRACT
Free radical scavenging activity of natural solar salt of Goa was evaluated by the DPPH method. It ranged between 0.0793 µg AAE /g and 0.145 µg AAE/g. Our results showed that this antioxidant activity of salt was contributed by extremely halophilic *Alteromonas*, *Gluconobacter* and members of haloarcheal genera such as *Halobacterium, Haloferax, Haloarcula, Halococcus, Halorubrum, Natrialba, Natrinema and Natronococcus,* associated with it. This is the first record of *Alteromonas*, *Gluconobacter*, *Natrialba*, *Natrinema*, and *Natronococcus* as free radical scavengers. A positive correlation of R = 0.89, p< 0.001 between the total antioxidant capacity and the phenolic content was observed in five Haloarchaeal genera selected for studies. Among which, *Haloferax* sp. GUSF-1 (KF796625) exhibited % DPPH RSA of 31.5 ± 0.43, total antioxidant capacity of 1.176 ± 0.75 mg AAE/g and phenolic content of 0.784 ± 0.004 mg GAE /g cells. This free radical scavenging potential of solar salts due to presence of these microbes, possibly reflects its use in moderation, as soil conditioner and fertiliser for coastal fruit bearing trees such as C*ocos nucifera, Mangifera indica* and *Artocarpus heterophyllus* in Goa-India.

KEYWORDS – Antioxidant, DPPH, extreme halophiles, Haloarchaea, phenolic content.

INTRODUCTION

Globally, solar salt plays an important role in all forms of life (Aral *et al*., 2004). Goa situated on the west coast of India, is engaged in natural salt production through green process of salt farming for the past 1500 years (Furtado and Fernandes 2009). This solar salt is widely used in moderate quantities as fertiliser and soil conditioner in Goa and in South Asian countries (Mani *et al*., 2012; Magat 2000). Both in biota as well as in the environment, oxidation reactions produce free radicals which are atoms, molecules or ions with unpaired electrons that are highly unstable and charged (Lü *et al*., 2010). These free radicals react with the first available oxidisable substrate causing damage and in case of cells, death. This process is however, averted by antioxidant molecules which scavenge free radicals as- (i) preventers: by preventing formation of free radicals; (ii) scavengers: by removing radicals and halting further propagation of chain reactions and (iii) repairers: which ameliorate oxidative damage to a cell (Lü *et al*., 2010). In recent years, researchers are keen to unveil antioxidant substances from plants, animals, microbes and natural substances with an ability to fight oxidative stress (Yevgenia *et al*., 2013). In the present study, we screened and quantitatively assessed free radical scavenging activity of extremely halophilic microbes retrieved from solar salts of Goa- India (Braganca and Furtado 2009; Fernandes and Furtado 2005; Sequeira 1992).

MATERIALS & METHODS

Chemicals and reagents

All chemicals and reagents used were of analytical grade. 1, 1-diphenyl-2- picrylhydrazyl (DPPH), (Sigma, USA). Ascorbic acid, gallic acid, ammonium molybdate, sodium phosphate, sodium carbonate and Folin- Ciocalteu reagent, (Himedia laboratories, India). Solar salt recovered from salt farming facilities in Goa was used in this study. Methanol and other solvents, sulphuric acid (Sd-fine chemicals, India). Ultrafltration unit M Direct – Q3, Millipore- USA was used for pure water.

Free radical scavenging activity of solar salt

Saturated solutions of each variety of solar salt was prepared by gradually adding sterile ultrafiltered water such that at each addition of water to 40 g of solar salt a super saturation state was maintained throughout the preparation of the super saturated solution of solar salt. Each of the saturated solutions were individually filtered through Whatman No 1 filter paper, and analyzed separately for its ability to decolourize DPPH. $MgSO_2.7H_2O$, $MgCl_2$, NaCl, $CaCl_2.2H_2O$ were also individually analysed for DPPH decolourization. The DPPH decolourization was assayed by a method previously described (Brand-Williams *et al*., 1995). 0.5 ml of individual solutions of solar salt and other salts were added to 1 ml of 0.1 mM methanolic DPPH reagent, mixed vigorously with minimum light exposure, incubated in the dark at room temperature (28 ± 30 ºC), for 30 min and monitored at 517 nm, using the UV- vis spectrophotometer (UV–1601, Shimadzu, Kyoto,Japan) against methanol as blank. The stability of the DPPH solutions was also monitored separately by checking the absorbance of 0.1 mM methanolic solution of DPPH, every 5 min, at 517 nm, for over an hour.

Demonstration of free radical scavenging microbes in solar salt

Extremely halophilic microbes in solar salt

An aliquote of 0.1 ml of the sediment free super saturated solution of solar salt was inoculated in a 50 ml Erlenmeyer flask containing 10 ml of NTYE broth (Braganca and Furtado 2009). The composition of the medium was as follows (g/liter): $MgSO_2.7H_2O$ 20, KCl 5, $CaCl_2.2H_2O$ 0.2, Tryptone 5, Yeast extract, 3; NaCl, 250 and pH adjusted to 7, using 1N NaOH. The flask was incubated at 42 ºC, 150 rpm using an incubator (REMI CIS-24 PLUS-India). After 7 days, an aliquote of 0.1 ml of this culture broth was then spread plated onto NTYE agar plates and incubated at 42 ºC in light, till well developed colonies were seen.

Development of Agar-growth-DPPH method

The fully formed colonies on NTYE agar were very carefully exposed to freshly prepared 0.1, 0.2 and 0.5 mM methanolic DPPH by layering the DPPH solution just to cover the surface of the agar and the colonies on it. Plates were incubated in the dark for 40 min at room temperature (28-30 ^{o}C), and those colonies surrounded by clear colourless/straw colour haloes against a purple background were scored and recorded as positive free radical scavengers.

Screening of free radical scavenging ability of extremely halophilic isolates retrieved from crude solar salt

A total of 101 extremely halophilic microbes isolated from crude solar salt produced in salt pans, located at Arambol (A), Agarwada (Ag), Arpora (Ar), Nerul (N), Ribandar (R) and Siridao (S) in Goa- India, maintained on NTYE agar at the Haloarchaeal repository, Department of Microbiology, Goa University, India (Braganca and Furtado 2009; Fernandes and Furtado 2005; Sequeira 1992). These were spot inoculated onto NTYE agar using a template, allowed to grow at 42 ºC for a period of 7 days and then subjected to Agar-growth-DPPH method.

Determination of free radical scavenging activity of extremely halophilic isolates retrieved from crude solar salt

Culturing and preparation of Methanolic Extract of Cells (MEC)

Each of the above 101 isolates on agar-slopes was inoculated, into a set of three large sterile tubes with 5 ml, of sterile NTYE broth. All tubes were incubated at 150 rpm on the orbital shaker (REMI CIS-24 PLUS- India) at 42 ºC. After 6 days, cells were harvested at 10000 rpm, 4 ºC for 10 min by using an Eppendorf centrifuge 5804 R. Cells washed with 15% NaCl and adjusted to a wet weight of 100 mg were treated with 1 ml of methanol and kept standing at 4 ºC in the dark, overnight and recovered by centrifuging at 10000 rpm, for 10 min and the resulting MEC stored in the dark at 4 °C.

