SALT INDUCIBLE-PROTEINS AND CONJUGAL GENE TRANSFER OF HALOTOLERANT *Staphylococcus* ISOLATED FROM SALINITY SOIL

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Halotolerent bacteria are those that can tolerate a broad range of NaCl concentrations (0-32% w/v) (Hezayen *et al.*, 2010). So, there are different categories of halotolerant microbes: not tolerate, those which tolerate only a small concentrations of salt about 1%, slightly tolerant (6-8%), moderately tolerant (18-22%) and extremely tolerant, those microbes that grow over the whole range of salt concentrations (0-32%) (Larsen, 1986). Recently, Parthiban *et al.* (2010) classified halophilic bacteria according to their salt requirement and growth pattern to slight halophiles which growth at 2-5% NaCl, moderate halophiles which growth at 5-20% NaCl and extreme halophiles which growth at 20-30% NaCl. Extreme halophiles are microorganisms that grow under hostile to most organisms. Some of them, such as bacteria which thrive in hypersaline environments have been recognized for their use in biotechnological remediation applications. The applied of halophilic bacteria include recovery of saline soil by directly supporting of growth of vegetation thus indirectly increasing crop yields in saline soil. The other application of halophilic bacteria was in food and pharmaceutical industries, production of enzymes, polymers and various cosmetic products. The possibility of application of halophilic bacteria in soil is recovery and the importance of microbial diversity in soil (Kannika, 2003).

Haophilic microorganisms respond to high salt external environment by accumulating osmotic in their cytosol, which protects them from cytoplasmic dehydration. Osmophily refers to the osmotic aspects of life at high salt concentrations, especially turgor pressure, cellular dehydration and desiccation. Halophily refers to the ionic requirements for life at high salt concentrations. Halophilic microorganisms usually adopt either at the two strategies of survival in saline environments: compatible solute strategy and salt-in strategy. Compatible solute strategy is employed by the majority of moderately halophlic and halotolerant bacteria, some yeasts, algae and fungi. In this strategy, cell maintains low concentrations of salt in their cytoplasm by balancing osmotic potential through the synthesis or uptake of organic

compatible solutes. Hence these microorganisms are able to adapt to a wide range of salt concentrations. The compatible solutes include polyols such as glycerol, sugars and their derivatives, amino acids and their derivatives as well as quaternary amines such as glycine betaine and ectoines. Compatible solutes display a general stabilizing effect by preventing the unfolding and denaturation of proteins caused by heating, freezing and drying (Ventosa *et al.,* 1998). The salt-in strategy is employed by true halophilies, including halophilic archaea and extremely halophilic bacteria. These microorganisms are adapted to high salt concentrations and cannot survive when the salinity of the medium is lowered. They generally do not synthesize organic solutes to maintain the osmotic equilibrium. This adaptation involves the selective influx of K^+ ions into the cytoplasm. All enzymes and structural cell components must to be adapted to high salt concentrations for proper cell function (Shivanand and Mugeraya, 2011).

Extreme environments such as acidic, thermophilic, hypersaline, and arid regions, are important 'hot spots' of microbial 'megadiversity'. These are habitats of microorganisms which have the genetic and physiological capacity to survive and grow under these harsh or extreme conditions (Woese, 1987; Olsen *et al.*, 1994). Extensive studies have been made in recent years into hyper saline environments resulting in a large number of new halophilic species being isolated e.g. *Oceanobacillus aswanensis,* it was isolated from salted fish sauce in Aswan city, Egypt (Hezayen *et al.*, 2010), *Paenibacillus chungwensis* which isolated from Marakanam salterns in India (Parthiban *et al.*, 2010). Gram-negative halophilic (*Vibrio, Alteromonas*, *Acinetobacter*, *Marinomonas* and *Pseudomonas*) (Prado *et al.,* 1991) and Gram positive halophilic (*Staphylococcus*, *Marinococcus, Sporosarcina Salinococcus* and *Bacillus*) have been recovered from saline soils, salterns and activated sludge (Farrow *et al.,* 1992; Ajibola *et al.,* 2005; Olukanni *et al.,* 2006; Elisangela *et al.,* 2009). *Staphylococcus* spp., *Micrococcus* spp. and *Bacillus* spp. have been isolated from sea water and tropical marine fish but little information has been reported on the species level identities or specific sources of these bacteria (Surendran *et al.,* 1989; Uddin *et al.,* 2001; Rao and Surendran, 2003; Swaminathan *et al.,* 2007; Jeyasekaran *et al.,* 2008).

The main objective of this study was to (1) isolation and characterization of some salt tolerant bacteria from salinity soil in Sharkia Governorate, (2) determine the protein pattern in halophilic bacteria and (3) studying the ability of salt tolerant gene(s) to transfer by natural gene transfer mechanisms.

