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BIOACTIVE POTENTIAL OF HALOPHILIC ARCHAEA ISOLATED FROM SALTPANS AROUND SAURASHTRA REGION

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ABSTRACT

Halophilic archaea are the microorganisms best adapted to life of extremes of salinity on earth. The desideratum to survive in extreme environments condition has provided haloarchaea with a sequence of components especially acclimated to work in such many conditions. Extreme environments occurrence several microbes producing novel bioactive compounds. The feasible application of bioactive molecules or compounds in pharmaceutical products and industrial fields has obtained increasing attention; however, there were many potential bioactivities of haloarchaea are still poorly explored. Here is the estimation of the antioxidant and bioactive potential of halophilic archaea. The acetone extract taken from the incipient isolated haloarchaea strain expressed the highest antioxidant activity, while the acetone extracts from isolated strains showed vigorous antimicrobial activity, especially against another halophilic group of microorganisms. The aqueous protein-rich extracts obtained from all haloarchaea express an important role in the activity of the acetylcholinesterase enzyme which is involved in the hydrolysis of cholinergic neurotransmitters and related to many neurological diseases.

Keywords: Antioxidants, haloarchaea, bioactive potential

INTRODUCTION

Halophilic archaea or haloarchaea are a group of extremophilic microorganisms that prohibit hypersaline environments, such as salt lakes and salterns [1]. Marine soil has been widely found as the source of microorganisms, possessing a high number of bioactive molecules [2]. Halophiles are a group of microorganisms that can live in saline environments and are economically consequential because they engender several bioactive compounds which are serviceable for many pharmaceutical industries. They thrive in harsh and rugged conditions for life including low water availability, and high salt concentration [3]. there are One of the primary differences between the halophilic archaea and halophiles in the other domains of life is that exclusively used the salt in strategy [4] as all other known halophiles use the according to solutes strategy. Interest in the haloarchaeal group was increased because of their facility to engender a wide types of compounds such as exopolysaccharides, carotenoids, and proteins habituated to work in such extreme conditions. The toleration of these enzymes is to ascended saline concentrations and temperatures, and these high antioxidant power and therapeutic potential of haloarchaeal carotenoids [5, 6]. Haloarchaea are concretely opportune for the engendering of extremozymes, which can abide high temperatures and saturating salt

concentration, conditions required in pabulum, detergent and textile sector [7, 8]. Moreover, the most haloarchaea can engender and accumulate carotenoids, which are responsible for red color [9]. In addition to their antioxidant activity carotenoids have shown the capability to stimulate cell to cell communication and demonstrate anticancer properties. Extremely halophilic archaea generating red colored colonies produce phytoene, lycopene, β -carotene, and its precursors such as iso pentenyl dehydro rhodopin (IDR), bisanhydrobactrioruberin (BABR) and monoanhydrobactrioruberin (MABR) [10]. Carotenoids are cell reinforcement compounds with an important part as human well-being enhancers and it is unmistakable that their ordinary utilization benefits to confine numerous degenerative sicknesses, like neurodegeneration, malignant growth, cardiovascular illnesses, macular degeneration, and waterfalls. Furthermore, many natural carotenoids are utilized as aliment preservatives and pharmaceutical and cosmetic compounds due to their antioxidant and provitamin properties [11]. Antioxidants block the process of oxidation by neutralizing free radicals. The antioxidants themselves become oxidized. Antioxidant activity is not concluded predicated on a single antioxidant test method. There are few in vitro test methods

for assessing antioxidant activities with the sample of interest. In general, in vitro antioxidant tests utilizing free radical traps are relatively straightforward to perform. Predicated on the synthetic response required between the antioxidant compounds and the free radicals, antioxidant capacity assays are broadly relegated into two types, 1. Hydrogen atom transfer (HAT) reaction predicated assays 2. Electron transfer (ET) reaction predicated assays. HAT and ET based assays: DPPH free radical scavenging assay, Ferric iron reducing antioxidant power (FARP), Superoxide anion radical scavenging assay, ABTS scavenging assay, NO radical inhibition assay, Hydroxyl radical scavenging activity, Reducing power assay.