Evaluation of free radical scavenging efficiency of MEC

Evaluation of free radical scavenging activity was carried out by a method of Brand-Williams *et al*., 1995. The degree of decolourization by cell extracts of individual isolates was monitored by measuring changes in the absorbance at 517 nm. Ascorbic acid (0.1%) was used as the positive control and also as the standard for the calibration curve. Experiments were carried out in triplicates. Results averaged and expressed as mean ± SD of ascorbic acid equivalent µg AAE/ g cells.

Computation of % DPPH RSA

% DPPH RSA was computed using the generalization;

% DPPH RSA = $(A_{control}–A_{test})/A_{control}) \times 100$,

where $A_{control}$ is the absorbance of control and A_{test} is the absorbance of the crude MEC. Results were expressed as mean ± SD of % DPPH RSA.

Statistical analysis

The experimental data of DPPH RSA was analysed using IBM-SPSS 23 statistical software. Analysis of variance and significant differences among means of different isolates were performed with one-way analysis of variance (ANOVA).

Identification of extremely halophilic isolates retrieved from crude solar salt

Morphological characterization

Eighty four isolates from different solar salt samples, testing positive for free radical scavenging activity were individually subjected to various tests. Gram staining using acetic acid fixed smears was performed by a method previously reported (Khandavilli *et al* 1999).

Biochemical analysis

Isolated and purified cultures were pre-grown on NTYE agar slants for 6 days at 42 ^{o}C. l ml suspension of each of the isolates was made in sterile 20% NaCl. 3 ml of Norberg & Hofstein's media 1969, supplemented with 20% NaCl, containing 0.1% phenol red was distributed into test tubes and sterilized (Norberg and Hofstein 1969). Each of the sugars (10%) were sterilized separately and an aliquot of 0.5 ml was added to test tube containing the media just before inoculating 100 µl of the inoculum. Growth and colour change was observed visually. The change in colour from yellow to red was recorded as indicative of acid production.

Chemotaxonomic analysis

Pigment analysis of cells

All eighty four isolates were individually grown at 42 °C, in NTYE broth shake culture. Cultures were centrifuged at 10000 rpm using a cooling centrifuge (Eppendorf centrifuge 5804 R), at 4 °C, for 20 min. Pellet was washed twice with 20% NaC1 solution. Cellular pigment was extracted in methanol using sonicator (B. Braun Biotech International, USA) at 0.7cycles/sec and the methanolic extract were scanned between 190- 800 nm.

Evaluation of the presence of glycerol diether moieties (GDEM) in cells

Glycerol diether linked lipids (GDEM) were extracted from cells by the method previously described (Ross *et al*., 1985). Inorder to reflect the phenotypic relationship between the retrieved isolates, dendograms were generated using the individual characteristics of every isolate recorded and computed to attain a hierarchical rearrangement of cultures through paired UPGMA, based on Jaccard distance using the software PAST3 v.1 and expressed as a phenogram.

Total antioxidant capacity of select haloarchaeal genera

Five isolates namely, GUSF-1 *Haloferax* sp. (KF796625), $GUFF_{188}$ *Haloarcula* sp. $GUFF_{179}$ *Halococcus* sp. $GUFF_{72}$ *Halorubrum* sp. and $GUFF_{233}$ *Natronocccus* sp. were selected assessment of total antioxidant capacity by the method previously described (Prieto *et al*., 1999). Ascorbic acid was used as a positive control and also as the standard. Experiments were carried out in triplicates, results averaged and expressed as ascorbic acid equivalent (mg AAE/g cells).

Total phenolic content of select haloarchaeal genera

The total phenolic content was determined spectrophotometrically as previously described (Singleton and Rossi 1965). Gallic acid was used as a positive control also as the standard for the calibration curve Experiment was carried out in triplicates, results averaged and the content of phenolics in the extracts was expressed in terms of gallic acid equivalent (mg GAE/g).

Statistical analysis

The correlation between antioxidant activity and phenolic content for five promising cultures was analysed using Pearson's correlation test (Pearson 1900).

Diversity studies

The generic level occurrence and diversity of extremely halophilic eubacteria and haloarchaea associated with solar salts from different locations of Goa were calculated as:

a) Simpson's Index of Diversity D' (Simpson 1949).

The index of extremely halophilic eubacteria and haloarchaea was calculated by applying the generalization:

$D' = N(N-1)/\Sigma n(n-1)$

D' = Diversity index

n = number of individuals of each genera found in solar salt of a salt pan

N = Total number of individual of all genera found in solar salt of a salt pan

b) The generic diversity in community was carried out by Shannon-Weiner's Diversity Index H' by using the generalization (Shannon *et al*.,1949);

$H' = \Sigma [(n/N)\ln(n/N)]$;

Where n=number of individual isolates; N= total number of all isolates.

RESULTS & DISCUSSION

Antioxidant activity of solar salt

Solar salts from six different salt pans, namely Arambol (A), Agarwada (Ag), Arpora (Ar), Nerul (N), Ribandar (R) and Siridao (S) could be categorized visually by the naked eye into: white with greyish tinge and the other white with brownish tinge. The tinge of colours was merely due to the colour of the soil of evaporation pans, wherein sea water is allowed to stagnate during solar salt crystallization stage. The conventional, well tested and highly employed method of DPPH was used to quantify the antioxidant ability of solar salt and of the extremely halophilic microbes retrieved from it. Saturated solutions of brownish solar salt and the greyish solar salt with a methanolic solution of DPPH resulted in decolourization of the purple colour of DPPH with a corresponding drop in absorbance at 517 nm. A drop in absorbance at 517 nm observed for the solar salt with the greyish tinge was 0.063 nm corresponding to 0.0793µg AAE / g, whereas for the solar salt with a brownish tinge was 0.083 nm corresponding 0.145µg AAE / g when compared to the DPPH solution whose absorbance did not decline over a period of 1 hour. This clearly indicated that solar salt had ability to scavenge free radicals and hence possessed antioxidant activity. This is the first time that free radical scavenging activity is checked and reported directly using solar salt. As the Goan crude solar salt is prepared through natural fractional crystallisation it essentially consists of NaCl with traces of $MgCl_2$ (Mani *et al*., 2012). Individual solutions of $MgSO_2.7H_2O$, $MgCl_2$, NaCl and $CaCl_2.2H_2O$ however failed to decolourize DPPH reagent.

Demonstration of free radical scavenging microbes in solar salt

Retrieval of extremely halophilic microbes from solar salt

Aliquots of saturated solutions of solar salt when spread out onto nutrient rich agar with 25% NaCl and incubated at 42 ^{o}C, developed into colonies in 8 days. As recorded in figure (1A) a variety of colonies grew ranging from pin heads to 1-1.5 mm in size, butyrous to dry, with even and uneven margins, colourless and others having yellow, orange and red pigmentation, grew within a week on NTYE, but none on TYE agar which did not have any NaCl. The absolute requirement of high concentration of NaCl for growth suggested that growth was either of members of heterotrophic halobacteria or of extremely halophilic eubacteria, known to dominate hypersaline econiches having salts upto saturation concentration (Grant and Larsen 1989). Such microbial growth has been reported from solar salts of Goa (Aguiar and Furtado 1996; Braganca and Furtado 2009; Fernandes and Furtado 2005; Khandavilli *et al*., 1999; Sequeira 1992).