MATERIALS AND METHODS

This study was carried out in Microbial Genetics Lab., Genetics Dept. and Bacterial Lab., Agriculture Micro-biology Dept., Faculty of Agriculture, Zagazig University, Egypt.

Soil samples

Soil samples were collected from salinity soils of Sharkia Governorate (El-Hessenia and Abo-Kibeer).

Saline solution

The saline solution consisted of 9 g/per liter NaCl (Miller, 1992).

Synthetic medium (SM)

The synthetic medium (SM) consisted of the following components (per liter): NaCl (117 g), KCl (2.0 g), $CaCl_2$ (0.2 g), disodium citrate (3.0 g), tryptone (3.0 g), yeast extract (5.0 g) and distilled water (1L). A 1.5% agar was added when required the medium solidified. The media was adjusted to pH 7.0 with NaOH or HCl and then autoclaved at 121°C for 20 min.

Luria-Bertani (LB) broth

The Luria-Bertani broth consisted of the following components (per liter):tryptone (10.0 g), yeast extract (5.0 g), sodium chloride (5.0 g) and distilled water (1L). The media was adjusted to pH 7.0 with NaOH or HCl and then autoclaved at 121ºC for 20 min (Davis and Byers, 1980).

Antibiotics

All the antibiotics were used in the current study are commercially and purchased from pharmaceuticals. The tetracycline and septazoal were prepared with concentration from 50-500 µg/ml while the streptomycin, ampcillin, chloramphenicol and penicillin were prepared with concentration from 500-2000 µg/ml

Acridin orange

A ten mg/ml concentration of acridin orange was used according to Guha *et al.* (1997).

Phosphate buffer

Phosphate buffer was prepared from 1/15 M potassium phosphate and 1/15 M disodium phosphate.

Bacterial isolation and identification

About 25 g of the soil samples was added to 225 ml of sterile saline solution in sterile flask. The flasks were placed on magnetic stirrer for 15min then serial dilutions were made. A 0.1ml from the above dilutions was spreaded on SM agar plates and incubated at 30ºC for 72 h. A colony of each morphology on SM plates was selected for purification and identification. Identification was carried out according to morphological and biochemical characters according to Krieg and Holt (1984) given in Bergey's manual. The isolates were identified to the genus level by Gram staining and testing for oxidase, catalase, respiratory activity and glucose/lactose oxidation-fermentation and then by their reaction in *API* systems (BioMérieux, Lyon, France) systems). Then confirmed some isolates by using Biolog Microlog1 4.20 system at Cairo Mircen (Cairo Microbiological Resources Center), Ain Shams Univ., Egypt.

Growth/survival rate of Staphylococcus spp. Isolates under different salt concentrations.

The isolates were inoculated in LB medium and incubated overnight at 30°C, 1 ml from each isolate was inoculated in 50 ml SM with different concentrations of NaCl, 11.7, 17.6, 23.4, 29.3 and 35.1% NaCl (w/v)). After interval time (1.0, 2.0, 4.0, 7.0 and 10.0 days), serial dilutions were made to determine the total viable counts of the isolates (Moukhtar *et al.,* 2005). Control was carried out using LB medium.

Sensitivity of Staphylococcus spp. isolates to some antibiotics

Different concentrations of six common antibiotics were used. Loop of liquid culture of each isolate was streaked onto surface of SM agar plates containing antibiotic and the control without antibiotics. All the plates were incubated at 30°C for 72 h.

Lysogenicity test of Staphylococcus spp. isolates

Each strain of *Staphylococcus* (1.0 ml) was inoculated into 50 ml of LB medium, and then placed on a shaker incubator for overnight at 30°C. UV induced agent was used at 5, 10 and 15 min. Centrifugation was carried out at 10,000 rpm for 15 min of UV treated and non-treated bacterial suspension. The supernatant was removed and passed through a sterile membrane (0.45 µm) and spotted onto layer of bacterial cells (Howaida *et al.,* 2007).

Plasmid curing of Staphylococcus spp. isolates

To determine the location of salt tolerant gene (s), plasmid curing was carried out by acridin orange. The isolates were grown overnight in 5 ml LB medium. Next day, 0.2 ml of cultures were added to 5 ml SM liquid medium and incubated for overnight. A 0.2 ml of the previous cultures was added to 2 ml of LB medium containing 10 mg/ml acridin orange. All the tubes were incubated in the dark at 30°C for overnight. A 0.1 ml from the above tubes were spread over LB agar plates and incubated at 30ºC for 24 h. A total of 70 colonies were selected and replicated onto LB and SM agar by replica plate technique (Chin *et al.,* 2005; Ajaz *et al.,* 2009; El-Deeb, 2009).

