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ISOLATION OF SALT TOLERANCE GENE(S) FROM SOME HALOTOLERANT *STREPTOMYCES* SPECIES USING POLYMERASE CHAIN REACTION

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ABSTRACT:

Sixteen halotolerant *Streptomyces* strains were tested for their salt tolerance as an attempt to isolate some salt tolerance genes *via* polymerase chain reaction (PCR). A group of these strains were isolated from Sedi-creer (*S. niveus* Sc-2 and *S. sendenensis* Sc-11); El-Malahat (Alexandria) (*S. graminofaciens* Ma-13); Qaroon's lake (*S. albovinaceus* Qa-44, *S. luteofluorescens* Qa-51, *S. albidoflavus* Qa-53 and *S. erthraeus* Qa-84). The other group was isolated from different soils from Damaaita (*S. violans* Da-3), Ismailia (*S. alboflavus* Is-10), Port Said (*S. bobili* Ps-12) and Sinai sandy soil (*Streptomyces* species Si-1, *S. tuirus* Si-4, *S. lateritius* Si-6, *S. hawaiiensis* Si-8, *S. muavecolor* Si-9 and *S. melanogenes* Si-11). Results showed that these strains varied in their salt tolerance range, in particular, with increasing NaCl concentration in the growth medium up to 140g/L. It was also noted that all the applied *Streptomyces* strains tolerated NaCl concentrations up to 70g/L. When NaCl concentration was raised to 105g/L, strains except *S. melanogenes* Si-11, gave moderate growth. On the contrary, NaCl concentration of 140g/L inhibited the growth of 50% of strains under investigation, but the other 50% of these strains gave moderate growth. On the molecular level, PCR was successfully used for isolating the *mtlD* (1150 bp) and *P5CR* (831 bp) genes from three (*S. albovinaceus* Qa-44, *S. albidoflavus* Qa-53, *S. erthraeus* Qa-84) and four (*S. albovinaceus* Qa-44, *Streptomyces* species Si-1**,** *S. luteofluorescens* Qa-51 and *S. lateritius* Si-6**)** strains, respectively. In addition, the fructan-accumulating (*sacB*) gene was detected in *Streptomyces* species Si-1 by amplification of a fragment of a size of about 1665 bp. These results confirmed the ability to use of PCR for isolation or detection of any gene based on its nucleotide sequencing in any microorganism. Furthermore, one can recommend the use of the applied halotolerant *Streptomyces* strains, based on their antimicrobial activities for biologically controlling the phytopathogenic fungi in saline soils.

INTRODUCTION:

Streptomyces is a gram-positive bacterium that undergoes morphological differentiation. In a nutritionally favorable condition, *Streptomyces* spores germinate to give rise to vegetative hyphae, which are characterized by filamentous, multi-genomic cells called mycelia (Kwak and Kendrick, 1996). The occurrence of nonpigmented halotolerant bacteria was probably first mentioned in 1919 by LeFevre and Round in their study of the microbiology of cucumber fermentation brines. One of the bacterial group isolated grew in the range of 0 to 15% NaCl, whereas other bacteria studied exhibited growth over the range of 5 to 25%. Research on the halophilic and halotolerant bacteria often seems to be less glamorous than the study of the archaea, with their unique adaptations, including a highly saline cytoplasm,

specialized salt-requiring proteins, and the unique light-driven proton and chloride pumps bacteriorhodopsin and halorhodopsin (Kushner, 1989).

Approaches to the study of genetic processes have recently been developed for several moderate halophiles, opening the way toward an understanding of haloadaptation at the molecular level (Prior *et al*., 1996; Antón *et al*., 2000; Roessler and Muller, 2002; Gadda and McAllister-Wilkins, 2003; Bellier and Mazodier, 2004; Varela *et al*., 2004; and Tokunaga *et al*., 2004).

