Al-Azhar Bull. Sci. Vol. 18, No. 2 (Dec.): pp. 37-58, 2007.

BACTERIOLOGICAL SYSTEMS AS A NEW APPROACH FOR DESALINATION OF SALTY WATER

BAYOUMI, R.A.; LOUBOUDY, S.S.; EL-GAMAL, M.S.; ABDELWAHED, M.A.
Botany and Microbiology Dept., Faculty of Science(Boys), Al-Azhar University, Madinet Nasr , Cairo, Egypt. P.N. 11884.

Abstract

Forty eight halotolerant microbial isolates were isolated from water and soil samples collected from Mediterranean sea (Alexandria), Ein-Helwan (Helwan), Red Sea (Hurghada), and Qarun lake (El-Fayoum). Soil samples were collected from Alexandria, Ein-Helwan, Qaron, El-Toor, and Sant-Katren. Three halotolerant bacterial isolates were chosen as the most potent halotolerant bacterial isolates for bacterial desalination of sea water. These isolates were identified as *Sporohalobacter marisomartui* BEW45, *Marinococcus hispanicus* BEW47 and *Halomonas elongata* BAW48. Combination of the three potent halotolerant bacterial isolates exhibited high desalination percentage. The highest desalination percentage (%) was achieved at 37 ºC, pH 6, for168 h., inoculum size 2.5 ml (each ml contain 67 x 10^7 cells, CFU), no tested carbon sources and yeast extract as best nitrogen source. The best bacterial desalination of sea water was performed by repeated recycling the sea water three times, by subjecting water to there potent halotolerant bacterial strains. The desalination percentage (%) of Ca^{++}, Mg^{++}, Cl^-, Na^+ and K^+ reached up to 88, 78, 79, 76 and 63% respectively. The resulted desalinated sea water was used in irrigation of *Hordeum vulgare* Giza 2. This study recommend the possibility of the selected strains to desalinate of salty water by designing of desalination plants similar to wastewater treatment plants to be used in irrigation of some economic crops and woody forest trees.

Introduction

Water is the most important of life sources that we need for our survival. It is necessary for growth of plants, and hydration of animals, which in turn are consumed by us. Human body contains approximately 60 % water. The total amount of water on earth has remained almost constant over billions of years. Water in rivers is only about 1/10.000 of all earth's water. Yet, rivers are the source of most daily used water. High percent of the world water on earth is saline water. As water is a precious commodity, as wise that we try to conserve and limit our use as much as possible (Fetter, 2001). Desalination processes remove dissolved salts and other materials from sea water and brackish water. The principal desalination technologies in use are reverse osmosis(RO) and distillation. Electrodialysis and vacuum freezing also have some applications. Fresh water scarcity is already posing major problem for more than a billion people around the world, mostly in arid developing countries. Spang(2006) explores the global potential for integrity wind power and desalination

technologies to increase water supplies. The World Heath Organization(HWO) predicts that by mid-century, four billion of us-nearly two-thirds of the World's present population-will face severe fresh water shortage. More than 11.000 desalination plants are in operation through the world producing more than 20 million cubic meters(roughly six billion gallons) of water per day and the number is growing. It has been estimated that there will be a 100% increase in the active installed capacity of desalination plants during the 2005-2015(Cotruvo, 2004;2007).In recent time, the pollution of seawater appears to be at a much higher level than in the past because of the explosion in industrial growth and the population observed all over the world. Considering this situation Yoshitaka *et al*., (2005) developed biological system, which is a new technology developed to overcome these pollution problems. The biological methods of desalination can be used in different area specially arid desert and island areas where large amounts of energy are not required. The cost of this methods is too little in comparison to other well established methods that need large amounts of energy, complex designs, technology with high costs**.** In the present study, we describe isolation, purification, characterization of the selected potent halotolerant bacterial isolates. Studying the parameters controlling the desalination process of sea water were also discussed.

Materials and Methods

I- Media used:

The following media were used for isolation, and purification of halotolerant microorganisms collected from water and soil samples.

1. Modified Lockhead's Skim milk agar medium (SMA) (Ammar et al., 2000):

This medium was used for isolation of halotolerant bacteria. It has the following composition (g/l): Skim milk powder, 50; NaCl, 100; $MgSO_4.7H_2O$, 20; $Ca(NO_3)_2$, 2.5; yeast extract, 1 and agar, 20.Minerals were dissolved in the distilled water and sterilized. Yeast extract and milk were also sterilized. pH was adjusted at 7.5 before sterilization. The two solutions were mixed together after sterilization. Total volume was completed up to 1000 ml.

2. Medium routinely used in H. Larsen's laboratory for axenic culturing of halobacteriaceae (HCM) (Larsen, 1981):

This medium has the following composition (g/l): NaCl,100; $MgSO_47H_2O$, 10; KCl, 5; $CaCl_2\,6H_2O$, 0.2; yeast extract, 5; tryptone, 5; agar, 20 and distilled water up

to 1000 ml. Salts and organic substances were sterilized separately and pH was adjusted at 7.

3. Sea water media:

Three types of sea water media were used in this study viz. 1% peptone sea water, 1% yeast extract sea water and 1% sodium nitrate sea water media.