SDS-PAGE of Staphylococcus spp. isolates proteins

Total cellular protein was extracted as described by Laemmli (1970) and subjected to SDS-polyacrylmide gel electrophoresis. The isolates were inoculated in LB medium and incubated overnight at 30ºC. A 1 ml from each isolate was inoculated into 50 ml SM with different concentrations of NaCl (11.7, 17.6, 23.4, 29.3 and 35.1%), the control was LB medium. All cultures were incubated at 30°C for 24 h. At the end of experiment, bacterial cells were separated from all treatments by centrifugation at 13400 rpm for 10 min then dispersed in saline solution (8.5 g NaCl/L). An aliquot of 50 µL of the bacterial suspension was

combined with 50 µL of the sample buffer (1 M Tris-HCl, 50% glycerol, 10% SDS, 10% β-mercaptoethanol, 0.1% Bromophenol blue, pH 6.8) and heated at 100°C for 10 min then loaded in 12% SDS-PAGE**.** After electrophoresis, the gel was stained in 50 ml of staining solution for 30-45 min with shaking at room temperature and distained in distaining solution. The gel was dried for 20 h and photographed. The photographs were analyzed using Gel Analyzer (Egygene) Software.

Bacterial conjugation

The salt tolerant *Staphylococcus* isolates were used as donors (streptomycin resistant and ampecillin sensitive). The non-tolerant *Micrococcus* sp. was used as recipient (streptomycin sensitive and ampecillin resistant). Overnight cultures of donors and recipient (0.2: 0.2 ml) were mixed and spread onto LB agar plates. The plates were incubated at 30°C for overnight; the mixtures were collected and re-suspended in phosphate buffer. Serial dilutions were made and plated onto selective media for donor (SM agar and str), recipient (LB agar and amp) and transconjugants (SM agar and str and amp).

RESULTS AND DISCUSSION

Quantitative and qualitative assessment of the isolates

Isolation was performed on SM agar. In total 100 isolates out of 60 (60%) of the Gram-positive bacteria were identified in this study and the other 40 isolates (40%) was gram negative bacteria. The gram-positive isolates were distributed in 7 genera: *Staphylococcus, Micrococcus, Bacillus*, *Corynebacterium, Streptococcus, Enterococcus* and *Sporosarcina*. Fifty percent of the Gram-positive bacteria were not identifiable because bacterial growth was insufficient to allow identification. The Gram-negative isolates were distributed in 3 genera: *Pseudomonas*, *Enterococcus* and *Escherichia*. The gram negative isolates were not identifiable to species levels.

Speciation and diversity of Gram-positive bacteria in salinity soil

Using the *Staphylococcus* API test- and Biolog Microlog1 4.20 system at Cairo Mircen, 20 of the isolates were confirmed to be *Staphylococcus* spp. Seven of 20 *Staphylococcus* isolates were identified to species level with 5.0 isolate identified as *S. xylosus*, two as *S. kloosii,* and 5.0 as *S. arlettea*, one as *S. equorum*, four as *S. saprophyticus*, two as *S. gallinarum* and one as *S. succinus*. *Staphylococcus* species accounted 66.67% of the identified Gram-positive bacteria. *Staphylococcus arlettea, S. saprophyticus* and *S. xylosus* were the most frequent species (70% prevalence) representing 25%, 20% and 25% of *Staphylococcus* species respectively. *Staphylococcus* species are known as halotolerant bacteria which habitat in different saline environments such as salted fish (Rifky, 1993), seawater (Kakizaki *et al*., 2008), activated sludge (Elisangela *et al*., 2009),

vegetable pickled plants (Abou-Elela *et al.,* 2010) and from marine fish (Al Bulushi *et al*., 2010). In total, five isolates were identified as *Micrococcus* by conventional biochemical tests. *Micrococcus* species accounted for 16.67% of the identified Gram-positive bacteria. *Micrococcus luteus* was the most dominant *Micrococcus* species. About five isolates of *Bacillus* bacteria were identified to genus level by conventional biochemical tests; these accounted for 16.67% of the identified Gram positive bacteria.