Scoring of growing colonies as free radical scavenger

Careful exposure of these bacterial colonies to methanolic DPPH directly, while still on agar resulted in some of the colonies exhibiting a light yellow halo around themselves, against a purple colour of DPPH retained by the agar (Fig. 1B) thus indicating the ability of some colonies to decolourize DPPH and others not. Such DPPH decolourization by microbial cultures has been conventionally evaluated in cultures, by spraying with DPPH, a Whatman filter paper, having the replicate of growth on agar plate (Velho-Pereira *et al*., 2015). The decolourization of DPPH, herein observed by us of colonies on agar is taken as an indicator of free radical scavenging potential and a measure of antioxidant activity of the corresponding colony, similar to that observed with the filter paper replicates. This method, herein referred by us as 'Agar-growth-DPPH method', is direct, easy and gives easy reproducible antioxidant scoring efficiency. Hence, the antioxidant potential of solar salt was not an attribute of its chemical constituents, but of the microbes associated with it (Fig. 1A and 1B). These results support and confirm our presumption that tons of locally produced Goan solar salt, used as soil conditioner and fertilizer carried the free radical scavenging potential.

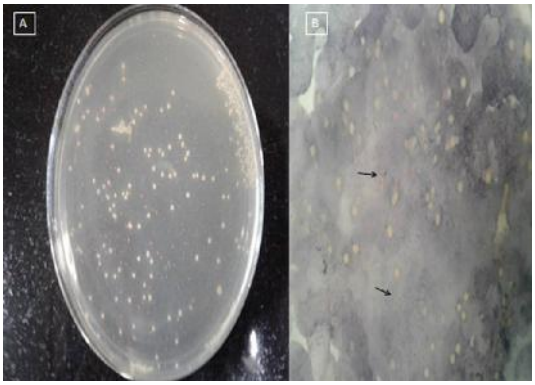

FIGURE 1A: Growth on NTYE agar on plating aliquot of saturated solution of solar salt.
FIGURE 1B: Arrows indicating colonies showing free radical scavenging activity with a halo around it, against a purple stained NTYE plate.

Screening for free radical scavenging activity among extremely halophilic isolates from solar salt/ brine isolates of Goa

Having established that the free radical scavenging ability of solar salt is associated with microbes growing in nutrient rich medium with 25% NaCl, we screened out 101 extremely halophilic microbes retrieved during earlier studies for their ability to scavenge free radicals. Eighty four of these isolates gave white-yellow zones around their colonies, by the 'Agar- growth – DPPH' method and were recorded as free radical scavengers. Analysis of MEC of each of these 84 extremely halophilic isolates further confirmed their ability to decolourize the purple coloured solution of DPPH to varying shades of purple, with a corresponding decrease in absorbance at 517 nm. As recorded in figure (2)**,** the degree of DPPH decolourization, varied in isolates retrieved from different solar salts. Further as recorded in figure (2) the % DPPH RSA ranged from a minimum of 6.06 ± 0.19 exhibited by the isolate coded as $GUFF_3$ to a maximum of 31.5 ± 0.43 shown by GUSF-1. The isolate GUSF-1 from Siridao salt gave the highest activity and was followed by $GUFF_{188}$, $GUFF_{179}$, $GUFF_{66}$, and $GUFF_{72}$ of Arambol, Arpora, Siridao, Ribandar respectively. A low activity of less than 10% was shown by 7 ($GUFF_{20,35,88,90,146,148\ 187}$), 1 ($GUFF_{95}$), 3 ($GUFF_{26,98,51}$), 4 ($GUFF_{28,56,82,83}$), 5 ($GUBF_{2,3}$, $GUFF_{3,61,168}$) and 2($GUFF_{36,76}$) obtained from Arambol, Agarwada, Arpora, Nerul, Ribandar and Siridao respectively. A highly significant variation ($F = 563.627$; $p<0.001$) of antioxidant values was observed within the free radical scavengers from different salt samples as per the one-way ANOVA obtained using the IBM SPSS software.

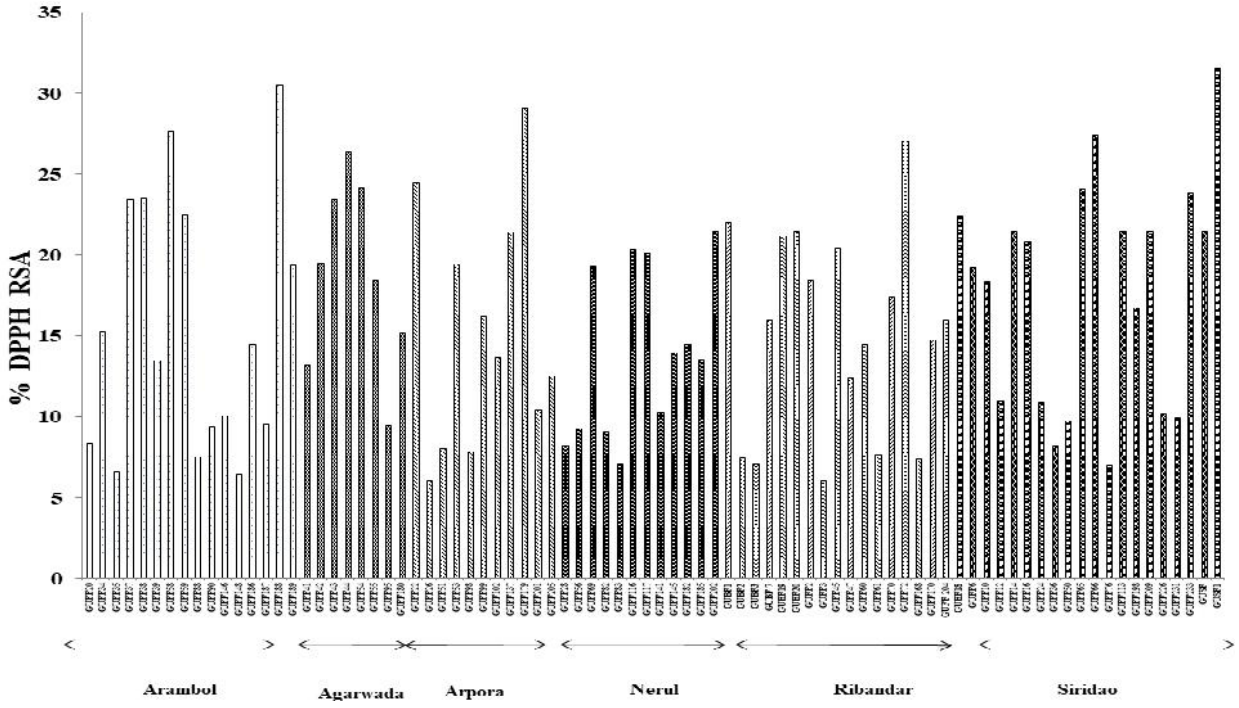

FIGURE 2: Decolourization of DPPH by methanolic extracts of individual extremely halophile isolates retrieved from different solar salts of Goa.
Isolate code: GUB/F/SF (Goa University/ Braganca/Fernandes/Sequeira Furtado).

Genera level identification of free radical scavengers retrieved from solar salts.