A number of investigations concerning the halophilic or halotolerant actinomycetes were done (Hussein and Abbas, 1986; Saleh *et al*., 1990; Zaki *et al*., 1991; Zaki *et al*., 1993; Mohamed *et al*., 2000). In Iraq, Hamdi *et al*. (1980) isolated *Micro-monospora lutea* nov Sp. (The isolate could tolerate up to 7 % NaCl), while, Diab and Al-Gounaim (1986) isolated a new *Streptomyces* species from soil of Kuwait that tolerated 7.5-10% NaCl.

Tarczysnki *et al.* (1992) showed that a bacterial gene encoding mannitol-1-phosphate dehydrogenase, *mtlD*, was engineered for expression in higher plants. Vander-Meer *et al.* (1994) investigated the significance of the metabolism of fructans (polyfructosylsucroses) in plants by studying the advantages of fructan-metabolizing plants over those unable to synthesize and degrade these nonstructural storage carbohydrates using two constructs containing the fructosyltransferase genes of either *Bacillus subtilis* (*sac*B which encodes levansucrase) or *Streptococcus mutans* (ftf). Qiu-DongLiang *et al.* (2002) described several mechanisms related to salt tolerance in plants, i.e., the accumulation of micromolecular osmotic substances (such as proline, betaine, polyol, polyamine and fructan), the synthesis of macromolecular proteins (such as late embryogenesis abundant protein, osmotin, water channel protein, K^+ channel protein), the activity of ATPase and related gene expression. Saito *et al*. (2003) reported that the gene encoding a 2,6-beta-D-fructan 6-levanbiohydrolase (LF2ase) (EC 3.2.1.64) that converts levan into levanbiose was cloned from the genomic DNA of *Streptomyces exfoliatus F3-2*. The gene encoded a signal peptide of 37 amino acids and a mature protein of 482 amino acids with a total length of 1560 bp**.**

This study is aimed to use the polymerase chain reaction (PCR) to isolate some salt tolerance genes from some *Streptomyces* species isolated from salt water and Sinai sandy soil in Egypt.

MATERIALS AND METHODS:

***Streptomyces* isolates source:** In a trial to isolate the salt stress genes from *Streptomyces* (for the first time in Egypt), Sixteen identified strains (Table 1) were used.

Table 1: Sources of halotolerant *Streptomyces* species.

Sources of species	Governorates	Locations	*Streptomyces* species
Marine ecosystem[1]	Alexandria	Sedi-creer	*S. niveus* Sc-2
			S. sendenensis Sc-11
	El-Fayoum	El-Malahat	*S. graminofaciens* Ma-13
		Qaroon's lake	*S. albovinaceus* Qa-44
			S. luteofluorescens Qa-51
			S. albidoflavus Qa-53
			S. erthraeus Qa-84
Soils[2]	Damaaita	Damaaita	*S. violans* Da-3
	Ismailia	Ismailia	*S. alboflavus* Is-10
	Port Said	Port Said	*S. bobili* Ps-12
	Sinai	Sinai	*Streptomyces* species Si-1
			S. tuirus Si-4
			S. lateritius Si-6
			S. hawaiiensis Si-8
			S. muavecolor Si-9
			S. melanogenes Si-11

[1]: As refered by (Saleh *et al.*, 1990). **[2]:** As referred by Mohamed *et al.* (2000) and Mohamed *et al.* (2001).

Detection of salt-tolerance range: To detect the ability of the *Streptomyces* strains to grow on increasing salt concentrations, the method given by Saleh *et al*. (1990) and Mohamed *et al*. (2000) were followed. In the experiment, different concentrations of NaCl [(0.05 (normal salt concentration of the medium), (3.5, 7, 10.5 and 14 %)] were separately added to starch nitrate agar medium (Waksman and Lechevalier, 1961). The inoculated plates were incubated at 28°C±2 up to 15 days to ensure the growth of the tested isolates. The growth of *Streptomyces* strains was determined and recorded as recommended by Mahfouz and Mohamed, (2002). Plates containing salt-free medium were used as control.