Effect of salinity on growth/survival rate of Staphylococcus species

To assess bacterial salt tolerance, the growth/survival behavior of the bacteria was analyzed in response to increasing salt concentration in. As shown in Fig. (1), little or no bacterial growth could be observed at high salt concentrations (29.3 and 35.1 NaCl) during two days. After two days, the growth of *Staphylococcus* species were reduced to reach under the detection limit except *S. kloosii* (1) which survival under 29.3 or 35.1% NaCl up to 10 days of incubations. Halophilic microorganisms usually adapt to survival in saline environments (Shivanand and Mugeraya, 2011). The optimal growth was occurred at 11.7 to 17.6% NaCl for all *Staphylococcus* species after two days. *S. xylosus, S. kloosii* (1), *S. arlettea* and *S. equorum* are extreme halophiles which growth at 23.4% NaCl. However, other tested bacteria are moderately halophilic which growth at 11.7-17.6% NaCl). This result agreed with the described for moderately halophilic bacteria which suggests the requirement of salt at concentration ranging from 2.9% up to 14.6% NaCl in culture medium (Ventosa *et al.,* 1998; Echigo *et al*., 2005). According to Parthiban *et al.* (2010), the above isolates are moderate halophiles which growth at 5-20% NaCl concentrations. Some of them, such as bacteria which thrive in hyper-saline environments have been recognized for their use in biotechnological remediation applications (Kannika, 2003).

Biochemical genetic variation of cell protein content

Protein contents of *S. xylosus*, *S. kloosii* (1)*, S. arlettea*, *S. equorum*, *S. saprophyticus*, *S. gallinarum*, *S. succinus* and *S. kloosii* (2) cells grown in free salt LB medium and SM medium with different concentrations of NaCl were compared by SDS electrophoretical analysis (Fig. 2 and Table 1). It was observed that, *S. saprophyticus*, *S. gallinarum*, *S. succinus* and *S. kloosii* (2) showed early fading out of most bacterial protein when treated with high salt concentrations (23.4, 29.3 and 35.1%). This observation was as well as with *S. kloosii* (1)*, S. arlettea* and *S. equorum* at 29.3 and 35.1%. Some proteins were disappeared [35 kDa of *S. xylosus,* 119 kDa of *S. arlettea,* 31 kDa of *S. equorum*,120, 93, 66 kDa of *S. saprophyticus*, 30 kDa of *S. gallinarum*,

34 kDa of *S. succinus* and 62, 32 kDa of *S. kloosii* (2)] under salt stress. The others proteins were appeared (58, 97, 77, 44, 78 and 51 kDa of *S. kloosii* (1), *S. arlettea*, *S. equorum*, *S. saprophyticus*, *S. gallinarum*, *S. succinus,* respectively). The intensity of some proteins was also observed (121 and 48 kDa for *S. xylosus,* 58 and 51 kDa for *S. kloosii* (1), 155 and 55 kDa for *S. equorum*, and 43 kDa for *S. kloosii* (2). These results indicate differences in protein content of whole bacterial cells under different salt stress conditions. There was induction of some new proteins not present in the free salt medium and inhibition of production of some others proteins that were produced in the free salt medium. There was also an increase, as well as a decrease in the level of expression of some proteins. All these differences may be directly associated with the bacterial response of to salt stress. The induction of novel proteins or the increased production of already existing proteins, which are only produced under stress conditions, is responsible for stress responses. The decease in production or the inhibition of production of certain proteins is most probably the result of high levels of proteins modification or gene regulation, caused by a decrease in metabolic activity (Faiza *et al.,* 2011).

Antibiotic resistant pattern

An antibiotic resistant is one of the most important criteria for persistence and competition of target bacteria with other bacteria in habitat. Furthermore, pattern of antibiotics is important for identification and scrimination of bacteria. As well as antibiotic resistance could be used as a selectable marker. So, the strains of *Staphylococcus* were tested for resistance of some of common antibiotics. Table (2) shows that, all *Staphylococcus* species were sensitive to ampicillin, chloro-amphenicol and penicillin at all used concentrations (500-2000 µg/ml), except *S. arlettea* and *S. equorum* were resistant to chloroamphenicol at 500 µg/ml so, these 3 antibiotics are most efficiency against the tested strains. On the other hand, all strains were resistant to streptomycin at all concentrations except *S. saprophyticus* and *S. kloosii* (2) at 500 µg/ml only. *S. xylosus, S. arlettea* and *S. equorum* were resistant to septazol at all concentration and tetracycline at 50 or 100 µg/ml. In the past, penicillin, erythromycin, clindamycin, and/or gen-tamicin were used for treating form staphylococcal infections. However, owing to many factors, including the extensive use of these antibiotics, staphylococci have developed resistant populations (Varaldo, 1989). The part of this development may be due to mutations and the other to transfer of plasmids between not only different strains or species (Dale, 1998) but also between genera (Hassan, 2010). It is early known that the plasmids carry a number of different antibiotic resistance genes (Dale, 1998). The resistance of tested strains to antibiotics indicated that these strains have one or more plasmid. The confirmed existence of plasmids in halophilic

bacteria cell has been represented (Arvanitis *et al.*, 1995, Moukhtar, 2005; Ghosh *et al*., 2010).

