Exploring the microbiota of solar saltern of Mulund, Mumbai, India

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Solar salterns around the world are extreme hypersaline environments and typically contain high numbers of Halophiles. In the present study Archaeal and bacterial diversity of the salterns located at Mulund (19°10'12''N, 72°57'18''E), Mumbai was investigated. Fifty Seven Halophiles were isolated from the brine samples collected. Out of these, forty five belonged to bacteria and twelve belonged to archaea. All isolated archaea were shown intensely red pigmentation. Out of these, eleven salt tolerant organisms were characterized using morphological, biochemical and 16S rRNA analysis. Chemosystematic analysis of selected two isolates was also carried out. Overall analysis showed domination of members of Halomonadaceae family in this ecosystem. Presence of industrially important hydrolytic enzymes and carotenoids producing halophiles was also recorded.

[Keywords: Diversity, Halomonadaceae, Phylogeny, FAME, Solar saltern, 16S rRNA]

Introduction
Solar salterns found around the world are extreme hypersaline habitats that contain thriving microbial ecosystems. Many investigations of the archaeal, bacterial and eukaryal inhabitants in these environments are carried out using both culture dependent and 16S rRNA sequencing. Vast majorities of these microorganisms are obligate extreme halophiles. All these halophiles form dense communities, which differ worldwide in its structure due to local geographical conditions and represent a precious natural resource in terms of microbial diversity. Salterns originating microorganisms bear biotechnological potential for the production of hydrolytic enzymes, exopolysaccharide, carotenoid pigments etc. Besides this compatible solutes produced in response to an osmotic stress by halophiles are also equally important. In this halophilic organisms mainly accumulate organic compounds like sugars, polyols, amino acids and their derivatives. These nonionic, highly water soluble compounds help to maintain osmotic equilibrium and stabilizes cell proteins in presence of high salt concentration and therefore called as compatible solutes. These solutes are commonly used as enzyme and or cell protectants. Halophilic bacteria and archaea have also been used to study haloviruses. Potential importance of halophiles in different industrial areas such as the leather industry, food preservation food colorant production is also evident. In the present study an attempt has been made to study the diversity of cultivable halophilic microorganisms from interconnected multipond solar saltern of Mulund (E.) Mumbai (19°10’12”N, 72°57’18”E). These ponds are situated along western coast in Arabian Sea. No one has reported community structure of these ponds till the date. Our investigation therefore aimed at characterization of prokaryotic biodiversity of these ponds. We have characterized the isolates from these salt pans using a polyphasic approach.

Materials and Methods

Physicochemical Analysis of brine sample
Samples from seven shallow interconnected ponds were collected aseptically from multipond solar salterns of Mulund (E) (19°10’12”N, 72°57’18”E) Mumbai, India in Feb 2010. Samples from each pond were the average of 10 samples spanning the whole pond. Samples were filtered and stored in a refrigerator during investigation. Temperature was measured at the time of sampling. Various physicochemical parameters were determined for seven samples individually, viz total solids, total dissolved solids, and total suspended solids were determined as described in APHA. Salinity was measured with a Refractometer (Erma Tokyo).
Electrical conductivity (EC) and pH were measured with a conductivimeter (VSI-01C, VSI Electronics Pvt ltd, India) and pH meter respectively. Dissolved oxygen was measured by an oxygenometer (YSI 57, India), Ca++, Mg++ and total hardness was determined by EDTA titrimetric method. The K⁺ and Na⁺ were determined with flame photometry (CL 361 ELICO, India)\(^{18}\).

**Isolation of Organisms**

Different media were used for cultivation of microorganisms such as Nutrient agar with 20% NaCl (Hi media, India), SW agar\(^{19}\), MGM agar\(^{20}\), SG medium\(^{21}\), Sabouraud agar\(^{22}\) Skimmed milk agar with 20% NaCl and marine agar 2216 (MA). All the seven samples were mixed in equal proportion and used for isolation of halophiles. Aliquots of 100 µL of composite hypersaline samples were plated on solid media. Plates were observed after 14 days of incubation and number of colonies appeared and colony characteristics were recorded.