Isolation of salt stress genes: Fifty ml in 250-ml conical flask of starch nitrate broth medium (Waksman and Lechevalier, 1961) supplemented with 3.5% NaCl, were inoculated separately with each of the *Streptomyces* strains. After incubation at 28°C±2 for 6 days on a rotary shaker (160-rpm), the mycelium was collected and pulverized in liquid nitrogen (Abdel-Fattah, 2005). To extract, purify and adjust the DNA concentration to 100 ng/µl, the method given by Mahfouz and Mohamed (2002) was followed.

Six oligonucleotide primers specific to three salt tolerance genes (Table 2) were kindly provided by Prof. Dr. A.M. Bahieldin, of Genetics Dept. Ain Shams University, Cairo, Egypt and senior Scientist of Environmental Stress Laboratory, AGERI, ARC, Giza, Egypt. PCR was conduced in a volume of 50 µl (Mohamed *et al*., 2001). Amplification was performed in a Perkin-Elmer (Gene Amp PCR System 2400) Thermocycler for 35 cycles after

initial denaturation for 4 min at 95°C. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min. The primer extension was extended to 7 min at 72°C in the final cycle. The PCR amplified products were detected by electrophoresis on 1.5% agarose gel in 1X TAE buffer at 80 volts for 1 hour (Sambrook *et al*., 1989). PCR fragments were visualized by staining gels with ethidium bromide (0.5 µg/ml) and photographed under UV light using a Polaroid camera.

Table (2): The nucleotide sequences of six oligonucleotide primers used for isolation of three salt tolerance genes.

Primers	Sequences (5'----------3')	Sizes (nt)	EPP (bp)
	P5CR gene		
P1	GGA GAT CTA ACA ATG GAG ATT CTT CCG ATT CCG GCG G	34	831
P2	GGG ATA TCT TAG CTC TGT GAG AGC TCG CGG C	31	
	mtlD gene		
P3	CGA GAT CTA ACA ATG AAA GCA TTA CAT TTG GCG C	34	1150
P4	GGG ATA TCT TAT TGC ATT GCT TTA TAA GCG G	31	
	sacB gene		
P5	CCA GAT CTA AAG AAA CGA ACC AAA AGC C	28	1665
P6	CCG ATA TCT TAT TTG TTA ACT GTT AAT GTC C	31	

EPP: Excepted PCR products.

RESULTS AND DISCUSSION:

Bacterial halophiles are abundant in environments such as salt lakes, saline soils, and salted food products. Most species keep their intracellular ionic concentrations at low levels while synthesizing or accumulating organic solutes to provide osmotic equilibrium of the cytoplasm with the surrounding medium **(**Kushner, 1989**).**

To describe microorganisms according to their behavior toward salt, different classification schemes have been devised. Although, several classification schemes or categories have been proposed (Vreeland, 1987; Ramos-Cormenzana, 1989; and Trüper *et al*., 1991), the most widely used is that of Kushner, who defined moderate halophiles as organisms growing optimally between 0.5 and 2.5 M salt (Kushner**,** 1978). Bacteria able to grow in the absence of salt as well as in the presence of relatively high salt concentrations (e.g., 8% in the case of *Staphylococcus aureus*) are desig-nated halotolerant (or extremely halotolerant if

growth extends above 2.5 M). A rare case of a bacterium that requires 2 M salt at least (optimal growth at 3.4 M), such as the action-mycete *Actinopolyspora halophila* (Gochnauer *et al*., 1975) is considered a borderline extreme halophile (Kushner**,** 1978; Johnson *et al*., 1986).

Regarding the use of bacterial genes for plant transformation, Ebskamp *et al.* (1994) pointed that fructan, a polyfructose molecule is a storage compound in a limited number of plant species. Usually these species accumulate fructan with a low degree of polymerization (DP) and most of these plants have properties, which preclude their use as a fructan source. Therefore, they modified the *sacB* gene from *Bacillus subtilis*, which encodes levansucrase, and introduced it into tobacco plants.