II- *Determination of some ions in sea water samples: chlorine, sodium, potassium, calcium and magnesium ions in sea water samples were determined as the following:-*

a- Determination of chloride (Cl^-) ion was carried out by the Mohr's method (Jeffery *et al*., 1989):

Mohr's method was based on titration of halide, such as NaCl with $AgNO_3$ solution in the presence of $K_2Cr_2O_2$ as indicator. The end point of the titration was the point at which the color of the suspension changes from pure yellow to reddish brown.

b- Determination of sodium (Na^+) and potassium (K^+) ions:

Sodium (Na^+), and potassium (K^+) ions were measured using a flame photometer model Corning 400.

c- Determination of calcium (Ca^{++}) ion was carried out according to Jeffery *et al.*, (1989):

Calcium ions were determined by using muroxide as indicator. In this method the saline water sample must changed to alkaline by addition of (1N)NaOH and titrated with standard ethylene diamine tetra acetic acid (EDTA).

d- Determination of magnesium (Mg^{++}) ion was carried out according to Jeffery *et al.*, (1989):

Magnesium water sample become alkaline by the addition of ammonia buffer mixture and titration was carried out with standard EDTA. The indicator, eriochrome black T (EBT) forms wine-red complexes with Ca^{++} and Mg^{++} ions. At the end point, the wine-red colour of the solution changes to blue colour. The volume of EDTA consumed in this method was equivalent to Ca^{++} and Mg^{++}, but the volume of EDTA consumed in determination of Mg^{++} is equal to the difference between total volume EDTA consumed in determination of calcium and magnesium and the volume of EDTA consumed in determination of calcium only.

III- Selection of the best halotolerant microbial isolates:

*Growth of halotolerant microorganisms on medium containing high salt concentration***:** The total microbial isolates were grown on the surface of isolation media (HCM and SMA media) containing 10% NaCl. Growth of total microorganisms were tested on the same medium but containing different concentrations of NaCl viz.15, 20, 25 and 30 % to select the potent halotolerant microorganisms that could tolerate high level of NaCl.

***VI- Desalination by using the best halotolerant bacterial isolates*:** Three saline water samples collected from different localities (Hurghada, Qarun, and Alexandria) were inoculated by the most halotolerant bacterial isolates to proof the tolerance of these bacteria towards high salt levels of saline water samples. Yeast extract sea water medium was inoculated by the potent bacterial isolates, and incubated at 37ºC for 7 days. At the end of the incubation period, the concentrations of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were determined before and after desalination process of sea water samples. Concentration of Ca^{++}, Mg^{++} and Cl^{-}, were determined by titration (Jeffery *et al.,* 1989) method while Na^{+} and K^{+} were determined by flame photometer (Muharram,2000). Bacterial protein content was also determined by Folin-Phenol reagent (Lowry *et al.,* 1951).

***VII- Effect of combined action among potent bacterial isolates on the desalination of sea water*:** Four combinations among potent bacterial isolates were performed by washing the culture with saline solution under aseptic conditions. Yeast extract sea water medium was inoculated by each combination. At the end of each incubation period, rates of Ca^{++}, Mg^{++} and Cl^{-}, were determined by the titration method while Na^{+} and K^{+} were determined by flame photometer.

***VIII-Identification of the three potent halotolerant bacterial isolates*:** Three potent halotolerant bacterial isolates were identified on of basis of various morphological, physiological, and biochemical characteristics following the criteria laid down in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

***IX- Parameters controlling desalination process and optimization of some nutritional and environmental conditions for the three most potent halotolerant bacterial isolates*:-** Following each factor performance, protein content was determined by Folin-Phenol reagent method (Lowry *et al.*,1951)and concentration of

Cl^-, Ca^{++} and Mg^{++} were also determined by titration which finally Na^+ and K^+ were determined by flame photometer.

1- Effect of different incubation periods was carried out by allowing the potent bacterial isolates to grow on yeast extract sea water medium incubated at different incubation periods.

2- Effect of different incubation temperatures was carried out by inoculating yeast extract sea water medium with the three potent halotolerant bacterial isolates separately or in combination and were incubated at different incubation temperatures.

3- Effect of different pH values:

Range from 3 – 10 was designed by using (1N) NaOH and (1N) HCl for sea water and inoculated by bacterial suspension.

4- Effect of inoculum size:

Different inoculum sizes of bacterial suspension were incubated individually or in combination among the most tolerant bacterial isolates.

5- Effect of different nitrogen sources:

Different nitrogen sources were added at a level of (1%,w/w) to sea water and inoculated by the optimal inoculum size suspension of the potent combination among the three bacterial isolates. The nitrogen sources that were applied included ammonium oxalate, ammonium nitrate, ammonium sulphate, urea, ammonium acetate, peptone, potassium nitrate, ammonium chloride, ammonium dihydrogen orthophosphate and yeast extract.

6- Effect of different carbon sources:

Different carbon sources were added to sea water at concentration (1%,w/w). The carbon sources were represented by glucose, lactose, galactose, fructose, raffinose, starch, cellobiose, cellulose, sucrose, glycerol, sorbitol, mamito, and xylose.

7- Effect of aeration conditions:

This experiment was carried out to investigate the effect of shaking or static condition on the desalination of sea water by the bacterial isolates.

XI-Effect of different doses of UV irradiation on sea water desalination:

The most halotolerant bacterial isolates were exposed to UV radiation at 365 nm for 2, 5, 10 and 15 min. Sea water medium was inoculated by bacterial isolates after UV treatment and incubated for 7 days under all optimal conditions.

***XII- Bacterial desalination of sea water*:** Sea water was treated by the most potent bacterial combination under all optimal conditions, this was carried out by repeating desalination process for three times. In the first step of treatment, sea water medium was inoculated by the potent bacterial combination and incubated under all optimal conditions for 7 days. At the end of incubation period, treated sea water was centrifuged to remove bacterial cells, the supernatant sea water was used in the second step of treatment. In this step, pre-treated sea water was also inoculated by the same potent bacterial isolates. At the end of incubation period treated sea water was centrifuged to be prepared for the third desalination treatment step. During the third treatment step, 100 ml of the treated water which was obtained from second treatment was re-inoculated, then incubated as in the previous two treatment steps. Concentration of Cl^-, Ca^{++}, Na^+, K^+ and Mg^+ were determined at the end of the last incubation period.