Among the Seven media used for cultivation the medium that showed highest diversity and supported faster growth was selected in further investigation. Using the selected medium minimum salt requirement for growth was determined by varying NaCl concentration in the range of 0 to 30%. Different types of colonies were observed and colony characters were recorded. Gram staining of isolates was performed using Dussalt’s method\(^{23}\).

**Biochemical tests**

Out of 78 screened microorganisms 57 salt resistant microorganisms were selected for further analysis. Indole production was detected by growing cells in medium supplemented with 1% (w/v) tryptophan and adding Kovacs’ reagent to the culture supernatant. To determine amylase production test, starch agar plates were inoculated and grown cultures were flooded with iodine solution. Production of catalase was determined by adding a 3% (w/v) H₂O₂ solution to colonies on agar. Gelatinase production was detected on agar plates supplemented with 1.5% (w/v) gelatin. Grown cultures were examined for the presence of clear zones around the colonies by flooding the plates with 15% HgCl₂ in 20% HCl. Presence of oxidase was determined by observing the formation of a violet colour by inoculating culture on a piece of filter paper moistened with 1% tetramethyl-p-phenylenediamine dihydrochloride. Protease production test was determined by observing the formation of clear zones around colonies on agar media amended with 2% (w/v) casein. Formation of turbid zones around colonies on agar medium supplemented with 0.1% (v/v) Tween 80 and 0.01% CaCl₂·2H₂O were considered as positive lipase test\(^{24}\). Appropriate positive and negative controls were used in all these tests\(^{25}\).

Effect of pH was determined by inoculating screened selected isolates in nutrient broth having various pH in the range of 5 to 12 and pH showing highest growth was selected for further investigation. Effect of temperature was determined by inoculating isolates in nutrient broth and incubating at various temperatures in the range of 25°C to 65°C, temperature showing highest growth was selected for further investigation. Optimum incubation time at predetermined pH and temperature was calculated by incubating culture and recording optical density after every 12 hours up to 168 hours.

Antibiotic susceptibility was tested by disc diffusion method using disc containing Bacitracin (10 U/disc), Polymyxin (300 µg/disc), Ciprofloxacin (5 µg/disc), Gentamycin (10 µg/disc), and Tetracycline (30 µg/disc), (Hi media Mumbai, India)\(^{25}\).

**16S rRNA sequencing of selected isolates**

The isolates were grown in saline nutrient broth. After centrifugation at 4500 g, 10 min, at 4°C and twice washing with distilled water, the pellets were selected for PCR amplification. Bacterial DNA was extracted by standard Phenol Chloroform extraction method\(^{26}\). Partial sequence of the 16S rRNA gene was amplified by PCR using the universal prokaryotic primers 530F (5′-GTGCCAGCAGCCGCGG-3′) and 1392R (5′-ACGGGCGGTGTGTAC-3′) which amplify a ~870 bp region of the 16S rRNA gene. PCR was performed in a final volume of 50 µL containing PCR amplification buffer (1X), Taq DNA polymerase (2.5 U), dNTPs (4 mM), primers (0.4 µM) and template DNA (4 ng). Amplification conditions were as the following: initial denaturation was carried out at 94°C for 3 min, 30 cycles at 94°C, 30 s at 52°C and 90 s at 72°C, followed by a final 7 min extension at 72°C. PCR products were electrophoresed in 1% (w/v) agarose gel containing ethidium bromide (1 µg mL⁻¹). The PCR products were purified and directly sequenced on the Applied Biosystems model 3730 XI (96 capillary) DNA sequencer (Applied biosystems, Inc., Foster City, Calif., USA). Sequence similarity searches were done using the BLAST program available at NCBI database. Alignment of
sequences was carried out with CLUSTAL W program version 2.1. Phylogenetic trees were constructed using the neighbor joining method. Tree files were generated by PHYLIP and viewed by TREEVIEW program. Bootstrap analysis was also carried out.