Lysogenecity test of Staphylococcus species

Lysogenicity test of halotolerant *Staphylococcus* species was carried out to detection of spontaneously released or UV induced bacteriophages (Table 3). No phage was detected, so no strain was lysogen under this current study. The failure of halotolerant *Staphylococcus* species to be lysogens may be due to the effect of salt on the relationship between phage and bacterial cell. Growing lysogenic cells in media containing NaCl concentrations progressively increased the amount of bacteriophage released. So the salt affects on lysogen stability by stimulating mutagenesis of the repressor gene. The salt stress alters the ability of phages to maintain the lysogenic state (Shkilnyj and Koudelka, 2007). These findings are consistent with the ideas that, an increased external NaCl increases Na^+ within the cell, this led to decreases lysogen stability by disrupting repressor DNA interactions. In contrast, other studies showed that successfully isolation of bacteriophages from halophilic bacteria obtained from soil (Calvo *et al*., 1988; Ventosa *et al*., 1998).

Plasmid curing of Staphylococcus species

To determine the location of salt tolerant gene(s), plasmid curing was carried out by acridin orange. It was suggested previously that all isolated strains must be contain plasmid(s) because it were resistant to one or more of antibiotics. Plasmid curing experiments were performed to recognize the role of plasmid in salinity resistance. After curing the strains were placed on SM medium, it found that all selected colonies (70) were tolerated to salt. These results indicated that plasmid(s) do not carry any salt tolerant genes in the used strains. So, the tolerant genes should be carried on chromosomal DNA. These results are in agreement with the results of Moukhtar (2005), it showed that salt tolerant genes are located on chromosomal DNA of *Halococcus salifodinae* and *Bacillus pasteurii*.

Bacterial conjugation

The salt tolerant *Staphylococcus* species were used as donors (streptomycin resistant and ampecilline sensitive). The non-tolerant *Micrococcus* sp. was used as recipient (streptomycin sensitive and ampecilline resistant). Table 4 shows that the number of transconjugants was ranged between 1.19 X 10^3 and 3.28 X 10^3 and conjugation frequency was ranged from 3.3 X 10^{-7} to 9.1 X 10^{-7}. The transconjugants cells were detected on selective media containing 11.7% NaCl salt concentrations, so, all colonies that could grow onto this media must be salt tolerant. These results indicated that the salt tolerant gene(s) were able to transfer by conjugation. Conjugation considered the only genetic transfer mechanism described for the bacterial halophiles.

Natural transformation has not been reported and approaches such as electro-portion or $CaCl_2$ treatment have either been unsuccessful or given no reproducible results (Ventosa *et al.,* 1998). Although some bacteriophages have been described for halophiles, transduction methods have not yet been developed. A majority difficulty in the use of halophiles for genetic transfer experiments is that at their optimal salinity they generally tolerate high concentrations of most antimicrobial agents (Nieto *et al.,* 1993). However, a decrease of the salinity resulted in an enhanced sensitivity of *Halomonas, Chromohalobacter* and *Salinivibrio* strains to many antimicrobials (Coronado *et al.,* 1995). Transfer frequency was affected by many factors such as cell growth phase, mating time, donor-to-recipient ratio and composition as well as salinity of the mating medium (Vargas *et al.,* 1997).

In the future study, these strains may be used to detection and isolation of salt tolerant gene(s) and transfer it to plants. This may be attempting to solve problems of saline soil that reduced agriculture production in over the world. Both physical and chemical methods were not cost-effective for soil reclamation of the reservoir.

SUMMARY

In this study some isolates of Gram positive and negative bacteria were isolated from salinity soil of Sharkia Governorate (EL-Hessenia and Abo-Kibeer). The Gram-positive isolates were distributed in 7 genera: *Staphylococcus, Micrococcus, Bacillus*, *Corynebacterium, Streptococcus, Enterococcus* and *Sporosarcina*. The Gram-negative isolates were distributed in 3 genera: *Pseudomonas, Enterococcus* and *Escherichia*. *Staphylococcus* species had the highest prevalence, at 66.67%, of the identified Gram positive bacteria, followed by *Micrococcus* and *Bacillus* species at 16.67% of each. *Staphylococcus* isolates were identified at species level to *S. xylosus*, *S. kloosii, S. arlettea*, *S. equorum*, *S. saprophyticus*, *S. gallinarum* and *S. succinus***.** The optimal growth of *Staphylococcus* species occurred at 11.7 to 17.6% NaCl concentration. The SDS PAGE analysis of the proteins contents of cells indicated the appearance of high and low molecular weight new proteins (58, 97, 77, 44, 78 and 51 kDa of *S. kloosii* (1)*, S. arlettea*, *S. equorum*, *S. saprophyticus*, *S. gallinarum*, *S. succinus* respectively). The intensity of some proteins was also observed (121 and 48 kDa for *S. xylosus,* 58 and 51 kDa for *S. kloosii* (1), 155 and 55 kDa for *S. equorum,* and 43 kDa for *S. kloosii* (2)). These differences may be directly associated with the bacterial response of to salt stress. *Staphylococcus* species were appeared different susceptibility to tested antibiotics. No one strain of *Staphylococcus* species was lysogen, so no phage was isolated from them. Plasmid curing indicated that plasmid(s) do not carry any salt tolerant