RESULTS

Twenty two and twenty five bacterial isolates were isolated from water and soil samples respectively on HCM medium. Only one fungal isolate was isolated from soil sample FQS24 on SMA medium. Code numbers, source and localities and the halotolerant isolates are recorded in table (1). All forty-eight halotolerant microbial isolates grew well on HCM and SMA media supplemented with 10% NaCl. Thirty two isolates succeeded to grow on HCM medium containing 25% NaCl. The results showed in table (2) also that twenty two microbial isolates thrived at 30% NaCl. Fifteen halotolerant bacterial isolates were selected as the most halotolerant isolates which showed good growth at 25% NaCl and moderately or weakly grew at 30% NaCl.

Eight halotolerant bacterial isolates were selected for the previously fifteen which grew at 25 and30 % NaCl according to their high total protein content and vigorous growth. Six isolates were selected due to their ability to grow on peptone sea water and yeast extract sea water media, these isolates were BQW27, BHW34,

BEW40, BEW45, BAS47 and BAW48. Yeast extract sea water medium gave high bacterial protein content as compared to peptone sea water medium. Results recorded in table (3) showed that the maximum amount of protein content was obtained by BEW45 growing on sea water of Alexandria followed by the same isolate on sea water of Qarun lake, whereas BQW27 exhibited significant growth when grown on Red sea water, followed by bacterial isolate BEW40 and BHW34 growing on Red sea water (Hurghada) medium. Three bacterial isolates were chosen as the potent halotolerant bacterial isolates viz. BEW45, BAS47 and BAW48 which gave the highest reduction value of most salt ions in sea water (removal percentage %). Desalination value by BEW45 as Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} concentrations percentage in case of Qarun water were recorded values up to 63,61,47,61, and 20 % respectively. BAS47 recorded removal percentage (%) were 72,11,11,51 and 49% respectively, while in case of BAW48 showed removal percentages (%) 45, 19,0, and 0 % respectively. In case of Mediterranean sea water, desalination by BEW45 as Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} salt ions removal percentage(%)obtained at levels of 83,47,36,26, and 25 % respectively. Results recorded in table (4) and presented graphically in figure (1) showed that, the combination of BEW45, BAS47, and BAW48 was the most optimum combination favorable for sea water desalination. At this combination Ca^{++}, Mg^{++}, Cl^{-}, Na^{+}, and K^{+} concentrations recorded as 401,2575,16437,and 676 mg/l, respectively and ion removal percentage (%) achieved 34,11,8,5 and 46% respectively.

***Identification of the three potent halotolerant bacterial isolates*:** Preliminary examination of the three potent halotolerant bacterial isolates on the bases of cell shape and arrangement, Gram reaction, and oxygen requirement were divided into three groups as follow: Group (A): Aerobic Gram-positive bacilli; Group (B): Aerobic Gram –positive cocci and Group (3): Aerobic Gram –negative bacilli. Using Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994), these halotolerant bacterial isolates were suggested to be belonging to *Sporohalobacter marismortui* ; *Marinococcus hispanicus* and *Halomonas elongata*, thus they could be given the tentatively identified as *Sporohalobacter marismortui*BEW45; *Marinococcus hispanicus*BAS47**;** and *Halomonas elongata* BAW48.

Parameters controlling desalination process and optimization of some nutritional and environmental conditions for the three potent halotolerant bacterial isolates:

Since *Sporohalobacter marismortui* BEW45, *Marinococcus hispanicus* BAS47 and *Halomonas elongata* BAW48 proved to be the potent bacterial strains, they were selected purposely for investigating some factors affecting desalination of sea water under laboratory conditions. Such factors included: incubation period, incubation temperature, pH, inoculum size and supplementation of different carbon and nitrogen sources and then evaluating the sea water desalination by applying all the previously determined optimal conditions. Optimum incubation period was 168 hr. At this incubation period, the concentration of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} in sea water were 414, 2911, 19619, 10533 and 1451 mg/l respectively in comparable with control and total protein content (mg/ml) was 0.426±0.036. Also, the salts reduction value (%) of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were 19, 7, 0.3, 8, and 45 respectively. Interestingly, the fact that this bacterial combination which was allowed to grow on sea water samples exhibited its maximum ability to sea water desalination by incubation at 37ºC, at this particular temperature the concentrations of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were 321, 2623, 19674, 11204 and 998 mg/l respectively and the total cell protein content (mg/ml) was 0.411±0.028, where the ions removal (%) for Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were 43, 15, 4, 12 and 7 respectively. Also, it was found that pH 6.0 was the optimum pH for desalination by combination among the three potent bacterial strains. At this particular pH, the concentrations of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+}, and K^{+} were 400, 2255, 16661, 12692 and 870 mg/l respectively, and bacterial protein content was 0.337±0.023, where the removal of ions (%) for Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were 49, 24, 15, 2 and 20 respectively. It was found that the most optimum inoculum size enhancing sea water desalination by combination among the three potent bacterial isolates was 2.5 ml of the bacterial suspension. At this particular inoculum size, the concentrations of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were in the range of 500, 3015, 18788, 11785 and 882 (mg/l) respectively. The highest ions reduction value (%) of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+}, and K^{+} were also found to be 43, 11, 8.6, 7 and 7.3 respectively. Total protein content (mg/ml) was 0.364±0.016 (mg/ml) with inoculum size 2.5ml. In addition, it was found that the optimum nitrogen source was yeast extract by combination among *S. marismortui* BEW45, *M. hispanicus* BAS47, and *H. elongata* BAW48 for desalination of sea water which gave higher reduction value of sea water ions than other nitrogen sources. The concentrations of