Accession numbers
The 16S rRNA sequences determined in this study were deposited in the Genbank sequence database under the accession numbers: JQ307193- JQ307200 and JF302664.1- JF302666.1. (Fig.1 & 2)

Chemo systematic characterization of selected isolates
Fatty acids of selected isolates were determined as described by Sasser 1990, by using the microbial identification system (MIDI Microbial ID) MIDI Sherlock, USA using cells grown on nutrient agar (pH 7.2) at 35°C for 3 days.

Results and Discussion
Physicochemical analysis of brine sample
Interconnected ponds of solar salterns of Mumbai were sampled in Feb 2010. Physicochemical analysis of brine samples collected is presented in Table 1. Brine samples were pale yellow in colour and viscous. Gradual rise in salinity was recorded from initial pond to crystallizer pond. Six fold rises in salinity of crystallizer pond was recorded. Remarkable reduction in dissolved oxygen was recorded as we go from initial to crystallizer pond and it was lowest in crystallizer pond. Obvious rise in TS, TSS and Mg²⁺ was recorded as evaporation proceeds. The results obtained indicate that suitable conditions existed for the occurrence of extremely halophilic microorganisms.

Isolation and identification of halophiles
Seven different media were used for isolation of halophiles. Out of these Seven, marine agar showed highest diversity and supported fast growth of halophiles as well. CFU count on marine agar was 2230 per 1 mL, while CFU count for nutrient agar was 1750 per 1 mL. We have selected 57 isolates grown on various media based on distinct colony appearance and cell morphology for further analysis. Out of the 57 isolates, 6 colonies were red, 2 colonies were pink, 9 colonies were yellow, 8 colonies were cream, 8 colonies were white, 8 colonies were dirty white 9 colonies were orange 2 were brown and 5 were of golden yellow in colour. Out of 57 isolates 17 were grams positive and 40 were gram negative. Out of the 57 isolates 11 were selected for further identification due to their high salt tolerance and designated as AGS 7, APP 8, APP 9, APP 10, APP 11, APP 12, APP 13, APP 14 APP 15, AGS 18 and AGS 30. Out of these eleven isolates, nine were gram negative and 2 were gram positive rods. Except APP

![Fig. 1—Phylogenetic tree showing the relationship among 16S rRNA gene sequences of archaea from Solar saltern, Mulund, Mumbai, India obtained in this study. The tree was constructed using neighbor joining tree.](image-url)
Remarkable diversity was recorded in sugar utilization pattern of isolates. In this study, APP 9, APP10, APP 11, APP 12, APP 14 and AGS 30 have used glucose as carbon source. APP 10, APP 11 and APP 14 used sucrose and APP 10, APP 11, APP 12 and APP 15 used fructose while APP 11 and APP 12 used arabinose as carbon source. However none of the isolates used xylose, cellobiose, etc.

Fig. 2—Phylogenetic tree showing the relationship among 16S rRNA gene sequences of firmicutes and γ-proteobacteria from Solar saltern, Mulund, Mumbai, India obtained in this study. The tree was constructed using neighbor joining tree.