In this work, it is of importance to mention that the applied *Streptomyces* stains were isolated from saline water and soil as reported in Table 1. These strains also had antibiosis activities against some bacteria, fungi and candida (Saleh *et al*., 1990; Mohamed *et al*., 2000 and Mohamed *et al*., 2001)**.**

Data in Table 3 show the salt tolerance range of sixteen halotolerant *Streptomyces* strains used for isolation of some salt stress genes *via* the polymerase chain reaction (PCR). Results showed that these strains varied in their salt tolerance range, in particular, with increasing NaCl concentration in the growth medium up to 140 g/L. It was also noted that all the *Streptomyces* strains had grown abundantly by (+++) at NaCl concentrations of 5, 35 and 70 g/L. When NaCl concentration was raised to 105 g/L, strains except *S. melanogenes* Si-11 gave moderate (++) growth. On the contrary, 50% of strains under investigation (*S.niveus* Sc-2, *S sendenensis* Sc-11, *S.graminofaciens* Ma-13, *S. albovinaceus* Qa-44, *S. luteofluorescens* Qa-51, *S. albidoflavus* Qa-53, *S. erthraeus* Qa-84 and *S. violans* Da-3) tolerated NaCl concentration up to 140 g/L and gave moderate growth, but the rest could not gow. This result was in agreement with that of Mohamed *et al*. (2000), who isolated halotolerant actinomycetes from sandy soil of different locations in Egypt. The highly halotolerant isolates with the ability to grow on 15-18% NaCl were *Streptomyces violans* Da-3, *S. alboflavus* Is-10, *S. bobili* PS-12 and *S. hawaiiensis* Si-8. Kuznetsov *et al*. (1993) isolated *Streptomyces albiaxialis* sp. Nov., a new oil hydrocarbon degrading species of thermo- and halotolerant *Streptomyces*.

On the molecular level, a trial was done to detect the three salt tolerance genes (*P5CR*, *mtlD* and *sacB*) in the DNA extracted from the applied species using PCR. Results in Table (4) showed that the *P5CR* gene was detected in the DNA extracts of four species, namely, *S. albovinaceus* Qa-44, *Streptomyces* species Si-1, *S. luteofluorescens* Qa-51 and *S. lateritius* Si-6 (Figure 1), while, *mtlD* gene was found in the DNA extracts of *S. albovinaceus* Qa-44, *S.albidoflavus* Qa-53, *S. erthraeus* Qa-84 (Figure 2). In addition, the *sacB* gene as PCR fragments with sizes of about 831, 1150 and 1665 bp was amplified for the three genes under investigation respectively.

These results indicate the differences in the genetic make up of different species under study. They also indicate that different mechanisms are involved in

conferring salt tolerance in these *Streptomyces* species. In addition, species with ability to tolerate 140 g/L that lack the three genes under investigation (*S. niveus* sc-2) should possess other functioning genes for salt tolerance in its genome. This idea can be confirmed when targeting other candidate genes for salt tolerance in a consequent study.

Table -3: Salt tolerance range of sixteen *Streptomyces* strains isolated from salt water (1-7) and Sinai sandy soil (8-16).