genes in these strains, so this study suggest that salt tolerant gene(s) must be located on the bacterial chromosome. Conjugation considered the only genetic transfer mechanism described for the bacterial halophiles. It was used in this study to studying of the ability of salt tolerant gene(s) to transfer. The number of transconjugants resulted from conjugation between salt tolerant *Staphylococcus* and non-salt tolerant *Micrococcus* was ranged from 1.19 X 10^3 to 3.28 X 10^3, conjugation frequency was ranged from 3.3 X 10^{-7} to 9.1 X 10^{-7}. The salt tolerant gene(s) were able to transfer by conjugation between genera of gram positive bacteria (*Staphylococcus* and *Micrococcus*).

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Table (1): Analysis of protein patters of *Staphylococcus* species

Strains	Concentrations of NaCl %						No. of total bands
	0.0	11.7	17.6	23.4	29.3	35.1	
Staph. xylosus	4	3	3	3	2	2	4
Staph. Kloosii (1)	3	3	2	3	0	0	4
Staph. arlettea	3	2	3	2	0	0	4
Staph. equorum	3	2	2	2	0	0	4
Staph. saprophyticus	6	5	3	0	0	0	7
Staph. gallinarum	3	3	2	0	0	0	4
Staph. succinus	3	3	2	0	0	0	4
Staph. Kloosii (2)	4	2	2	0	0	0	4

Table (2): Sensitivity or resistance of *Staphylococcus* species for different antibiotics.

Antibiotics (µg/ml)	Tetracycline			Septazol			Streptomycin			Ampicillin			Penicillin			Chloromphenicol		
Strains	50	100	500	50	100	500	500	1000	2000	500	1000	2000	500	1000	2000	500	1000	2000
Staph. xylosus	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Staph. kloosii (1)	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Staph. arlettea	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-
Staph. equorum	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-
Staph.saprophyticus	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Staph.gallinarum	+	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Staph. succinus	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Staph. kloosii (2)	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

-: Sensitive +: Resistant

Table (3): Putative phage spontaneously released or UV induced from *Staphylococcus* spp.

Strains	Putative Phage released from							
	Staph. xylosus	*Staph. Kloosii* (1)	*Staph. arlettea*	*Staph. equorum*	*Staph. saprophyticus*	*Staph. gallinarum*	*Staph. succinus*	*Staph. kloosii* (2)
Staph. xylosus	-	-	-	-	-	-	-	-
Staph. Kloosii (1)	-	-	-	-	-	-	-	-
Staph. arlettea	-	-	-	-	-	-	-	-
Staph. equorum	-	-	-	-	-	-	-	-
Staph. saprophyticus	-	-	-	-	-	-	-	-
Staph. gallinarum	-	-	-	-	-	-	-	-
Staph. succinus	-	-	-	-	-	-	-	-
Staph. kloosii (2)	-	-	-	-	-	-	-	-

-: No phage detected

Table (4): Conjugation transfer between halotolerant *Staphylococcus* strains and non salt tolerant *Micrococcus* sp.

Mating		CFU/ml at 30ºC after 24 hour			Conjugation frequency
Donor	Recipient	Donor	Recipient	Transconjugants	
Staph. xylosus	*Micrococcus* sp.	$6.8 X 10^6$	$2.98 X 10^3$	$3.09 X10^3$	$8.6 X 10^{-7}$
Staph. Kloosii (1)	*Micrococcus* sp.	$6.9 X 10^6$	$1.48 X 10^4$	$3.28 X 10^3$	$9.1 X 10^{-7}$
Staph. arlettea	*Micrococcus* sp.	$7.0 X 10^6$	$3.56 X 10^3$	$1.19 X 10^3$	$3.3 X 10^{-7}$
Staph. equorum	*Micrococcus* sp.	$7.12 X10^6$	$1.15 X 10^4$	$3.16 X 10^3$	$8.7 X 10^{-7}$
Staph.saprophyticus	*Micrococcus* sp.	$7.15 X 10^6$	$3.11 X 10^3$	$2.68 X 10^3$	$7.4 X 10^{-7}$
Staph.gallinarum	*Micrococcus* sp.	$6.65 X 10^6$	$1.21 X 10^3$	$2.37 X 10^3$	$6.6 X 10^{-7}$
Staph. succinus	*Micrococcus* sp.	$8.19 X10^6$	$1.36 X 10^3$	$1.23 X 10^3$	$3.4 X 10^{-7}$
Staph. Kloosii (2)	*Micrococcus* sp.	$7.98 X 10^6$	$3.3 X 10^2$	$1.49 X 10^3$	$4.1 X 10^{-7}$