Table 1—Physico-chemical characteristics of the seven ponds studied

<table>
<thead>
<tr>
<th>Physico chemical parameter</th>
<th>Initial pond</th>
<th>2nd pond</th>
<th>3rd pond</th>
<th>4th pond</th>
<th>5th pond</th>
<th>6th pond</th>
<th>Crystallizer pond</th>
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<tbody>
<tr>
<td>Salinity (%)</td>
<td>6</td>
<td>7</td>
<td>12</td>
<td>15</td>
<td>19</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>pH</td>
<td>8</td>
<td>7.12</td>
<td>7.16</td>
<td>7.16</td>
<td>7.19</td>
<td>7.16</td>
<td>7.16</td>
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<tr>
<td>Temperature</td>
<td>25</td>
<td>27</td>
<td>29</td>
<td>33</td>
<td>35</td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>Dissolved O₂ (mg/l)</td>
<td>7.2</td>
<td>7</td>
<td>5.6</td>
<td>4.8</td>
<td>2.4</td>
<td>2.31</td>
<td>1.86</td>
</tr>
<tr>
<td>TS (g/l)</td>
<td>111</td>
<td>132</td>
<td>134</td>
<td>148</td>
<td>265</td>
<td>411</td>
<td>474</td>
</tr>
<tr>
<td>TDS (g/l)</td>
<td>65.25</td>
<td>64.1</td>
<td>87.1</td>
<td>129.6</td>
<td>181.6</td>
<td>365.9</td>
<td>376.4</td>
</tr>
<tr>
<td>TSS (g/l)</td>
<td>2.1</td>
<td>4.2</td>
<td>7.8</td>
<td>13</td>
<td>26</td>
<td>42.55</td>
<td>51.3</td>
</tr>
<tr>
<td>Hardness (g/l)</td>
<td>7.2</td>
<td>9.2</td>
<td>11.8</td>
<td>14.4</td>
<td>16.8</td>
<td>18.9</td>
<td>19.5</td>
</tr>
<tr>
<td>Ca²⁺ (g/l)</td>
<td>0.7</td>
<td>0.8</td>
<td>1.1</td>
<td>1.15</td>
<td>1.3</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Mg²⁺ (g/l)</td>
<td>2.1</td>
<td>5.8</td>
<td>7.4</td>
<td>10.6</td>
<td>16.7</td>
<td>11.6</td>
<td>9.8</td>
</tr>
<tr>
<td>K⁺ (g/l)</td>
<td>4.7</td>
<td>5.2</td>
<td>5.6</td>
<td>6.7</td>
<td>7.3</td>
<td>7.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Na⁺ (g/l)</td>
<td>71.3</td>
<td>67.3</td>
<td>89.7</td>
<td>81.9</td>
<td>99.3</td>
<td>98.9</td>
<td>91.4</td>
</tr>
</tbody>
</table>
sorbitol, mannitol and lactose as carbon sources. Enzyme profile of eleven isolates was also recorded. In this out of 11, APP 8, APP 9 and AGS 30 have secreted extracellular amylase. APP 8, APP 9 and APP 15 were recorded as potential producer of protease. APP 9 and APP 30 have secreted gelatinase. Remarkable salt tolerance was observed in the isolate APP 15 that optimally grew at 26% salt concentration followed by APP 9, AGS 7 and AGS 18 that optimally grew on 24% of salt concentration. Isolate AGS 30 and APP 8 have tolerated 22% of salt. APP 10, APP 11, APP 12 APP 13 and APP 14 were grown on up to 20% of salt.

All the 11 isolates were tested for antibiotic susceptibility. Isolate APP 9 and AGS 30 were sensitive to all antibiotics. Isolate APP 14, APP 10 and APP 11 showed sensitivity to all antibiotics except gentamycin. AGS 18 showed sensitivity to bacitracin and polymyxin while APP 8 showed sensitivity to bacitracin, ciprofloxacin and tetracycline. Isolate APP 12 showed sensitivity to polymyxin and ciprofloxacin.