Eighty four extremely halophilic microbial isolates capable of scavenging free radical were assigned to their taxonomic domains and genera. Chromatograms of hexane extracts of methanolysates of cells of 78 (93%) isolates gave distinct spot at Rf 0.2, corresponding to that of GDEM's, a characteristic of cellular core lipid in cells of Archaea (Fig. 3). Additionally, these 78 isolates were resistant to penicillin G, erythromycin and cyclohexamide,

known to inhibit bacteria and eubacteria, but not archaea. Halobacteria are also known for its resistance towards many antibiotics especially those of cell wall and protein synthesis (Purdy et al., 2004). Further the absolute requirements of high salt concentration of NaCl for growth and extreme sensitivity to bile salts affirmed the 78 isolates belonged to haloarchaea of Domain Archaea (Purdy et al., 2004). Bile salts has been known to cause lysis of halobacteria due to it's presence of high concentration of taurine conjugates of cholic acid (Purdy et al., 2004). Core lipids are completely absent in eubacteria and eukarya (Purdy et al., 2004). The remaining 6 isolates did not posess GDEM's, grew on 0-25% NaCl and showed resistance to bile salts. As GDEM is the main key for assigning affiliation of domains to isolates (Purdy et al., 2004). The former group of 78 isolates were assigned to the domain Archaea**,** and the latter group of six isolates were assigned to domain Eubacteria.

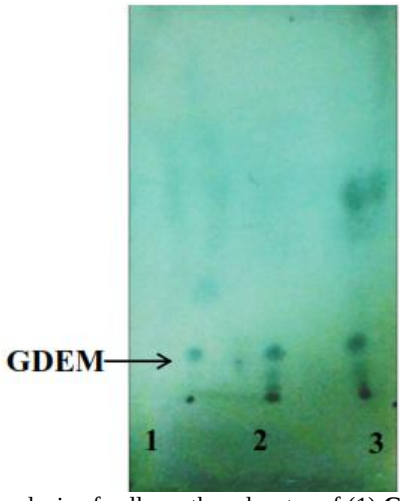

FIGURE 3: Thin-layer chromatographic analysis of cells methanolysates of (1) **GUSF-1** (2) **$GUFF_{233}$** (3) **$GUFF_{72}$**

These six eubacterial isolates, were gram negative and they were all classified to belong to Phylum Proteobacteria (Holt *et al*., 1994). Based on their biochemical characteristics, (Table 1), $GUFF_{76,83,95}$, were assigned to the class *Alphaproteobacteria*, order *Rhodospirillales*, family *Acetobacteraceae* and genera *Gluconobacter*. Because $GUFF_{141,145,146}$ did not grow on pyruvate and arginine hence referred to class *Gamaproteobacteria*, order *Alteromonadales*, family *Alteromonadaceae* and genus *Alteromonas*. The statistical software PAST3 v.1 also confirmed the biochemical differences as represented in the phenogram (Fig. 4) and sorted them as *Alteromonas* and *Gluconobacter* respectively. As yet there are no reports available on free radical scavenging activity within these two genera (*Alteromonas* and *Gluconobacter*). Hence, this is the first time free radical scavenging activity is reported in these two genera by us. However, such activity has been reported in *B. cereus* isolate from salt pan soil samples (Venkatajalapathi *et al*., 2016).

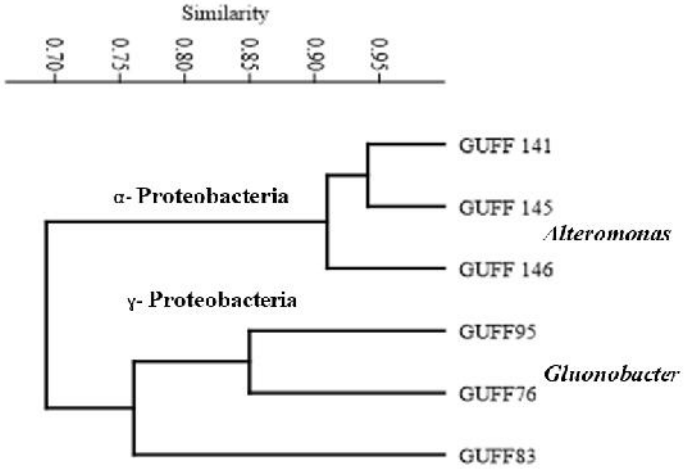

FIGURE 4: Phenogram depicting the sorting of eubacterial isolates to their respective genera based on biochemical characteristics using keys of Bergey's Systematic Bacteriology and the software **PAST3 v.1.**

TABLE 1. Free radical scavenging activity and biochemical potentials of extremely halophilic free radical scavengers from different solar salt samples

Solar Salt sample	Extreme halophilic isolates (GU)	Free radical scavenging activity	GDEM	Pigmentation	Gram character	Morphology					pH		Mg^{+2}		Biochemical potentials																				Genera
		µg AAE g^{-1} cells				Cocci (Co)	Cups (C)	Rods (R)	Triangles (T)	Pleomorphic (P)	Squares (S)	7	10	10 mM	790mM	Gelatinase	Protease	Lipase	Amylase	Mannitol	Glucose	Sucrose	Fructose	Galactose	Lactose	Arabinose	Citrate	Acetate	Malate	Pyruvate	Succinate	Lactate	Formate	Arginine	
	FF_{41}	105.8 ± 0.18	+	R	+	-	C	-	-	P	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	*Haloferax* sp.1
	FF_{42}	156.0 ± 0.24	+	O	+	-	C	-	-	P	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	+	*Haloferax* sp.2
	FF_{43}	188.1 ± 0.3	+	O	+	-	C	-	-	P	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+	*Haloferax* sp.3
	FF_{44}	211.6 ± 0.28	+	R	+	-	C	-	-	P	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	*Haloferax* sp.4
A	FF_{54}	193.8 ± 0.2	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.1
	FF_{55}	148.0 ± 0.48	+	O	-	-	-	R	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halobacterium* sp.1
	FF_{95}	76 ± 0.008	-	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	*Gluconobacter* sp.1
	FF_{180}	121.8 ± 0.16	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.2
	FF_{20}	64.2 ± 0.64	+	Y	-	-	C	-	-	P	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	*Haloferax* sp.5
	FF_{34}	122 ± 0.04	+	O	-	-		-	Tr	P	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Haloarcula* sp.1
	FF_{35}	52.8 ± 0.016	+	Y	-	-	-	R	-	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	*Halobacterium* sp.2
Ag	FF_{17}	187.5 ± 0.36	+	O	-	-	-	R	-	P	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-	-	+	*Natrialba* sp.1
	FF_{18}	188 ± 0.56	+	O	-	-	-	C	-	P	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+	+	*Haloferax* sp.6
	FF_{39}	107.6 ± 0.2	+	R	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	-	+	+		*Halococcus* sp.3
	FF_{84}	221.2 ± 0.5	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.4
	FF_{59}	180 ± 1.2	+	O	+	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.5
	FF_{88}	60 ± 0.14	+	O	-	-	-	R	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halobacterium* sp.3
	FF_{80}	74.4 ± 0.28	+	O	-	-	-	-	-	P	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halorubrum* sp.1
	FF_{146}	80.4 ± 0.62	-	R	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	*Alteromonas* sp.1
	FF_{148}	51.6 ± 0.02	+	P	-	-	-	R	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halobacterium* sp.4
	FF_{186}	115.4 ± 0.42	+	P	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Haloarcula* sp.2
	FF_{187}	76.4 ± 0.18	+	O	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Haloarcula* sp.3
	FF_{188}	243.8 ± 0.4	+	O	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Haloarcula* sp.4
	FF_{189}	155 ± 0.14	+	C	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Haloarcula* sp.5
	FF_{22}	196 ± 0.36	+	O	-	-	C	-	-	-	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	*Haloferax* sp.7
	FF_{26}	48.5 ± 0.04	+	Y	+	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.6
	FF_{51}	64.3 ± 0.1	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.7
	FF_{53}	155.5 ± 0.16	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.8
	FF_{98}	62.8 ± 0.2	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	*Halococcus* sp.9
Ar	FF_{99}	130.1 ± 0.36	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.10
	FF_{102}	109.5 ± 0.12	+	O	-	Co	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.11
	FF_{137}	171.5 ± 0.24	+	O	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	*Haloarcula* sp.6
	FF_{179}	233.4 ± 0.52	+	C	-	Co	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Halococcus* sp.12
	FF_{201}	83.3 ± 0.5	+	C	-	Co	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Halococcus* sp.13
	FF_{205}	100.5 ± 0.2	+	O	-	Co	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	*Halococcus* sp.14
	FF_{28}	65.6 ± 0.26	+	Y	-	Co	-	-	-	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-	*Halococcus* sp.15
N	F_{56}	74.3 ± 0.09	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.16
	FF_{69}	156 ± 0.6	+	O	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.17
	FF_{82}	36.5 ± 0.02	+	C	-	-	R	-	-	-	-	-	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	*Natrinema* sp.1