$3.6 X10^9$ CFU/ ml of recipient cultures at zero time

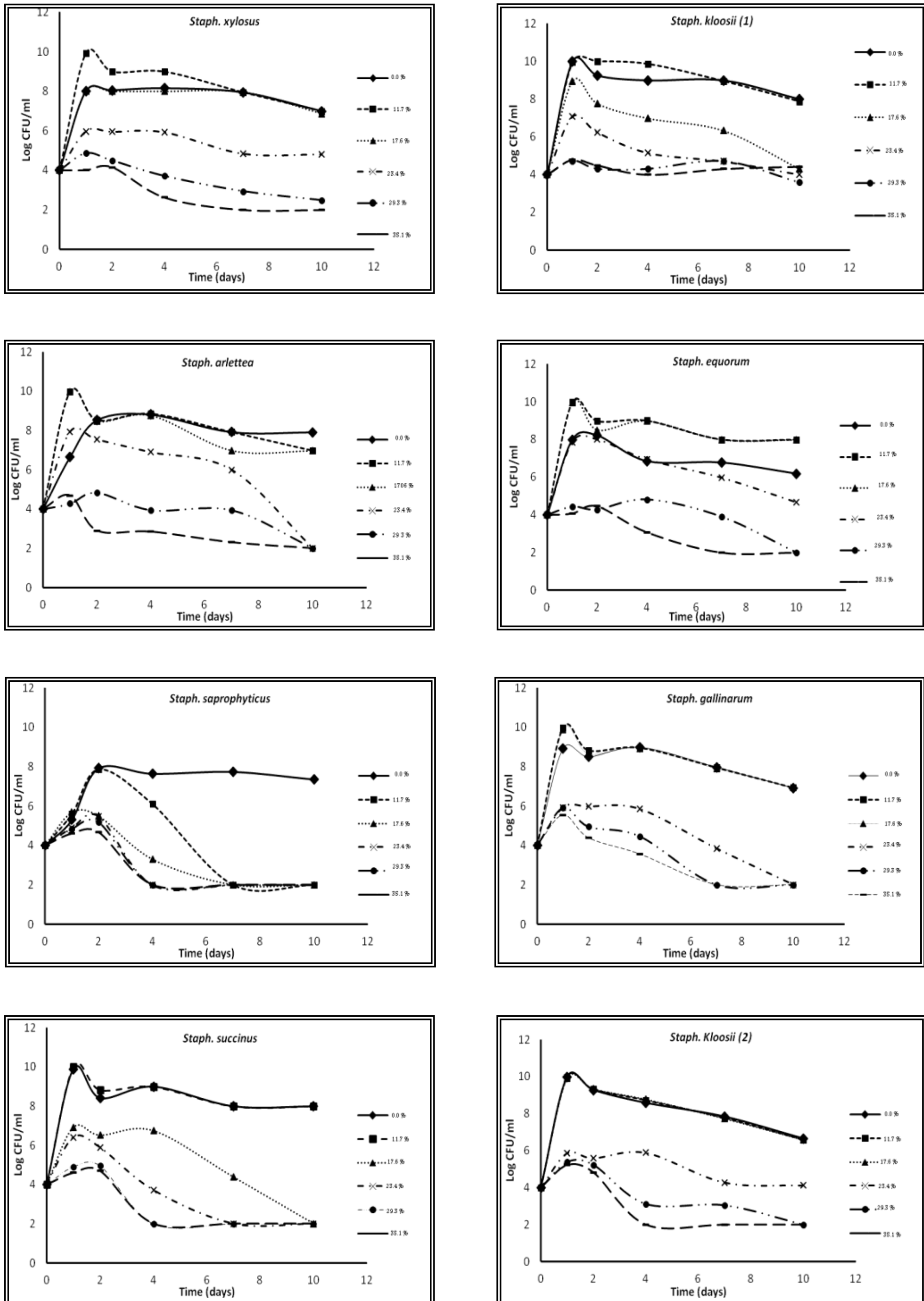

Fig. (1): Growth/survival of *Staphylococcus* species under different concentrations of sodium chloride (0.0, 11.7, 17.6, 23.4, 29.3 and 35.1% NaCl).