In present work, we describe the isolation of halophiles from saltpans of Saurashtra region (Gujarat, India) and the bioactive potential of their extracts. The result revealed that the extracts obtained with acetone shows remarkable antioxidant activities as well as ability to inhibit the acetyl choline esterase enzyme, involved in the hydrolysis of cholinergic disease becoming a promising source of metabolites with an applied interest in pharmacy, cosmetics and food industry [12, 13].

MATERIAL AND METHODS

Sampling site

The saltern present around Isolation was carried out from the salt pans of Saurashtra

region like Bhavnagar (Nari and Newport), Near Surajbari bridge (Kchha), and Mithapur, Gujrat. These sites are characterized by large amounts of sodium carbonate, or complexes of this salt.

Isolation

1 ml of brine and 1 gm of sediment sample were inoculated into 100 ml of TYES media containing (g L^{-1}) NaCl 250; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 20.0; KCl 5.0; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.2; Tryptone 5.0; yeast extract 5.0; [14] and incubated at 37°C for 7-10 days on a rotary shaker (Orbitek, LEIL) at 150 rpm. 100 μl of enriched samples were used to inoculate solid agar media described as above and incubated at 37°C for 8-10 days. Morphologically different colonies were subcultured for further study. The pH of the medium was adjusted to 7.0–7.2 with 1 N NaOH.

Identification of Archaeal isolate

The 16S rRNA gene of isolates was amplified using archaeal primers. The forward primer, F27: 5'ATT CCG GTT GAT CCT GCC GAAG3' (positions 6–27), was designed in the laboratory according to [15] and reverse primer, primer, R15215'AGG AGG TGA TCC AGC CGC AG3' (positions 1540–1521), was referred from [16]. Sequencing was done in SLS Research Pvt Lid, Surat. using DNA sequencing reaction of PCR amplicon was carried out with archaea specific primer ARCHEA_F/ ARCHEA_R using BDT v3.1

Cycle sequencing kit on ABI 3730xl Genetic Analyzer an automated DNA sequencer (SLS Surat, Gujrat). The sequences were analyzed by NCBI BLAST search and were submitted to the NCBI Gene Bank database.

Preparation of Archaeal Extract

Total eight pure culture were obtained after subculturing which were further used for extract Preparation. Culture from the stationary phase was centrifuged at 10,000 RPM for 10 min at 4°C. The obtained cell pellets were lysed by sodium hypo chloride

treatment. Superannuants were stored until analyzed for different assays.

Antioxidant Activity Assays

Different assays are conducted to test the antioxidant activity of archaea cell extracts and all the extracellular extracts obtained from the culture medium. The solvent employed to dissolve the extracts (PBS) was used as a negative control. The radical scavenging activity was expressed as the percent of inhibition relative to the negative control, following formula:

$$\% \text{ Scavenging activity} = 100 - \frac{\text{Abs sample} \times 100}{\text{Abs negative control}} \quad \text{Eq.1}$$

When the activity of the sample was higher than 65%, the concentration of the extract providing a 50% reduction of the radical scavenging was calculated by sigmoidal fitting of the data, using the GraphPad prism 6 program. These values were calculated from a graph built by plotting the log of the radical scavenging activity (%) against the normalized sample concentration, according to the concentration of protein or carotenoids in the extract.

DPPH Assay

DPPH assay (1,1-Diphenyl-2-picrylhydrazyl assay) was performed by the method of Sachindra *et al.* [15] Concisely DPPH (0.12 mM) solution was prepared in methanol. 88 µL of each extract solution

were mixed with 800 µL of DPPH solution. Absorbance at 517 nm was measured after incubation at 25°C for 30 min in the dark.

ABTS Assay

Free radical scavenging activity of each extract was determined by the ABTS+(2,2'-azino-bis(3-ethyl benzo thiazoline-6-sulfonic acid)) radical cation decolorization assay [16]. In which a solution of ABTS (7mM in water) and potassium persulfate (2.5mM) were kept in the dark at room temperature for 12-16 hours. Then ABTS solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After that 50µL of each extract solution was added din to 950µL of the diluted ABTS+ solution, the

absorbance was measured at 734 nm after incubated for 6 min in the dark.