Ca^{++}, Mg^{++}, Cl^{-}, Na^{+}, and K^{+} were 350, 2982, 20383, 11820 and 956 (mg/l) respectively and total bacterial protein content was 0.313±0.017. The ions reduction value (%) for Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were found to be 53, 8.5, 8.7, 9.1, and 1.1 respectively. All tested carbon sources not exhibited any increase in reduction value of ions in sea water. By incubation under shaking condition the concentrations of Ca^{++}, Mg^{++}, Cl^{-}, Na^{+}, and K^{+} in sea water were 437, 2492, 15302, 6982, and 665 mg/l respectively. The reduction value (%) for Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} were 51, 26, 26, 45, and 30 respectively and the total bacterial protein at shaking conditions was 0.341±0.008 (mg/ml) (Tables 5and6).

Effect of ultra violet (UV) irradiation on combination of *S. marismortui BEW45*, *M. hispanicus BAS47* and *H. elongata BAW48* on desalination of sea water:

In fact, all the present results proved that UV could be considered as mutagenic agent for a combination among *S. marismortui* BEW45, *M. hispanicus* BAS47 and *H. elongata* BAW48 without any significant changes for their ability on the desalination process of sea water (Figure 2).

Repeated recycling of sea water by a combination of halotolerant bacterial isolates:

Results recorded in table (6), and represented graphically in figure (3), after the first step of bacterial desalination Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} concentration of sea water were 489, 2458, 15638, 7247 and 668 (mg/l) respectively, and the reduction value (%) were 48, 35, 37, 39, and 32 respectively. After the second step of bacterial desalination Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} concentrations of sea achieved value of 197, 1247, 7943, 3445 and 423 (mg/l) respectively, and the reduction values (%) obtained were 79, 67, 68, 71 and 57 respectively. After the third step of bacterial desalination, Ca^{++}, Mg^{++}, Cl^{-}, Na^{+} and K^{+} concentrations of sea water were 113, 832, 5213, 2832 and 315 (mg/l) respectively, and the reduction value (%) were 88, 78, 79, 76, and 68 respectively. It was clear that the reduction value (%) for Ca^{++} was 48 and increased to 88% after the third desalination step, Mg^{++} was 35% and achieved of 78% by performance of the third step, Cl^{-} was 37% and reached up to 79% after carrying out the third desalination step, Na^{+} was 39% which give a value of 76% at the end of the third step, and K^{+} was 32% and reached up to 63% after the final bacterial desalination step.

Application of halotolerant bacterial desalinated of sea water strains in the irrigation of *Hordeum vulgare* in experimental fields **:-**

The desalinated water was used in irrigation of *Hordeum vulgare* Giza 2 which is characterized by growth in fewer amount of water under salt stress. As shown in table (8) the control shoot length was 5.7±0.264cm but in case of plants irrigated by recycled treated sea water were 4.863±123. The root length of control plant was 10.66±0.104 cm. but in case of plants irrigated by the treated sea water were 6.633±0.55. Chlorophyll (a) content for control plants was 7.7±0.173 mg/g whereas the same for test plants was 6.1±0.0404 (mg/g). Also chlorophyll (b) content for control plants was 4.43±0.02 (mg/g) while for treated plants was 4.41±0.242 (mg/g). Finally carotenoid content recorded 2.5±0.264 (mg/g) for control plants while for test plants was 2.173±0.125 (mg/g). It was found that irrigation with microbiologically desalinated sea water did not vary remarkably from that of the tap water.

Discussion

The population of the world has been greatly increasing water is the limiting factor for life in the universe. Although, covers about three quarters of the earth's surface, only 3% is fresh water from various sources. Thus, water treatment is usually needed, and the desalination process is the most efficient method for obtaining fresh water from sea water. Few years ago attempts were made for utilizing power plants in sea water desalination which was one of the modern trends to overcome the gap between the increasing water demands and the shortage in water resources in the world. There were few number of studies on microbiological desalination of salty water using a microbiological system. Accordingly the present study was conducted mainly to eliminate salts from saline water using a microbiological system. The most potent in the present study were identified as *Sporohalobacter marisomartui* BEW45, *Marinococcus hispanicus* BEW47 and *Halomonas elongata* BAW48. Das-Sarma and Arora(2001) mentioned examples of most widely distributed halophilic microorganisms include *Halobacterium* sp., *Marinococcus*, *Bacillus*, *Salinococcus* and *Tetragenococcus.* In a recent study, it become clear that the abundance of bacterial communities in the highly saline environments is comparable to those found in the normal marine zones. To be able

to live at high salt concentrations, halophilic and halotolerant organisms must maintain a cytoplasm that is osmotically isotonic with the outside medium (Dennis and Shimmin,1997; Ventosa *et al*.,1998).Halophilic posses many hydrolytic enzymes such as DNAses, lipases, amylases, gelatinases and proteases capable of functioning under conditions that lead to precipitation of most proteins (Das-Sarma and Arora,2001; Kirkar, 2004). Although the current commercial uses of the halophiles are quite significant and many novel and unique properties of many of these organisms, suggest that they have even greater potential for desalination biotechnology.