	FF_{83}	57 ± 0.02	-	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	*Gluconobacter* sp.1
	FF_{116}	183.5 ± 0.14	+	R	-	Co	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.18
	FF_{117}	161.5 ± 0.06	+	R	-	Co	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.19
	FF_{141}	82.3 ± 0.24	-	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	*Alteronomonas* sp.2
	FF_{145}	111.9 ± 0.19	-	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Alteronomonas* sp.3
	FF_{182}	115.9 ± 0.16	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.20
	FF_{185}	108.7 ± 0.15	+	P	-	-	-	-	Tr	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	*Haloarcula* sp.7
	FF_{202}	182.5 ± 0.64	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.21
	BF_1	176.5 ± 0.16	+	RO	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	-		*Haloferax* ATCC BAA 644
	BF_2	59.6 ± 0.1	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+		*Haloferax* ATCC BAA 645
	BF_3	56.8 ± 0.02	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+	+	-		*Haloferax* ATCC BAA 646
	BF_7	130.3 ± 0.12	+	O	-	-	-	R	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Haloarcula* sp.8
	BF_{19}	193.4 ± 0.08	+	RO	-	-	-	R	-	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+	+	+		*Halobacterium* sp.5
R	BF_{20}	172 ± 0.82	+	RO	-	-	-	R	-	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	-	-	-	-	+	+	+		*Halobacterium* sp.6
	FF_2	148 ± 0.16	+	O	+	C	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.22
	FF_3	48.5 ± 0.08	+	Y	-	-	-	-	Tr	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Haloarcula* sp.9
	FF_{45}	164 ± 0.08	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	*Halococcus* sp.23
	FF_{47}	99.6 ± 0.06	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.24
	FF_{60}	115.9 ± 0.34	+	P	-	-	-	R	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halobacterium* sp.7
	FF_{81}	61.2 ± 0.074	+	O	-	-	-	R	-	-	-	+	-	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halobacterium* sp.8
	FF_{70}	139.5 ± 1.32	+	SP	-	Co	-	-	-	-	-	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.25
	FF_{72}	56.4 ± 0.12	+	R	-	Co	-	-	-	-	-	+	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halorubrum* sp.2
	FF_{168}	59.4 ± 1.3	+	C	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	*Halococcus* sp.26
	FF_{170}	118 ± 0.02	+	O	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	*Halococcus* sp.27
	FF_{204}	111.8 ± 0.08	+	C	-	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	*Halococcus* sp.28
	BF_{18}	211.8 ± 1.24	+	O	-	-	-	R	-	-	-	+	-	+	-	+	+	+	+	-	+	+	+	+	+	-	-	+	-	+	+	+		*Halobacterium* sp.9
	FF_8	154.2 ± 0.14	+	O	+	C	-	-	-	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.29
	FF_{10}	147 ± 0.14	+	P	-	-	C	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Haloferax* sp.8
	FF_{12}	88.2 ± 0.57	+	C	-	-	-	R	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	*Natrialba* sp. 2
	FF_{14}	172 ± 0.3	+	O	+	Co	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.30
	FF_{16}	166.6 ± 0.6	+	O	-	-	-	-	Tr	-	-	+	-	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	*Halorcula* sp.10
	FF_{29}	87.2 ± 0.4	+	O	-	-	C	-	-	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	*Haloferax* sp.9
	FF_{36}	65.8 ± 0.52	+	O	-	-	-	-	Tr	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	*Halorcula* sp.11
	FF_{50}	78 ± 0.54	+	P	-	Co	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.31
	FF_{65}	192.6 ± 0.34	+	O	+	Co	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.32
S	FF_{66}	219.8 ± 0.016	+	O	+	Co	-	-	-	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	*Halococcus* sp.33
	FF_{76}	56.16 ± 0.16	-	C	-	Co	-	-	-	-	-	+	-	+	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	-	-	-	+	*Gluconobacter* sp.3
	FF_{113}	172 ± 0.52	+	O	+	Co	-	-	-	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.34
	FF_{198}	133.9 ± 0.26	+	P	+	Co	-	-	-	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.35
	FF_{209}	171.8 ± 0.22	+	W	+	Co	-	-	-	-	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halococcus* sp.36
	FF_{226}	81.5 ± 0.08	+	C	-	-	-	R	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	*Natrialba* sp.3
	FF_{231}	79.6 ± 0.12	+	C	-	-	-	R	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	*Natrialba* sp.4
	FF_{233}	191 ± 0.12	+	R	-	Co	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Natronococcus* sp.1
	SF	180 ± 0.36	+	O	-	-	-	R	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Halobacterium* sp.10
	SF-1	252.2 ± 1.14	+	O	-	-	C	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*Haloferax sp.* (KF796625)

- Isolate GU ; Goa University
- Solar salt samples; A- Arambol, Ag- Agarwada, Ar- Arpora, N- Nerul, R-Ribandar, S- Siridao.
- GDEM + present, - absent
- Colony pigmentation; C- cream, O- orange, R – red, RO- red orange, P – peach, SP- salmon pink, W- white, Y- yellow ;
- Gram character (+) positive (purple), (-) negative (pink).
- Morphology; Co- cocci,, C-cups, R- rods, P – pleomorphic, Tr- triangles, S –squares.
- Biochemical trait; + positive , - negative

