Isolation of Halophilic Microorganisms From salted soil in Jazan area
Thabet Ibrahim Qassem (200911926)
Biology Department -Faculty of Science- Jazan University

Abstract
Halophiles are a group of microorganisms that live in saline environments and in many cases require salinity to survive. Halophiles include a great diversity of organisms. At different concentrations of NaCl, 0.5, 10 and 15% (w/v) bacteria and fungi were isolated. The colony counts were increased at 5% (w/v) and then decreased with increasing salt concentration up to 15% (w/v). Different fungal species were isolate and identified at different under different concentrations of NaCl. *Aspergillus sp*, *Penicillium sp*, *Cladosporium sp*, *Rhizopus sp* and *Fusarium sp* were detected under different concentration.

Key words: Halophilic, Microorganisms, Soil

Introduction
Nearly 40% of world’s surface has salinity (Jadhav et al., 2010). Salinization of soil is a serious problem and is increasing gradually in many parts of the world, particularly in arid and semiarid areas. At present, out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares is affected by excess salt content (Evelin et al., 2009, Moradi et al., 2011). At high salinity level, it was found that treatments
supplied by biofertilization with yeast decreased the adverse effect of salinity. Halophilic microorganisms are already in use for some biotechnological processes, such as commercial production of carotene, polymers, enzymes, compatible solutes (Jadhav et al., 2010).

Kushner and Kamekura (1988) defined several categories of microorganisms on the basis of their optimal growth: (1) non-halophiles are those that grow best in media containing less than 0.2 M NaCl (1% salt). (2) slight halophiles grow best in media with 0.2 to 0.5 M NaCl (1-3% salt). (3) moderate halophiles grow best with 0.5 to 2.5 M NaCl (3-15% salt). (4) extreme halophiles show optimal growth in media containing 2.5 to 5.2 M NaCl (15-32% salt) (Ventosa, 2006). Ibekewe et al. (2010) reported that salinity and pH caused severe decrease in the rhizosphere bacterial population. The unfavorable effects of salinity on soil fertility are numerous. Its effects on uptake of nutrients, absorption of moisture and soil structure are well known. So we can use different halotolerant or halophile bacteria for production of biofertilizer for optimum use of saline land potential in agriculture. Halophiles are a group of microorganisms that live in saline environments and in many cases require salinity to survive. Halophiles include a great diversity of organisms, like moderately halophilic aerobic
bacteria, cyanobacteria, sulphur-oxidizing bacteria, heterotrophic bacteria, anaerobic bacteria, archaea, protozoa, fungi, algae and multicellular eukaryotes. Microorganisms that are able to grow in the absence as well as in the presence of salt are designated as halotolerant and those that are able to grow above approximately 15% (w/v) NaCl (2.5 M) are considered extremely halotolerant (Kushner et al., 1998, DasSarma and Halophiles, 2001). According to Kushner (1978), many marine organisms are slight halophiles (with 3% w/v NaCl in sea water). Moderate halophiles optimally grow at 3-15% w/v NaCl, extreme halophiles at 25% w/v NaCl.

Compatible solutes are low-molecular weight osmoregulatory compounds which are highly water-soluble sugars, alcohols, amino acids, betaines, ectoines or their derivatives. In addition to their stabilizing effects, they are used as salt antagonists, stress protective agents, moisturizers and therapeutics. They stabilize enzymes, DNA and whole cells against stresses such as freezing, drying and heating. They increase freshness of foods by stabilizing components. Induction of osmolytes in cells can increase protein folding and thereby improve salt tolerance which could be useful in agriculture and xeriscaping (Roberts 2005 and Detkova et al., 2007). Halophilic microorganisms usually adopt either of the two strategies of
survival in saline environments: ‘compatible solute’ strategy and ‘salt-in’ strategy (Ventosa et al., 1998). The work aimed to isolate halophilic or halotolerant microorganisms from salt soil.