Qasim (1999) and Qasim *et al*., (2000) showed that the removal of total dissolved solids from water was described as desalination, desalting or salt water-conversion. The desalination factories in Egypt are rare in comparison to Saudi Arabia and Kuwait; because costs of the desalination are generally higher than the costs of other water supply alternatives (e.g. water transfer and groundwater pumping). The growing potable water demand in Red Sea and Sinai resorts lead to organize a national plan for implementation of desalination technology. In accordance to our results, Kargi and Dincer, (1998) used halotolerant bacteria viz. *Halobacter halobium* supplemented with activated sludge culture in saline wastewater treatment in aerated rotating bidisc contactor. In addition, Ammar and Muharram (1998) and Ammar *et al*.,(2000) studied the application of microbial biotechnology in sea water desalination and used five different fungal isolates belonging to *Aspergillus fumigatus*. The present results were in agreement to some extent with those obtained by Gandbhir *et al.,* (1995) who mentioned that, *Haloferax mediterranei* required high salt concentrations for growth while *Halomonas elongata*, a halotolerant eubacterium was able to grow at any salt concentrations. Similar results were reported by Patel *et al.,* (1993) who found that halophilic *Halobacterium halobium* S9 grew well on media containing 25 % NaCl. It has been reported that, *Halomonas elongata* was able to live in a wide range of salt concentration (0.05- 3.4 M), because it versatile cell physiology in ways which increased the structural integrity of walls and increased amount of negatively charged lipids (Vreeland *et al.,* 1991). In agreement to the present results, Blum *et al.,* (2001) showed that proteins, peptones and some amino acids (asparagine, proline, and glutamic acid) supported good growth with halotolerant and halophilic bacteria. Similar results were obtained by Oren (1995) who found that the

respiratory activity was stimulated more than two folds by addition of glycerol, but not by the addition of any of other carbon source tested, including sugars, organic acids, and amino acids, or by addition of inorganic nutrients. A similar respiratory activity was also found by diluting the Dead Sea water samples with distilled water and incubation in light. In addition, Studdert *et al.*,(1997) used a medium containing 2 % yeast extract, and 20 % NaCl for studying haloalkalophilic bacterial species. Johnsen *et al.,* (2001) isolated some halophilic and halotolerant bacterial isolates utilizing peptides, yeast extract and proteins as carbon sources. Blum *et al.*, (2001) isolated some halotolerant isolates using some components of the yeast extract as electron donor. It was obvious that, the bacterial desalination process by recycling of the sea water several times through the three repeated desalination process was much better for desalination of sea water. In an attempts to discuss the resistance of bacteria to high salt concentration, several investigations showed that bacteria allowed growth at high salinity, accumulate one or more specific solutes in the cell. Thus bacteria contradict the out flow of water molecules by the accumulation of these solutes which are called compatible solutes or organic osmolytes, thus, bacteria can adapt themselves to high osmolarity environment. This stress reaction generally referred to as osmoregulation or osmoadaptation, and aimed to maintain turgor and volume within boundaries acceptable for normal cellular physiology. The primary response to osmotic stress in bacteria is mainly the accumulation of K^+ ions. Potassium is a major cytoplasmic cation in growing bacterial cell and play an important role in cell physiology. Cellular K^+ activates various cytoplasmic enzymes and is required for protein synthesis. For these physiological processes, bacteria had evolved diverse potassium transport system. It was reported that potassium and ammonium ions exert stimulatory effect upon glucose fermentation activity. Magnesium play several important metabolic functions in the production and transport of energy. Magnesium is involved in the synthesis of protein, and it assist in the functioning of some enzymes. All of these results throw light upon the significance of bacterial desalination of sea water as a good objective in sea water desalination. Due to the lack information's about the desalination of sea water through the microbial system, it is beneficial to perform further investigations in this vital field. After all, the sooner this technology is transferred from ideas on paper to projects in the field, the sooner that target populations can start to benefit from improved access to water.

Table (1): Halotolerant microbial isolates obtained from four water and five soil samples on HCM and SMA media.

Isolate no.	Water isolates.		Isolate no.	Soil isolates.	
	Code no.	Sample collection localities		Code no.	Sample collection localities
1	BAW48	Alexandria	23	BAS25	Alexandria
2	BEW39	Ein-Helwan	24	BAS47	
3	BEW40		25	BTS4	El-Toor
4	BEW41		26	BES9	Ein-Helwan
5	BEW42		27	BES10	
6	BEW43		28	BES19	
7	BEW44		29	BES20	
8	BEW45		30	BES21	
9	BEW46		31	BES23	
10	BHW31	Hurghada	32	BSS11	Saint-Katren
11	BHW32		33	BSS12	
12	BHW34		34	BSS13	
13	BHW35		35	BSS14	
14	BQW26	Qarun lake	36	BQS1	Qarun lake
15	BQW27		37	BQS2	
16	BQW28		38	BQS3	
17	BQW29		39	BQS5	
18	BQW30		40	BQS6	
19	BQW33		41	BQS7	
20	BQW36		42	BQS8	
21	BQW37		43	BQS15	
22	BQW38		44	BQS16	
			45	BQS17	
			46	BQS18	
			47	BQS22	
			48	FQS24	

BAW and BAS: Bacteria isolated from Alex. Sea water, and soil samples.
BQW and BQS: Bacteria isolated from Qarun lake water, and soil samples.
BEW and BES: Bacteria isolated from Ein-Helwan water, and soil samples.
BHW: Bacteria isolated from Hurghada sea water sample.
FQS: Fungus isolated from Qarun soil sample.
BTS: Bacteria isolated from El-Toor soil sample.
BSS: Bacteria isolated from Saint-Katren soil sample.