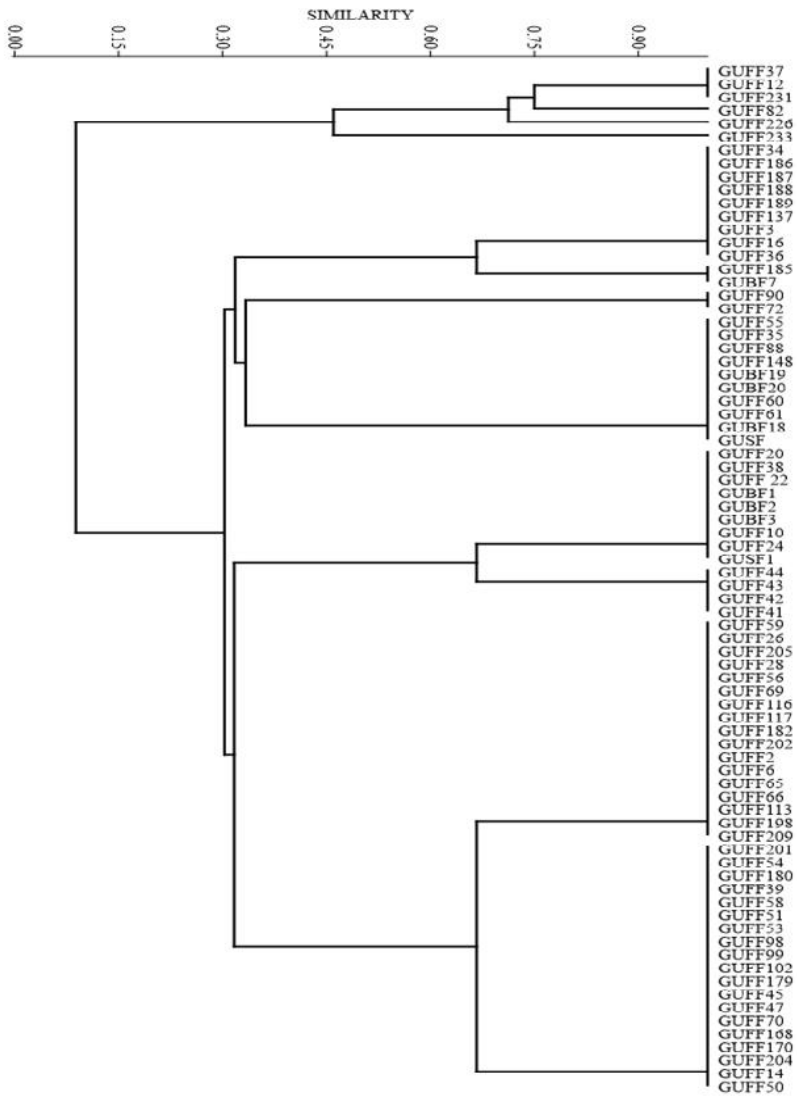

FIGURE 5: Phenogram depicting the sorting of Haloarchaeal isolates to their respective genera based on morphology, pH and Mg^{+2} ion requirements using the software **PAST 3.v.1**.

The 78 isolates belonging to the domain Archaea had a salt requirement above 15% wt/vol for growth and 25% for optimum growth. These were considered as extreme halophiles and hence were referred to Phylum Euryarchaeata, class *Halobacteria*, order *Halobacteriales*. Based on phenotypic test used were in accordance to minimal standards proposed for describing and assigning taxa in the order *Halobacteriales* (Oren *et al*., 1997). Based on gram character, cellular morphology, requirement of pH and Mg^{+2} ions (Table.1), the isolates from different salt samples were sorted out into eight genera; *Halobacterium*, *Haloferax*, *Haloarcula*, *Halococcus*, *Halorubrum*, *Natrialba*, *Natrinema*, *Natronococus* as in (Fig. 5) (Oren, A. 2012),. **$GUFF_{233}$** genus *Natronococcus*: Cells were non motile, cocci, arranged in irregular clusters, cells did not lyse in distilled water, utilized sucrose, fructose, glucose, acetate and lactose as sole source of carbon, had phosphatidyl glycerol and phosphatidyl glycerol phosphate methyl esters as polar lipids, grew at pH 10.5. The strain utilised citrate and resembled *Natronococcus* reported from Soda Lake (Roh *et al*., 2007). **$GUFF_{82}$** genus *Natrinema*: Colonies orange red, smooth circular, cells rod shaped, turned pleomorphic cells lysed at 1.5 M NaCl, Liquefied gelatin and did not hydrolyse starch. The isolate grew in the pH range of 6.0-8 (McGenity et al., 1998). **$GUFF_{12,\ 37,\ 231,\ 233}$** genus *Natrialba*: colonies were weakly cream to peach. Cells gram positive rods grew on media with NaCl upto 25% and pH 10.5 (Xu *et al*., 2001). **$GUFF_{72,\ 90}$** genus *Halorubrum*: Colonies orange red cells gram negative rods. Some cultures pleomorphic. Growth in medium containing 15-30% NaCl, pH 5-9 with optimum growth at pH 7. Starch, gelatin and casein were not hydrolysed. Arginine was not used as a sole source of carbon, nitrogen or energy, susceptible to bacitracin and novobiocin and hence resembled the *Halorubrum* isolated from saline soils (Ventosa *et al*., 2004). **$GUBF_{1,\ 2,\ 3,}$ $GUFF_{10,\ 20,\ 22,\ 24,\ 38,\ 41,\ 42,\ 43,\ 44}$ GUSF-1** genus *Haloferax*: cells gram-negative and

cup shaped. Cells lyse immediately in distilled water, isolates could tolerate salt as low as 1.0 M NaCl and high as 5.2 M NaCl, optimum pH for growth 7, resistant to rifampicin (Elshahed *et al*., 2004). **GUBF$_{18,19,20,}$ GUFF$_{35,55,60,61,88,148}$ GUSF** genus *Halobacterium*: cells gram negative and slender rods Colonies on agar plates containing 25% (w/v) total salts were red, elevated and round. pH for growth was 5.5–8.5, salt tolerance was from 2.7 to 5.2 M NaCl (Yang *et al*., 2006).

GUFF$_{2,6,14,26,28,39,45,47,50,51,53,54,56,58,59,,65,66,69,70,98,99,102,113,116,117,168,170,179,180,182,198,201,202,204,205,209}$ genus *Halococcus*: Cells were gram negative and coccoid. Colonies smooth-surfaced with clear edges. Susceptible to rifampicin and bacitracin (Wang *et al*., 2007). **GUBF$_{7}$, GUFF$_{3, 16, 34, 36, 137, 185, 186, 187, 188, 189}$** genus *Haloarcula*: cells were flat disk-shaped, round or triangular grew on simple carbon sources (glucose, fructose, sucrose, glycerol, acetate, succinate, and malate).Susceptible to bacitracin and novobiocin. These isolates resembled those isolated from the Dead Sea (Oren *et al*., 1990).

Diversity studies

Occurrence of free radical scavenging haloarchaea belonging to different genera differed from solar salt to solar salt (Fig. 6). A maximum of 6 genera were found in Agarwada, while a minimum of three haloarchaeal genera were found in Arambol, Arpora and Nerul. None of the solar salts had isolates belonging to all the 8 retrieved genera. Members of genus *Natrialba* were found in Agarwada and Siridao solar salts while *Natrinema* in Nerul and *Natronococcus* in Siridao. The frequency of ocurrence of free radical scavenging members was: *Halococcus* > *Haloarcula* = *Haloferax* > *Halobacterium* > *Halorubrum* = *Natrialba* > *Natrinema* = *Natronococcus*. Eubacteria retrieved although extremely halophilic was 10 times less than that of haloarchaea and was restricted to genus *Gluconobacter* and *Alteromonas*. Further, as accounted in (Fig. 6), the distribution of the two genera in Goan solar salts was very unequal. In Nerul members of *Gluconobacter* was 50% of *Alteromonas*. Siridao and Arambol had only *Gluconobacter*, whereas Agarwada had only *Alteromonas*. Antioxidation activity is widely distributed among members of eubacterial and archaeal domain retrieved from solar salt of Goa.