Nitric oxide (NO) Assay

The capacity of scavenging free radical NO was evaluated according to baliga *et al.* [17] Briefly, 500 μ L Of each extracts were added in 500 μ L of sodium nitroprusside (10 mM in PBS) Then 500 μ L of griess reagent was added after 90 min incubation. After that absorbance was measured at 546 nm.

Ferrocyanide Reducing Power (FRP)

This assay was performed as described by tundis *et al.* [18] In which 300 μ L of sample solution were mixed with, 300 μ L of distilled water and 300 μ L of potassium ferrocyanide (1%). After incubation of 20 min, 300 μ L of TCA (10%) and 60 μ L of FeCl₃ (0.1%) were added and incubated for

10 min before the measurement of absorbance at 700 nm.

α -Amylase inhibition test

The α -amylase inhibition assay was performed by lauk *et al* [19]. succinctly 200 μ L of each extract sample were added in 200 μ L of amylase solution (100 U mL⁻¹ in buffer) and then 200 μ L of starch solution (0.1% in buffer sodium phosphate buffer 0.1M pH 7) was mixed after which the solution was incubated for 10 minute at 37°C. After incubation 100 μ L of HCl (1M) and 500 μ L of iodide were added to the solution (5 mM I₂+ 5 mM KI, in dH₂O) and absorbance was taken at 580 nm. The inhibitory activity was calculated by the given formula:

$$\% \text{ inhibitory activity} = 100 \times \frac{\text{Abs of sample} - \text{Abs of blank}}{\text{Abs of negative control}} \quad \text{Eq.2}$$

RESULT AND DISCUSSION

Isolation and Identification

A total Eight pure isolates are selected for extract preparation. Out of which two potent isolate SUJ 8 and MIT-2 were identified by 16s rRNA sequencing. The BLAST analysis of the 16S rRNA gene fragment of the haloarchaeon strain wsp1 showed 99.80% similarity to *Haloferax alexandrinus* (Figure 1). The sequence was deposited in GenBank/DDBJ database with accession number OP458567. Suj-8 showed 100%

similarity with *Haloferax sp. strain YC-43* (Figure 1). The accession number of the sequence submitted in Genbank is OP458566.

The antioxidant potential of all the extracts was tested as radical scavenging assays, based on DPPH, ABTS, Nitric oxide (NO), and ferrocyanide reduction potential (FRP) assays.

DPPH assay

Figure 1 displayed DPPH assay, in which SUJ-6 showed maximum antioxidant

activity on 4th day which is 68.64%. while SUJ-8, SUJ-9 and SUJ-10 showed maximum antioxidant activity on fifth day which are 84.05% ,96.59% and 70.16% respectively. MIT-6, MIT-8 and SUJ- 11 showed maximum activity on sixth day which are 82.93%,83.95 and 84.34 respectively. While MIT-7 showed maximum activity 73.04% on 7th day. The scavenging activity of DPPH assay by all isolates is shown in **Figure 2**.

ABTS assay

Figure 3 shows ABTS assay, here MIT-8 showed Maximum scavenging activity on 5th day i.e. 29.45 which is the least among all isolates. While MIT-6, MIT-2, SUJ-10, SUJ-8 and SUJ-6 showed maximum activity on 6th day as 43.82%, 44.25%, 54.55%, 58.33% and 62.23% respectively.

Ferrocyanide Reducing Power (FRP) assay

Acetone extract of isolates exhibit antioxidant activity when assayed with FRP

assay. SUJ-8 showed maximum activity 35.69% on 3rd day, SUJ-9 on 4th day which is 52.31% while SUJ-6, MIT-6 and MIT-8 presented a maximum activity on 6th day as 64.88%,12.15 and 18.05 respectively. On the other hand MIT-2, SUJ-10, and SUJ-11 showed maximum activity on 7th day which is 65.69%, 55.33% and 60.42% respectively (**Figure 4**).