Table (2): Qualitative growth estimation of the forty eight halotolerant microbial isolates at different concentrations of sodium chloride (%).

No.	Microbial code number.	NaCl concentrations (%).					
		10	15	20	25	30	35
1	BQS 1	GG	GG	GG	GG	MG	NG
2	BQS 2	G	WG	NG	NG	NG	NG
3	BQS 3	MG	G	NG	NG	NG	NG
4	BTS 4	GG	GG	G	G	WG	NG
5	BQS 5	GG	MG	WG	NG	NG	NG
6	BQS 6	G	WG	NG	NG	NG	NG
7	BQS 7	GG	MG	G	NG	NG	NG
8	BQS 8	GG	MG	WG	WG	NG	NG
9	BES 9	G	WG	NG	NG	NG	NG
10	BES 10	GG	MG	WG	WG	NG	NG
11	BSS 11	MG	G	NG	NG	NG	NG
12	BSS 12	G	NG	NG	NG	NG	NG
13	BSS 13	GG	MG	WG	WG	NG	NG
14	BSS 14	GG	GG	G	G	NG	NG
15	BQS 15	GG	MG	WG	G	NG	NG
16	BQS 16	GG	GG	MG	MG	WG	NG
17	BQS 17	GG	MG	MG	MG	G	NG
18	BQS 18	MG	G	NG	NG	NG	NG
19	BES 19	G	WG	NG	NG	NG	NG
20	BES 20	MG	MG	MG	G	NG	NG
21	BES 21	G	WG	NG	NG	NG	NG
22	BQS 22	GG	GG	G	G	NG	NG
23	BES 23	G	NG	NG	NG	NG	NG
24	FQS 24	G	NG	NG	NG	NG	NG
25	BAS 25	GG	GG	GG	GG	WG	NG
26	BQW 26	GG	GG	MG	G	NG	NG
27	BQW 27	GG	GG	GG	GG	WG	NG
28	BQW 28	GG	GG	GG	GG	WG	NG
29	BQW 29	G	NG	NG	NG	NG	NG
30	BQW 30	GG	GG	MG	MG	G	NG
31	BHW 31	GG	GG	GG	GG	MG	NG

Table (2): Cont.

No.	Microbial code number.	NaCl concentrations (%).					
		10	15	20	25	30	35
32	BHW 32	GG	GG	GG	MG	G	NG
33	BQW 33	GG	GG	GG	GG	G	NG
34	BHW 34	GG	GG	GG	GG	G	NG
35	BHW 35	GG	GG	GG	MG	NG	NG
36	BQW 36	GG	GG	GG	GG	G	NG
37	BQW 37	GG	MG	NG	NG	NG	NG
38	BQW 38	GG	GG	MG	MG	WG	NG
39	BEW 39	GG	GG	MG	MG	WG	NG
40	BEW 40	GG	GG	GG	GG	WG	NG
41	BEW 41	GG	GG	GG	GG	G	NG
42	BEW 42	GG	GG	NG	WG	NG	NG
43	BEW 43	GG	GG	GG	GG	G	NG
44	BEW 44	GG	MG	G	NG	NG	NG
45	BEW 45	GG	GG	GG	GG	MG	NG
46	BEW 46	GG	GG	GG	GG	MG	NG
47	BAS 47	GG	GG	GG	GG	G	NG
48	BAW 48	GG	GG	GG	GG	WG	NG
Total number		48	44	34	32	22	0
Percentage (%).		100	91.66	70.83	66.66	45.83	0

GG: Good growth. MG: Moderate growth G: Growth. WG: Weak growth. NG: No growth.

Table (3): Determination of the bacterial protein content of the best six halotolerant bacterial isolates.

No.	Isolates	Total protein content (mg/ml)		
		Red sea (Hurghada)	Qarun lake (El-Fayoum)	Mediterranean (Alexandria)
1	BQW 27	0.456 ± 0.045	0.286 ± 0.023	0.280 ± 0.028
2	BHW 34	0.372 ± 0.009	0.190 ± 0.015	0.272 ± 0.019
3	BEW 40	0.386 ± 0.070	0.364 ± 0.021	0.246 ± 0.006
4	BEW 45	0.347 ± 0.023	0.480 ± 0.015	0.530 ± 0.027
5	BAS 47	0.279 ± 0.026	0.326 ± 0.124	0.324 ± 0.047
6	BAW 48	0.176 ± 0.029	0.308 ± 0.008	0.360 ± 0.025

Table (4): Determination of ions concentrations and bacterial protein content in sea water samples after desalination.

No.	Code number of bacterial isolate.	Ion concentration (mg/l).					Total protein content (mg/ml).
		Ca^{++}	Mg^{++}	Cl^{-}	Na^{+}	K^{+}	
	Control	611	2909	17902	8460	1262	0.0
1	BEW 45	421	2618	17364	8206	909	0.360 ± 0.03
2	BAS 47	513	2647	17364	8290	971	0.305 ± 0.01
3	BAW 48	439	2676	17185	8290	984	0.402 ± 0.02
4	BEW45 and BAS47	437	2610	17257	8290	857	0.603 ± 0.051
5	BAS47 and BAW48	434	2675	16980	8333	740	0.409 ± 0.008
6	BAW48 and BE45	457	2765	17164	8119	841	0.360 ± 0.038
7	BEW45, BAS47 and BAW 48	401	2575	16437	8037	676	0.413 ± 0.003

Table (5): Summary of the best reduction in ion concentration sea water and bacterial protein content of all optimal parameters for desalination by combination of most potent halotolerant bacterial strains viz. *S. marismortui* BEW45, *M. hispanicus* BAS47, and *H. elongata* BAW48.