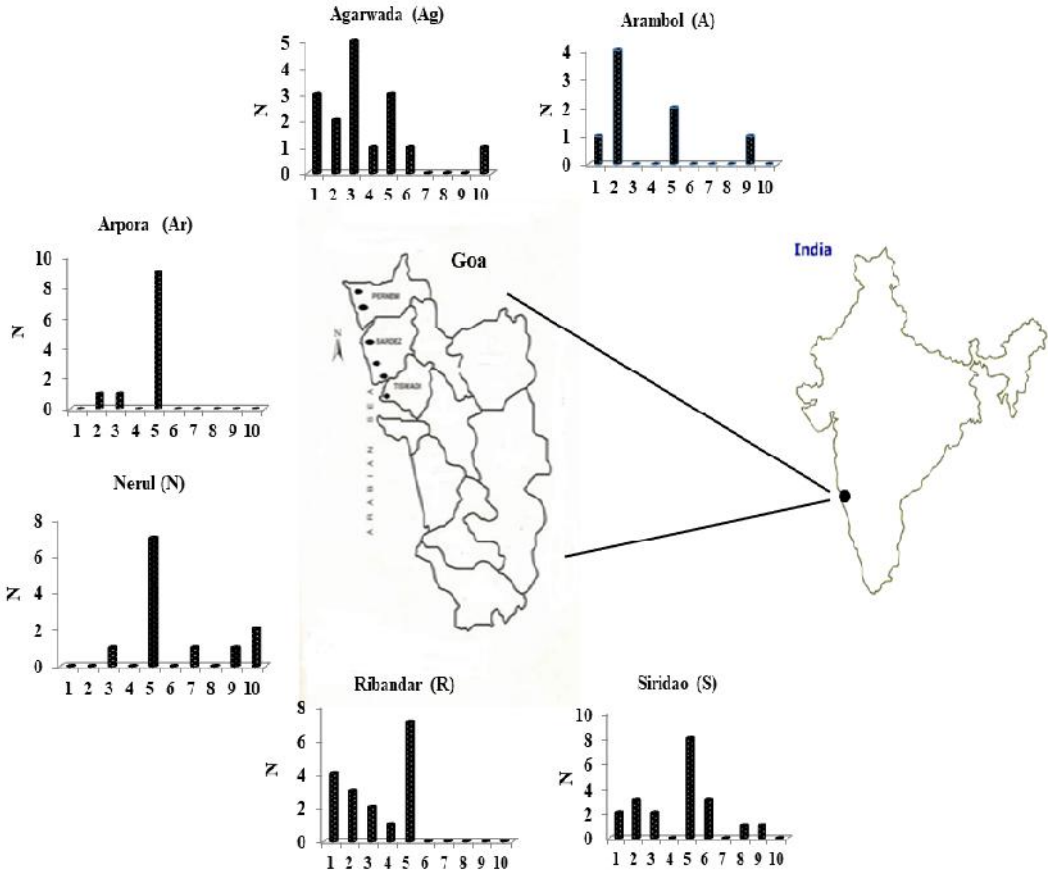

FIGURE 6: Number of free radical Haloarchaeal scavengers retrieved from solar salts of Goa:
A- Arambol; Ag- Agarwada; Ar -Arpora; N- Nerul; R- Ribandar; S- Siridao obtained from different geographical location of Goa- India.
X axis values indicate different retrieved genera; **1.** *Halobacterium*, **2.** *Haloferax*, **3.** *Haloarcula*, **4.** *Halorubrum*, **5.** *Halococcus*, **6.** *Natrialba*, **7.** *Natrinema*, **8.** *Natronocccus*, **9.** *Alteromonas*, **10.** *Gluconobacter*.
Y –axis indicate number of isolates present **(N)**.

Members of *Alteromonas* and *Gluconobacter* engaged in free radical scavenging as seen in Nerul (Fig. 7A) as per Simpson's Index of diversity whereas the diversity of haloarchaea associated with solar salts is Siridao> Agarwada= Ribandar>Arambol > Arpora >Nerul. The distribution of as per Shannon- Weiner's diversity index H' was Nerul > Arambol = Agarwada = Siridao. The diversity of haloarchaea (Fig. 7B) associated with solar salts as per Shannon-Weiner's Diversity Index H' was Agarwada > Ribandar > Siridao > Arpora> Arambol> Nerul. Only in the case of Ribandar, Shannon-Weiner's Diversity Index H' and Simpson's Index of Diversity, D' were nearly equal indicating that the dominance was in the following order; Siridao > Agarwada > Ribandar > Arambol > Arpora > Nerul. The antioxidant potential of eubacteria and haloarchaea associated with Goan solar salt is a reflection of constant exposure of these cultures to sunlight and radiations which is reported to trigger the formation of free radicals and reactive oxygen species (Abbes *et al*.,2013; Mandelli *et al*., 2012; Rodrigo-Baños *et al*., 2015; Sikkandar *et al*., 2013). (Pathak and Sardar 2012) have reported the antioxidant activity from *Halorubrum* sp. isolated from brine samples of solar salterns of Mumbai also evaluated through the DPPH assay.

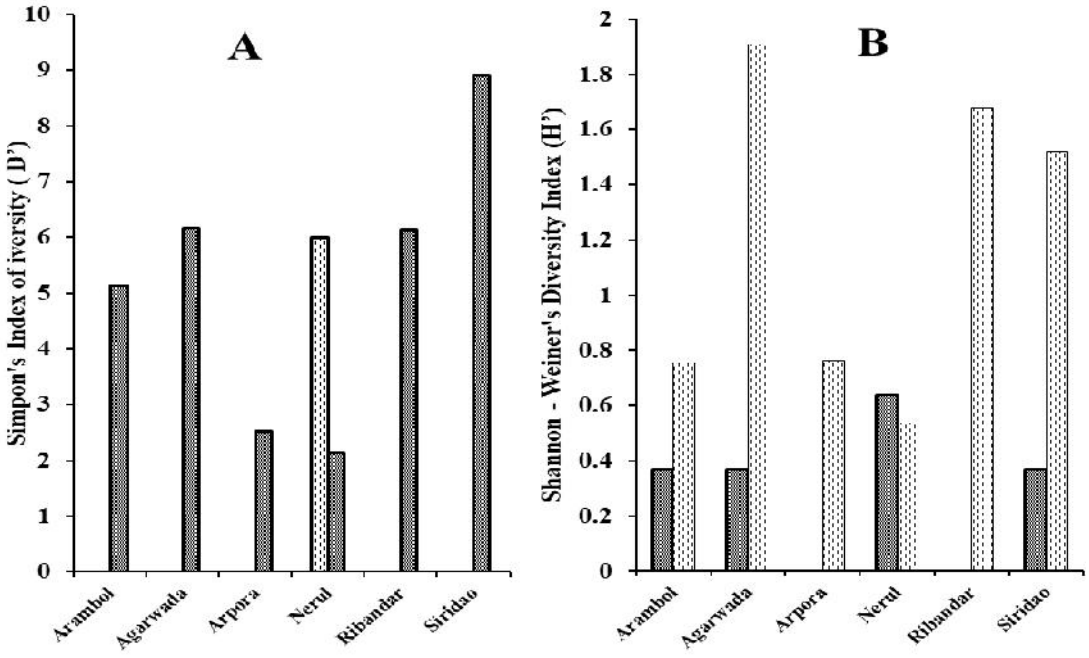

FIGURE 7: Diversity index, **A-** Simpson's Index of Diversity 'D': Eubacteria ▨, Haloarchaea ■
B - Shannon- Weiner's Diversity Index 'H': Eubacteria , Haloarchaea ▨