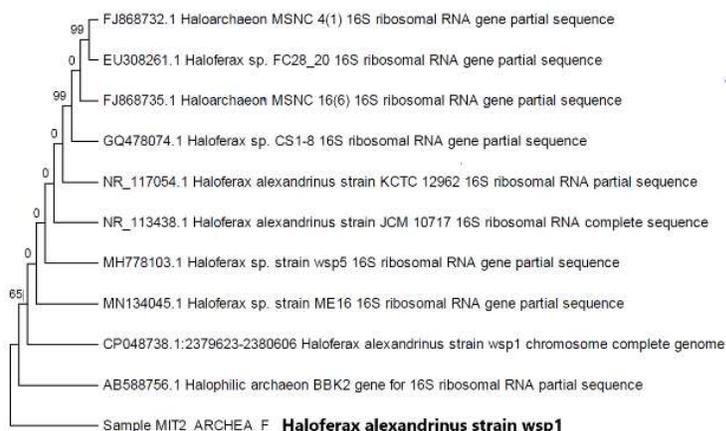
Nitric oxide (NO) assay

No isolates showed remarkable activity when assayed with NO assay.

α -Amylase inhibition test

The maximum inhibition activity showed by SUJ-8 which is 13.35%. Regarding the antidiabetic potential, the acetone extracts obtained from biomass exhibited α -amylase inhibitory activities as 77.25%(SUJ-9), 82.1% (SUJ-8), 70.09%(SUJ-10), 61.43% (SUJ-11), 96.55% (SUJ-6),87.98% (MIT-2), 92% (MIT-8), and 88.23% (MIT-6) (**Figure 5**).

Phylogenetic Tree:



Phylogenetic Tree:

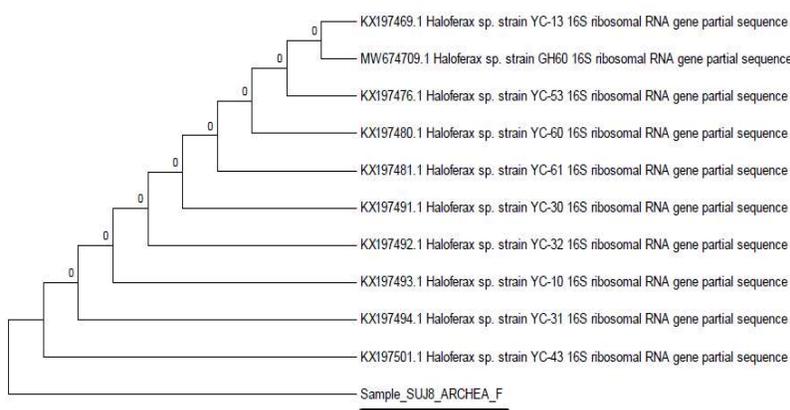


Figure 1: Phylogenetic tree MIT-2 and SUJ-8

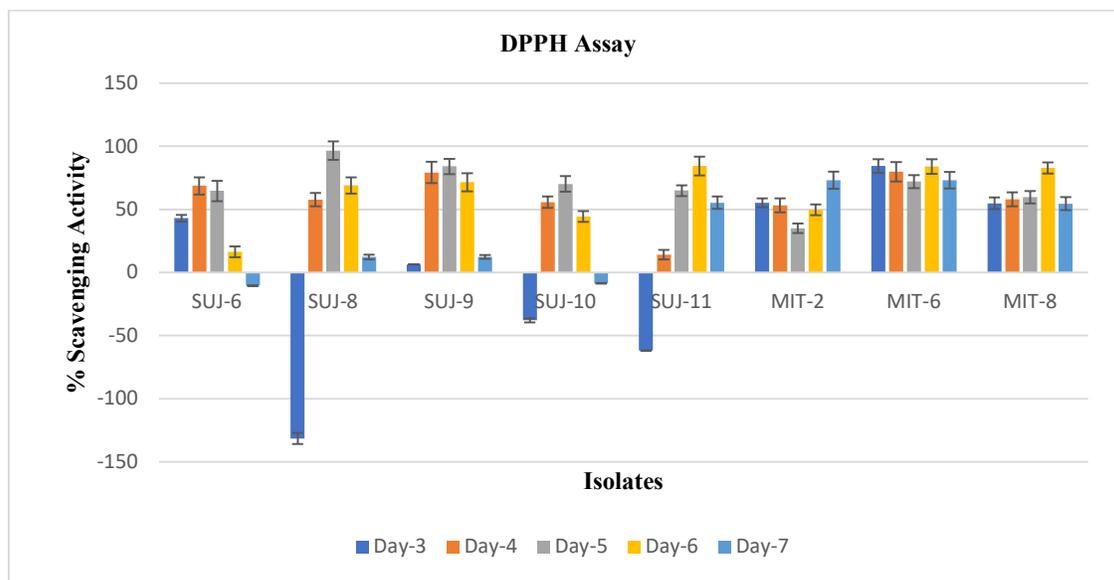


Figure 2: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

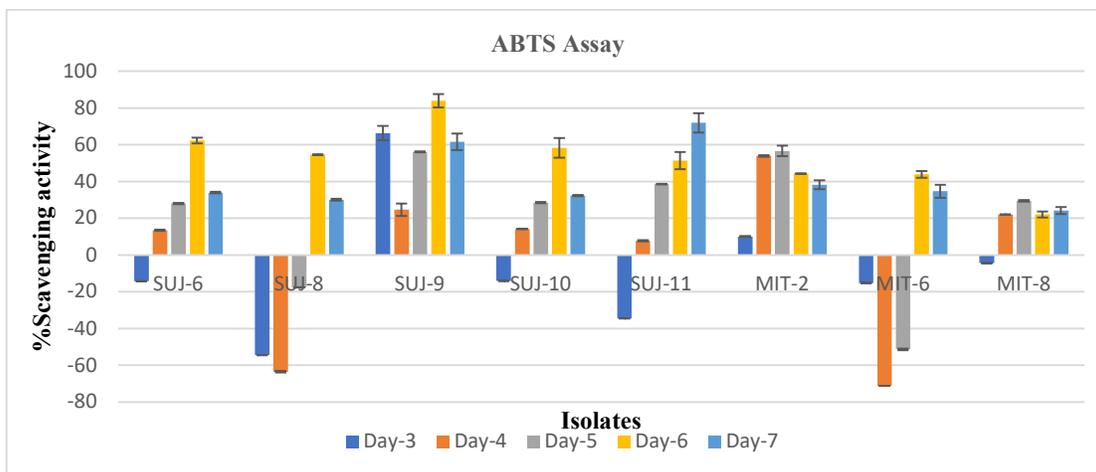


Figure 3: 2,2'-azino-bis(3-ethyl benzo thiazoline-6-sulfonic acid) ABTS Assay

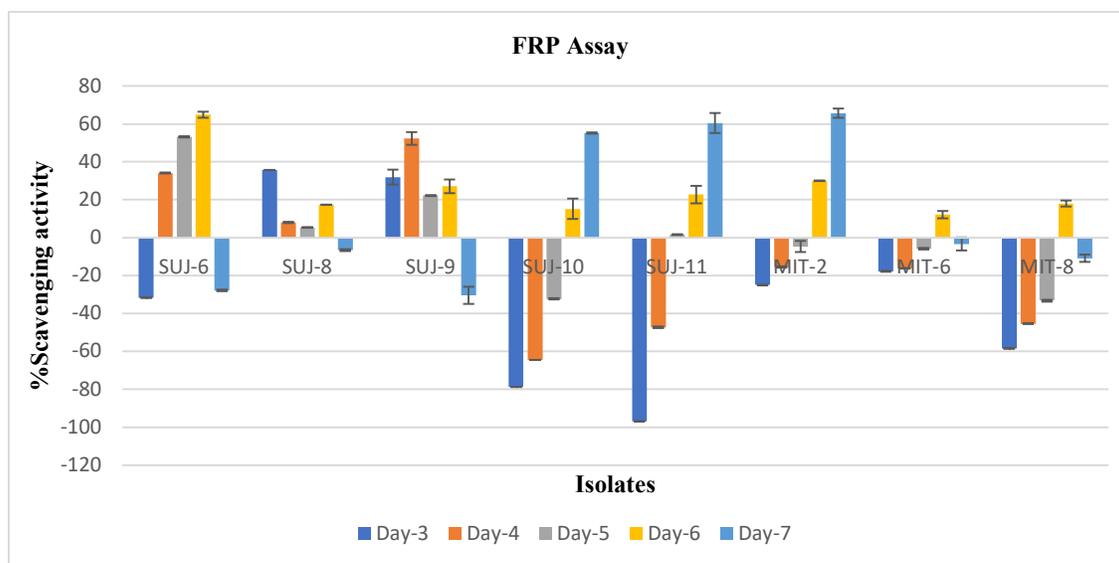


Figure 4: Ferrocyanide Reducing Power FRP Assay

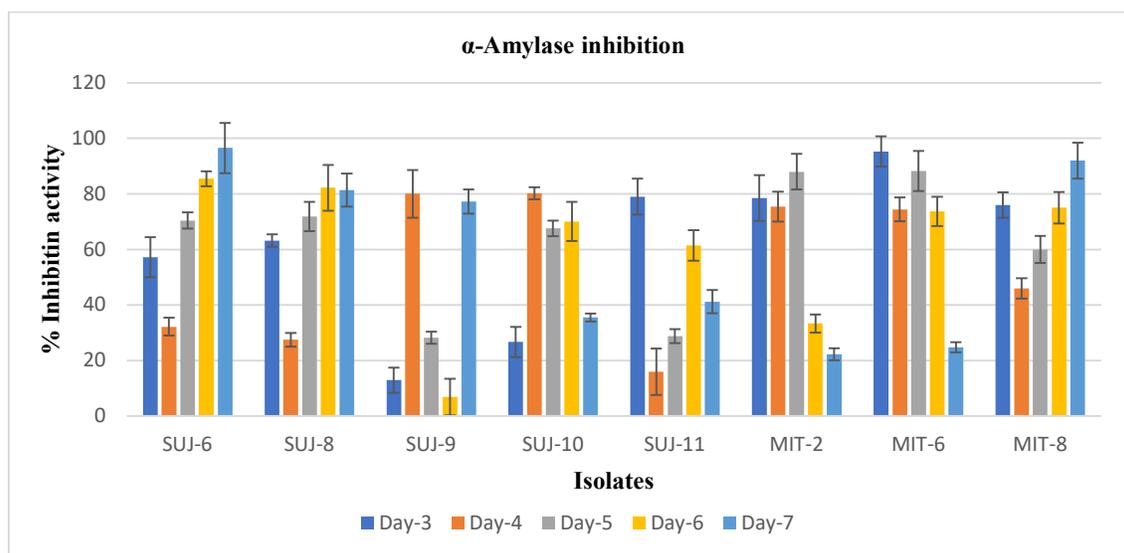


Figure 5: α-Amylase inhibition Assay

Table 1: Isolates showing Scavenging activity

ISOLATE	DPPH	ABTS	FRP	NO
SUJ 6	68.64	62.63	64.88	13.56
SUJ 8	96.59	54.55	35.69	14.35
SUJ 9	84.05	66.30	52.31	9.73
SUJ 10	70.18	58.33	55.18	14.30
SUJ 11	84.34	71.91	60.42	6.89
MIT 2	73.04	44.25	44.77	-275.07
MIT 6	84.33	43.82	2.15	-114.28
MIT 8	82.93	29.45	18.05	-128.65

CONCLUSION

The result obtained entail a complete study of antimicrobial activity about the haloarchaea as a wide range of antioxidant assays have been employed. As shown in Table 1 the extract of isolate SUJ8 gives a high antioxidant activity when assayed on DPPH which is 96.59%. While SUJ6 showed a least scavenging activity which is 68.64%. On other hand SUJ11 gives a high antioxidant activity when assayed on ABTS which is 71.71%. while MIT8 shows a lowest which is 29.45%. Ferrocyanide reduction is shown by the isolate MIT2 is 64.88% and minimum by MIT6 is 2.15%. None of isolates given a satisfactory scavenging activity of NO.

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