Parameter	Ion concentration (mg/l).										Protein content (mg/ml).	
	Ca^{++}		Mg^{++}		Cl^{-}		Na^{+}		K^{+}			
	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Cont.	Treat.	Control	Treat.
Incubation period (168 hours).	511	414	3119	2911	19674	19619	11475	10533	2665	1451	0.00	0.426±0.036
Bottle capacity (250 ml).	561	321	3087	2623	20384	19674	12687	11204	1074	998	0.00	0.389±0.011
Temperature (37°C).	561	321	3087	2623	20384	19674	12687	11204	1074	996	0.00	0.411±0.028
pH (6.0).	781	400	2954	2255	19674	16661	12968	12692	1096	870	0.00	0.337±0.023
Inoculum (2.5 ml) (Each ml contained $67X10^{7}$CFU)	882	500	3380	3015	20561	18788	12678	11785	951	882	0.00	0.364±0.016
Nitrogen source (yeast extract).	744	350	3258	2982	22333	20383	13002	11820	967	956	0.00	0.313±0.017
Shaking condition at 100 rpm	882	437	3380	2492	20561	15302	12678	6982	951	665	0.00	0.341±0.008

Table (6): Summary of reduction of ion values (%) in sea water under all optimal parameters for desalination by combination of potent halotolerant bacterial strains viz. *S. marismortui*BEW45, *M. hispanicus*BAS47, and *H. elongata*BAW48.

Parameter	Ions reduction value (%).									
	Ca^{++}		Mg^{++}		Cl^{-}		Na^{+}		K^{+}	
	Cont	Treat	Cont	Treat	Cont	Treat	Cont	Treat	Cont	Treat
Incubation period (168 hours).	100	19	100	7	100	0.3	100	8	100	45
Bottle capacity (250 ml).	100	43	100	15	100	4	100	12	100	7
Temperature (37°C).	100	43	100	15	100	4	100	12	100	7
pH 6.0	100	49	100	24	100	15	100	2	100	20
Inoculum size (2.5 ml).	100	43	100	11	100	8.6	100	7	100	7.3
Nitrogen source (yeast extract).	100	53	100	8.5	100	8.7	100	9.1	100	1.1
Shaking conditions at 100 rpm	100	51	100	26	100	26	100	45	100	30

Table (7): Determination of ions concentration after bacterial desalination of sea water by combination of *S. marismortui* BEW45, *M. hispanicus* BAS47, and *H. elongata* BAW48 for several treatments.

Treatment stage	Ions concentration (mg/l).				
	Ca^{++}	Mg^{++}	Cl^{-}	Na^{+}	K^{+}
Control	940	3781	24822	11880	983
First step	489	2458	15638	7247	668
Second step	197	1247	7943	3445	423
Third step	113	832	5213	2832	315

Table (8): Effect of repeated seawater bacterial desalination on the growth and pigments of *Hordeum vulgare* Giza 2 after 21 days of irrigation.

Parameter	Control (Tap water).	Desalinated sea water.
Shoot length (cm).	5.7 ± 0.264	4.863 ± 0.123
Root length (cm).	10.66 ± 0.104	6.633 ± 0.550
Plant weight (g).	0.75 ± 0.21	0.603 ± 0.011
Chlorophyll a (mg/g).	7.7 ± 0.173	6.1 ± 0.404
Chlorophyll b (mg/g)	4.43 ± 0.02	4.41 ± 0.242
Carotenoid. (mg/g).	2.5 ± 0.264	2.173 ± 0.125

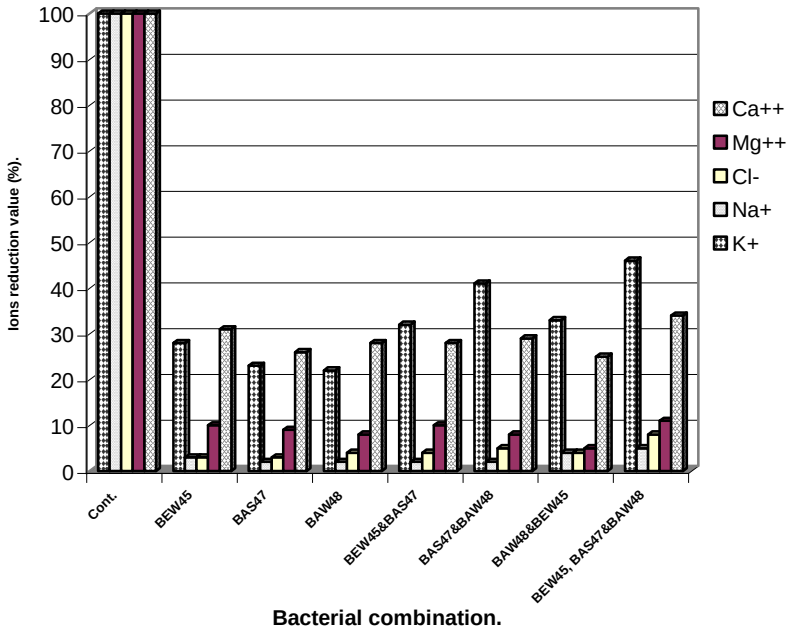

Figure (1): Determination of reduction ions (%) of ions in sea water by the combination of three most potent bacterial strains.