Total antioxidant capacity and Total phenolic content.
GUSF-1 *Haloferax* sp. (KF796625), $GUFF_{188}$ *Haloarcula* sp. $GUFF_{179}$ *Halococcus* sp. $GUFF_{72}$ *Halorubrum* sp. and $GUFF_{233}$ *Natronocccus* sp. were assessed for further analysis of total antioxidant capacity and total phenolic content. Addition of MEC (with absorption peaks between 300-600 nm) of each of the five cultures to phosphomolybdenum reagent, resulted in development of a bluish greenish colouration readable at 695 nm and was accompanied by the disappearance of MEC absorption peaks between 300-600 nm, thus confirming the involvement of MEC in free radical scavenging through reduction of Mo (VI) – Mo (V) to the bluish green phosphate complex, under acidic conditions (Huang *et al*., 2005). As recorded in (Fig.8) total antioxidant capacity varied from haloarchaea to haloarchaea. *Haloferax* sp. GUSF-1 (KF796625) gave the maximum of 1.176 ± 0.75 mg AAE/ g cells, whereas *Halorubrum* sp. $GUFF_{72}$ gave minimum of 0.167 ± 0.001 mg AAE/ g cells, which was nearly 10 times less. Addition of FC reagent to the MEC developed blue colour which gave an absorption maximum at 765 nm. The formation of blue colour is attributed to an oxido- reduction reaction occurring between MEC and FC reagent, wherein phenolic moieties of the MEC are oxidised with simultaneous reduction of the alkaline FC. The extract of each of the 5 cultures showed presence of phenolic content capable of reacting with the alkaline FC. *Haloferax* sp. GUSF-1 (KF796625) gave highest value of phenolic content of 0.784 ± 0.004 mg GAE/g cells (Fig.8). This was followed by *Halococcus* sp. $GUFF_{179}$ > *Haloarcula* sp. $GUFF_{188}$ > *Natronococcus* sp. $GUFF_{233}$. The MEC of *Halorubrum* sp. $GUFF_{72}$ showed the minimum content of 0.135 ± 0.001 mg GAE/g cells. A positive correlation was also observed between phenolic content and total antioxidant capacity ($R = 0.89$, $p < 0.001$). The extracts which gave absorption between 300-600 nm was involved in the FC reaction as these peaks are seen to be abolished during the reaction. The occurrence of non- pathogenic haloarchaea and extremely halophilic eubacteria in Goan solar salts with an ability to scavenge free radicals adds value and promotes the traditional use of natural solar salt, in moderation, as conditioner and fertiliser to soils bearing trees such as *Cocos nucifera*, *Mangifera indica*, *Artocarpus*

heterophyllus and others. The presence of phenolic moieties in these cells ensure their involvement in free radical scavenging of metals and hydrocarbons which are on increase as pollutants in coastal soils (Utkina *et al*., 2004).

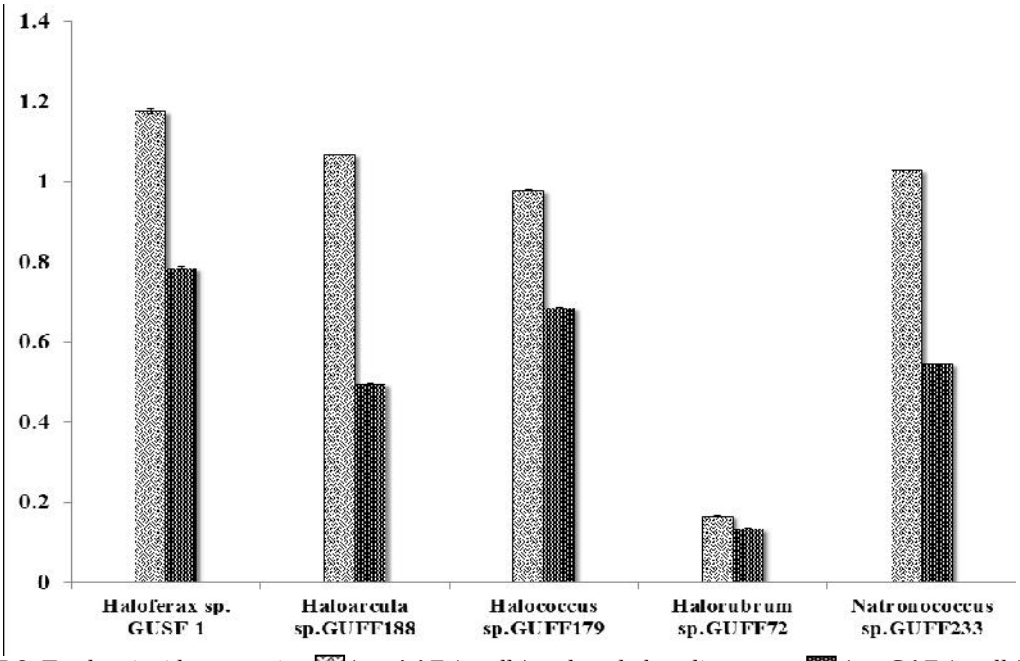

FIGURE 8; Total antioxidant capacity (mg AAE /g cells) and total phenolic content (mg GAE /g cells) of select isolates belonging to different genera

CONCLUSION

In conclusion, our study records that extremely halophilic eubacteria and haloarchaea are responsible for free radical scavenging activity exhibited by natural solar salt produced in salt pans of Agarwada, Arambol, Arpora, Nerul, Ribandar and Siridao in Goa - India. The study demonstrates for the first time the ability of DPPH to directly sort out colonies, while still on agar plate into free radical scavengers and non-scavengers. Two eubacterial genera namely *Alteromonas* and *Gluconobacter* and eight different haloarchaeal genera namely *Halobacterium, Haloferax, Haloarcula, Halococcus, Halorubrum, Natrialba, Natrinema and Natronococcus* retrieved from solar salts scavenge free radicals at varying degree. *Haloferax* sp. GUSF-1 (KF796625) (% DPPH RSA 31.5 ±0.43 / 252.2 ±1.14 µg AAE/g cell), having phenolic moieties as antioxidant principle, offers a promise for antioxidant harnessing. Interestingly, this is the first time *Alteromonas, Gluconobacter, Natrialba, Natrinema* and *Natronococcus* are reported with antioxidant capability. To our mind, this free radical scavenging potential of natural solar salts by microbes associated with it.

The bio-principles of these microbes make the crude solar salts chemo- reactive free radical scavengers. And possibly contributes to thus for scientifically unknown reason for traditional of solar salt Additionally, the phenolic antioxidant moieties of salt associated microbes is expected to ensure free radical scavenging of metals and hydrocarbon pollutants which are on increase in coastal soil.

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