Table 1—Closest relatives of our bacterial isolates from 16S rRNA library

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Isolates</th>
<th>Nearest neighbour</th>
<th>Accession No.</th>
<th>% Similarity</th>
<th>Phylum/Subphylum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AGS-7</td>
<td>Halobacterium sp. HA4</td>
<td>HM368366.1</td>
<td>86%</td>
<td>Archaea</td>
</tr>
<tr>
<td>2</td>
<td>AGS-18</td>
<td>Halorubrum sp. SS1-3</td>
<td>JN196470.1</td>
<td>96%</td>
<td>Archaea</td>
</tr>
<tr>
<td>3</td>
<td>AGS-30</td>
<td>Halobacillus halophilus</td>
<td>AB681790.1</td>
<td>93%</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>4</td>
<td>APP-8</td>
<td>Halobacillus sp. MM22</td>
<td>JN791376.1</td>
<td>99%</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>5</td>
<td>APP-9</td>
<td>Salicola sp. s3-2</td>
<td>JN196462.1</td>
<td>99%</td>
<td>γ-proteobacteria</td>
</tr>
<tr>
<td>6</td>
<td>APP-10</td>
<td>Halobacillus sp. 50B22_2</td>
<td>EU308363.1</td>
<td>98%</td>
<td>γ-proteobacteria</td>
</tr>
<tr>
<td>7</td>
<td>APP-11</td>
<td>Halobacillus sp. 50B22_2</td>
<td>EU308363.1</td>
<td>98%</td>
<td>γ-proteobacteria</td>
</tr>
<tr>
<td>8</td>
<td>APP-12</td>
<td>Halobacillus sp. 50B22_2</td>
<td>EU308363.1</td>
<td>99%</td>
<td>γ-proteobacteria</td>
</tr>
<tr>
<td>9</td>
<td>APP-13</td>
<td>Pseudomonas halophilia</td>
<td>FR746108.1</td>
<td>92%</td>
<td>γ-proteobacteria</td>
</tr>
<tr>
<td>10</td>
<td>APP-14</td>
<td>Pseudomonas halophilia</td>
<td>FR746108.1</td>
<td>99%</td>
<td>γ-proteobacteria</td>
</tr>
<tr>
<td>11</td>
<td>APP-15</td>
<td>Haloferax prahovense TL6</td>
<td>NR028165.1</td>
<td>99%</td>
<td>Archaea</td>
</tr>
</tbody>
</table>

16S rRNA sequencing

Partial sequences of 16S rRNA fragments were amplified from 5’ terminus. Phylogenetic analysis of these sequences revealed a range of identities to several group of bacteria (Table 3). The clones fell into three major lineages the archaea, firmicutes and γ-proteobacteria. Six sequences were placed into the γ-proteobacteria. Out of six, three were closely related to the genus Halomonas with 98 - 99% similarity. The isolates APP 10, APP 11 and APP 12 showed 98%, 98% and 99% identity with Halomonas sp. 50 B22_2 respectively. Halomonas were reported earlier from Almeria, Cadiz and Huelva (Spain)30, historic Dagong brine well, china31 and from alkaline Lonar soda lake, India32. Isolates APP 13 and APP 14 showed 92% and 99% similarity with P. halophila. It is earlier reported from Maras saltern Peruvian Andes33. Isolate APP 9 showed 99% similarity with Salicola sp. s3-2 which showed efficient protease production. It is previously reported from Maras saltern34. The isolate AGS 7, AGS 18 and APP 15 are placed in the phylum archaea. Amongst this AGS 7 showed 86% similarity with Halobacterium sp. HA4, AGS 18 showed 96% similarity with Halorubrum sp. SS1 and Haloferax APP 15 showed 99% similarity with Haloferax prahovense TL6. Halobacterium, Halorubrum and Haloferax sp. were earlier reported from salterns of Tamil Nadu11; Tunisian multipond solar salterns35 and from Puerto Rico and the Caribbean36. These bacteria are reported to synthesize carotenoids with potent antioxidant activity. The other isolates AGS 30 and APP 8 showed 93% and 99% similarity with Halobacillus halophilus and Halobacillus sp. MM22 respectively. We have identified three different lineages. Most of our clones from solar saltern of Mumbai are related to halophilic and haloalkalophilic organisms reported earlier from solar saltern of Tamil nadu11, Bhavnagar22, Puerto Rico36, Almeria and Tunisian multipond solar salterns35. Our study describes not only the existence of bacterial and archaeal diversity of solar salterns of Mulund, but also indicates presence of industrially important cultures like Halobacillus, Pseudomonas, Halomonas, Halorubrum, Halobacterium which could be used as efficient producers of carotenoids, hydrocarbon...
Table 3—Biochemical characteristics of Halophiles isolated and reported by us