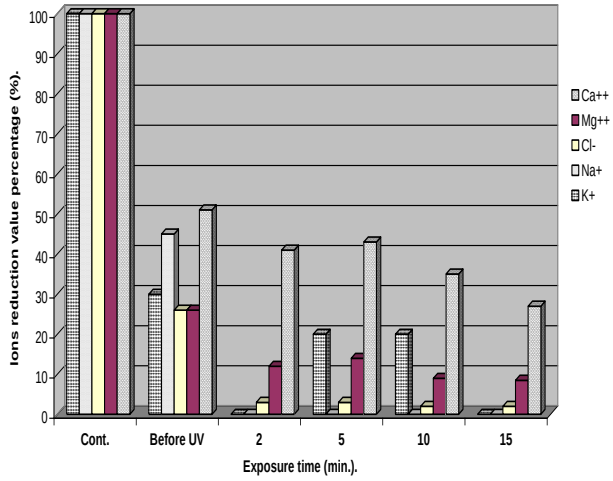

Figure (2): Determination of reduction (%) of ions in sea water by the combination of *S. marismortui* BEW45, *M. hispanicus* (BAS47), and *H. elongata* BAW48 due to UV-irradiation.

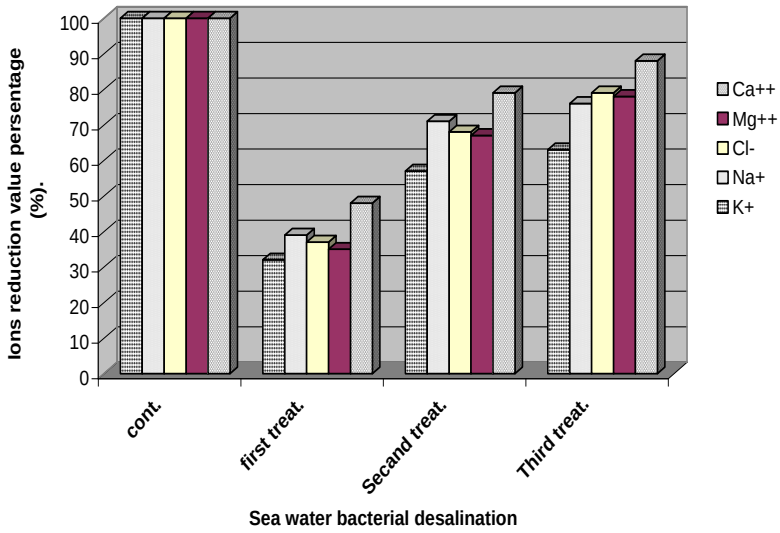

Figure (3): Determination of reduced ions (%) in repeated sea water by repeated desalination using the combination of *S. marismortui* BEW45, *M. hispanicus* BAS47 and *H. elongata* BAW48.

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نظم بكتيريولوجية كاتجاه جديد لإزالة ملوحة المياه المالحة

رضا احمد بيومى , سميرصلاح اللبودى, ممدوح سالم الجمل , محمد عبد الواحد

مـــن

قسم النبات والميكروبيولوجى– كلية العلوم(بنين)– جامعة الأزهـر– مدينة نصر–القاهرة–

الرقم البريدى: 11884.

تم عزل عدد 48عزلـه ميكروبيـة محبـة للملوحـة من عينـات من المياه والتربـة المالحـة تم جمعها من مياه البحر الأحمر (الغردقـة) والبحر الأبيض المتوسط (الإسكندرية) وحلوان (عين حلوان) والفيوم (بحيرة قارون). تم جمع عينات التربة من مناطق الإسكندرية وعين حلوان وبحيرة قارون و منطقة الطور وسانت كاترين بسيناء. تم اختيار عدد 3 عزلات بكتيريـة كأقوى عزلات محبة للملوحة لإزالة ملوحة مياه البحر التي أظهرت أفضل نسبة فـي إزالـة ملوحـة المياه المالحـة. تـم تعريـف العزلات الثلاثـة باستخدام المفـاتيح العلميـة العالميـة المعروفـة حيث تبين أن العزلات تنتمـــي إلـــى: ســـبوروهالويكتر مـــاريزمورتي *Sporohalobacter marismortui* BEW45 ومـارينوكوكس هيسبانيكس *Marinococcus hispanicus* BEW47 وهالومونـاس الونجاتـا *Halomonas elongata*BAW48. اظهر الخلط بين العزلات البكتيرية الثلاثة المحبة للملوحـة نسبة إزالة عالية للملوحة. أفضل نسبة ازالـة للملوحـة تم الحصول عليها بواسطة خليط البكتيريا عند درجة حرارة 37ºم واس هيدروجيني 6 وفترة تحضين 168 ساعة وباستخدام حقنـة من معلق خليط البكتيريا حجمها 2.5 مل الذي يحتوي علي 10×67^{7} CFU ومصدر نيتروجين مستخلص الخميرة وباستخدام الحضانة الهزازة (التهوية). تم التوصل إلـى نسبة إزالـة لملوحـة الكالسيوم إلـي 88% والماغنسيوم 78% والكلور79% والصوديوم 76% والبوتاسيوم 63%. تم ري نبات الشعير (جيزة 2) بالمـاء الذي تم إزالـة ملوحتـه بواسطة البكتيريـا . توصـى الدراسـة بإمكانيـة إزالـة ملوحـة المياه المالحـة باستخدام السـلالات البكتيريـة المختـارة عن طريـق تصميم محطـات لإزالـة الملوحـة مماثلـة لمحطـات معالجـة ميـاه الصـرف الصـحي لتسـتخدم فـى ري بعـض المحاصـيل الاقتصـادية وأشجار الغابات الخشبية.