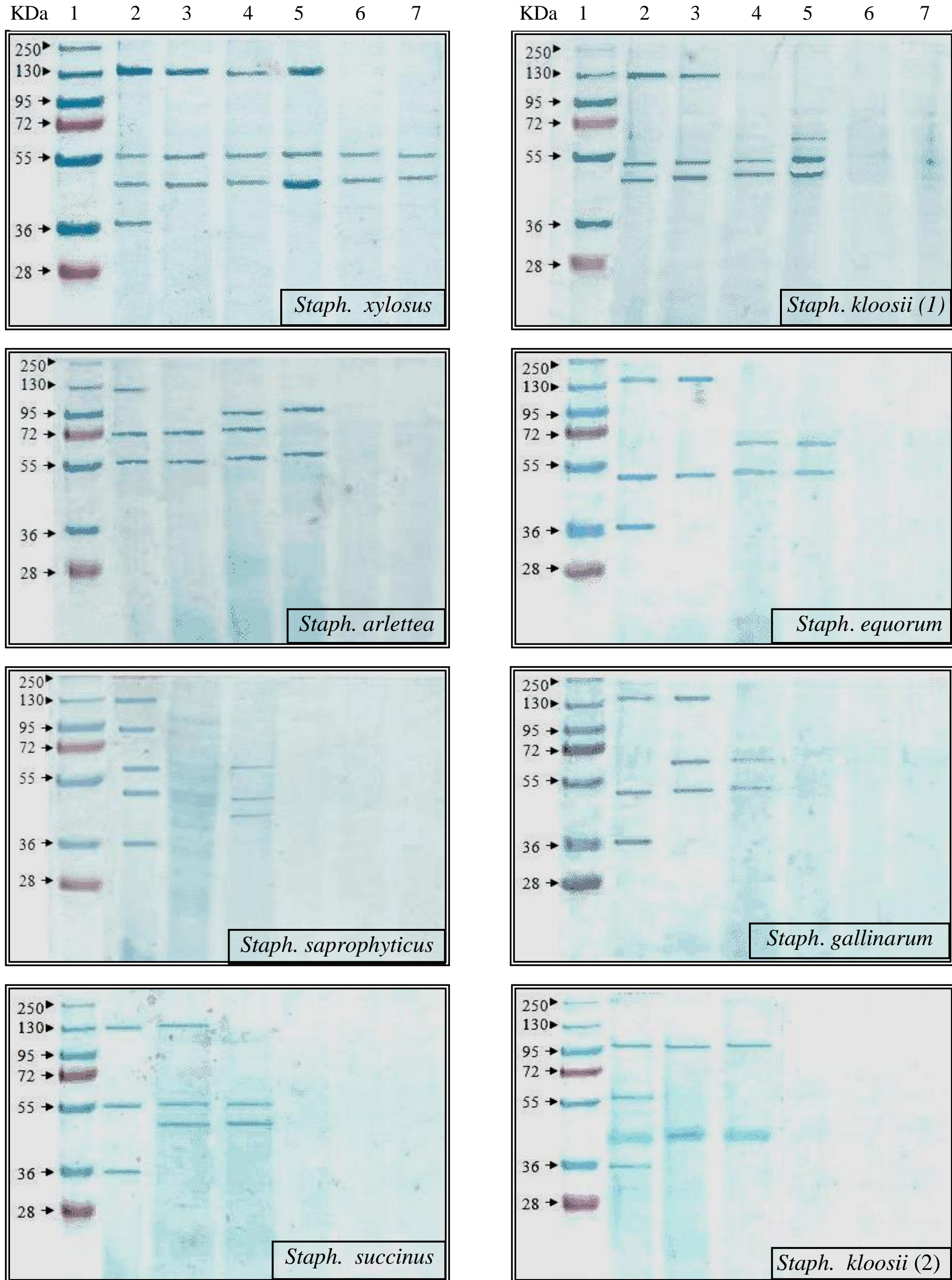

Fig. (2): Electrophoretic banding pattern of total protein of *Staphylococcus* spp. Total protein was separated using a 13% SDS-polyacrylamide gel. Lane 1, Molecular weight marker. Lanes 2, 3, 4, 5, 6 and 7 samples from cultures that were growth or survival in the medium containing 0.0, 11.7, 17.6, 23.4, 29.3 and 35.1% NaCl concentrations.