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AGS7</th>
<th>APP8</th>
<th>APP9</th>
<th>APP10</th>
<th>APP11</th>
<th>APP12</th>
<th>APP13</th>
<th>APP14</th>
<th>APP15</th>
<th>AGS18</th>
<th>AGS30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram nature</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>3x1</td>
<td>5x1</td>
<td>4x1</td>
<td>4x1</td>
<td>3x1</td>
<td>4x1</td>
<td>5x1</td>
<td>3x1</td>
<td>4x1</td>
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<td>4x1</td>
</tr>
<tr>
<td>Motility</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
<td>Motile</td>
</tr>
<tr>
<td>Colony pigmentation</td>
<td>Bright red</td>
<td>White</td>
<td>Yellow</td>
<td>Cream</td>
<td>Pale yellow</td>
<td>Cream</td>
<td>White</td>
<td>White</td>
<td>Orange</td>
<td>Bright red</td>
<td>Yellow</td>
</tr>
<tr>
<td>NaCl Range for growth (%)</td>
<td>10-22</td>
<td>10-24</td>
<td>2 to 20</td>
<td>2-20</td>
<td>2-21</td>
<td>2-20</td>
<td>4-20</td>
<td>10-26</td>
<td>10-24</td>
<td>2 to 22</td>
<td></td>
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<tr>
<td>Temp. optimum °C</td>
<td>35°C</td>
<td>35°C</td>
<td>35°C</td>
<td>35°C</td>
<td>35°C</td>
<td>35°C</td>
<td>35°C</td>
<td>42°C</td>
<td>35°C</td>
<td>35°C</td>
<td></td>
</tr>
<tr>
<td>pH Optimum</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Utilization of

- Glucose
- Sucrose
- Arabinose
- Lactose
- Maltose
- Fructose
- Mannitol
- Xylose
- Sorbitol
- Cellobiose
- Galactose

Indole production
- +  +  +  +  +  -  -  +  +  +

Enzyme Profile

- Catalase
- Amylase
- Gelatinase
- Urease
- Protease
- Cellulase
- Oxidase

Antibiotic Susceptibility

- Bacitracin (10unit/disc) ND S S S S S R S S ND S S
- Polymyxin(300mcg/disc) ND R S S S S S S ND S S
- Ciprofloxacin(5 mcg/disc) ND S S S S S S S ND R S
- Gentamycin(10mcg/disc) ND R S R R R R R ND R S
- Tetracyclin(30mcg/disc) ND S S S R R S ND R S

ND= Not detected,
S= Sensitive R=Resistant
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Chemo systematic Characterization of selected isolates

Whole fatty acid composition of AGS 7 and AGS 30 are shown in Table 4. AGS 7 has a cellular fatty acid profile containing large amount of straight chain fatty acid while AGS 30 has more amount of branched fatty acid. Major fatty acid detected in AGS 7 is C_{16:0} while AGS 30 has anteiso C_{15:0} types of chain.

Conclusion

Artificial solar salterns are known to harbor high number of taxonomically diverse halophilic organisms understanding of this ecosystem is therefore highly desirable. Present investigation first time reported presence of halophilic bacteria and archaea from solar saltern of Mulund, Mumbai, India. Isolates identified are also reported by different groups from solar salterns distributed worldwide. Microbiota reported in this work includes many of salt stable amylase, protease and carotenoid pigments producing Halophiles.

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References

31 Wenliang xiang, Jianhua Guo, Wei Feng, Min Huang, Hao Chen, Jian Zhao, Jie Zhang ,Zhirong Yang, Qun Sun, Community of extremely halophilic bacteria in historic Dagong Brine well in Southwestern China, World journal of microbiol biotechnol 24 (2008) 2297-2305
33 Maturrano Lenin, Fernando Santos, Ramon Rossello-Mora, and Josefa Anto’n, Microbial Diversity in Maras Salterns, a Hypersaline Environment in the Peruvian Andes, Applied And Environmental Microbiology, (2006) 3887–3895