**Material and Methods**

**Microbial Analysis:** Salted soil samples from Jazan were taken with the help of sterilized spatula at a depth of 5-15 cm. One gm of soil was mixed with 9 ml of sterilized water and mixed thoroughly. 1 ml from the solution was then mixed in 9 ml sterilized water to make $10^{-2}$ dilution of this solution and in the same pattern dilutions up to $10^{-7}$ were prepared to determine the microbial count. The total numbers of microorganisms (bacteria and fungi) was counted. The numbers of colony forming units (CFU) in the selective media were determined by means of the serial dilution technique and the spread plate method. Analyses were performed in three replicates. Viable counts for fungi were performed using Dox agar medium. Streptomycin was added after autoclaving at final concentration of 30 mg ml. The plates were incubated at 28°C and colonies were counted after 8 days. Viable counts for bacteria was determined using a Nutrient agar medium contained (per liter of water) Peptone, 5.0 g, Beef extract, 3.0g Sodium chloride 5.0g, agar 20.0g.
Results and Discussion

Halophilic and halotolerant microorganisms were studied with many authors (Janda-Ulfig et al., 2009, Soontharapirakkul and Incharoensakdi 2010, Shivanand and Mugeraya 2011). In our study, numbers of bacteria and fungi (CFU - colony forming units) increased with increasing NaCl concentration up to 5% (w/v) and then decreased with increasing concentration. Bacterial colonies did not appear at high concentration 15% (w/v); this result agrees with the results obtained by Ibekwe et al. (2010). On the other hand, six fungal colonies appeared at high concentration 15% (w/v) (Table 1 & Fig1). Basically, the effect of Na⁺ on the growth of different species of microorganisms will differ due to the growing water activity of each microorganism. Microorganisms under hypertonic environments either die or remain dormant except halotolerant and halophilic microorganisms (Chookietwattana, 2003). Different fungal species were isolated and identified at different under different concentrations of NaCl. *Aspergillus sp, Penicillium sp, Cladosporium sp, Rhizopus sp and Fusarium sp* were detected under different concentrations (Table 2).
Table (1) Numbers of Bacteria and Fungi (CFU - colony forming units) $\times 10^3$ / g dry salted soil at different concentration of NaCl % (w/v)

<table>
<thead>
<tr>
<th>NaCl Conc. % (w/v)</th>
<th>CFU - colony forming units of</th>
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<tbody>
<tr>
<td></td>
<td>Bacteria</td>
<td>Fungi</td>
</tr>
<tr>
<td>0</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td>24</td>
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</tr>
<tr>
<td>15</td>
<td>00</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
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</tbody>
</table>

Fig (1) Numbers of fungi (CFU - colony forming units) $\times 10^3$ / g dry salted soil at different concentration of NaCl % (w/v).

Fig (2) Numbers of bacteria (CFU - colony forming units) $\times 10^3$ / g dry salted soil at different concentration of NaCl % (w/v).
Table (2) Isolated fungus under different concentrations of NaCl

<table>
<thead>
<tr>
<th>Fungus growth</th>
<th>Na Cl concentration (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus sp</td>
<td>+</td>
</tr>
<tr>
<td>Penicillium sp</td>
<td>+</td>
</tr>
<tr>
<td>Cladosporium sp</td>
<td>+</td>
</tr>
<tr>
<td>Rhizopus sp</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium sp</td>
<td>+</td>
</tr>
<tr>
<td>Unknown species</td>
<td>+</td>
</tr>
</tbody>
</table>

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References


تم عمل تعداد كلي للكائنات الدقيقة البكتريا والفطريات من تربة ملحية في منطقة جازان عند تركيزات متحركة من ملح كلوريد الصوديوم (0، 0.5، 1، 5، 15، 50 % وزن/حجم) ووجد أن عدد المستعمرات الميكروبية تزداد عند تركيز 5% (وزن/حجم) ولكن تقل بزيادة التركيز، في نفس الوقت ظهرت مستعمرات فطرية عند تركيز 1% بينما فشلت في النمو عند تركيز 20 %، على الجانب الآخر لم تظهر مستعمرات بكتيرية عند تركيز 10 % وفي تلك الدراسة أيضاً تم عزل وتعريف الفطريات النامية عند تركيزات متحركة من الملح وذلك على مستوي الجنس ووجد أنها تنتمي لجنس الأسكرجيلس والبنسيليم والريزوباس والفيزاريوم والكلادوسوريم، أنواع أخرى لم يتم التعرف عليها، ووجد نوع من فطر البنسيليم ينمو عند التركيزات المختلفة من الملح حتى 15 % (وزن/حجم).