No.	*Streptomyces* strains	NaCl Concentrations (%)				
		0.05	3.5	7.0	10.5	14
1	*S. niveus* Sc-2	+++	+++	+++	++	++
2	*S. sendenensis* Sc-11	+++	+++	+++	++	++
3	*S. graminofaciens* Ma-13	+++	+++	+++	++	++
4	*S. albovinaceus* Qa-44	+++	+++	+++	++	++
5	*S. luteofluorescens* Qa-51	+++	+++	+++	++	++
6	*S. albidoflavus* Qa-53	+++	+++	+++	++	++
7	*S. erthraeus* Qa-84	+++	+++	+++	++	++
8	*S. violans* Da-3	+++	+++	+++	++	++
9	*S. alboflavus* Is-10	+++	+++	+++	++	-
10	*S. bobili* Ps-12	+++	+++	+++	++	-
11	*Streptomyces* species Si-1	+++	+++	+++	++	-
12	*S. tuirus* Si-4	+++	+++	+++	++	-
13	*S. lateritius* Si-6	+++	+++	+++	++	-
14	*S. hawaiiensis* Si-8	+++	+++	+++	++	-
15	*S. muavecolor* Si-9	+++	+++	+++	++	-
16	*S. melanogenes* Si-11	+++	+++	++	+	-

-: No growth. +: Weak growth. ++: Moderate growth. +++: Abundant growth.

Table -4: PCR detection of salt tolerance genes from the DNA extracts of sixteen *Streptomyces* species isolated from salt water (1-7) & Sinai sandy soil (8-16).

No.	*Streptomyces* strains	PCR detected genes		
		P5CR	***mtlD***	***sacB***
1	*S. niveus* Sc-2	-	-	-
2	*S. sendenensis* Sc-11	-	-	-
3	*S. graminofaciens* Ma-13	-	-	-
4	*S. albovinaceus* Qa-44	+	+	-
5	*S. luteofluorescens* Qa-51	+	-	-
6	*S. albidoflavus* Qa-53	-	+	-
7	*S. erthraeus* Qa-84	-	+	-
8	*S. violans* Da-3	-	-	-
9	*S. alboflavus* Is-10	-	-	-
10	*S. bobili* Ps-12	-	-	-
11	*Streptomyces* species Si-1	+	-	+
12	*S. tuirus* Si-4	-	-	-
13	*S. lateritius* Si-6	+	-	-
14	*S. hawaiiensis* Si-8	-	-	-
15	*S. muavecolor* Si-9	-	-	-
16	*S. melanogenes* Si-11	-	-	-

+: Detected-: Not detected.

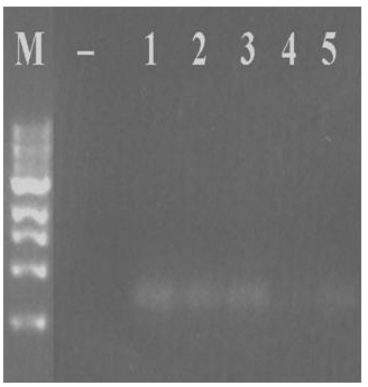

Figure 1. Agarose gel electrophoresis of amplified *P5CR* gene from the DNA extracted from four *Streptomyces* species, namely, *S. albovinaceus* Qa-44 (lane 1), *Streptomyces* species Si-1 (lane 2), *S. luteofluorescens* Qa-51 (lane 3), without sample (lane 4) and *S. lateritius* Si-6 (lane 5). -: Negative control (PCR mixture with no template). M: 1 Kb DNA ladder.

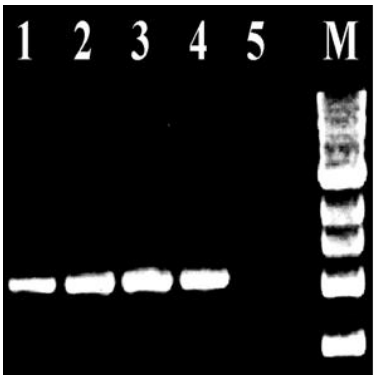

Figure 2. Agarose gel electrophoresis of amplified *mtlD* gene from the DNA extracted from *S. albovinaceus* Qa-44 (lane 1), *S. albidoflavus* Qa-53 (lane 2) and *S. erthraeus* Qa-84 (lane 3). Lane 4: Positive control. Lane 5: Negative control (PCR mixture with no template). M: 1 Kb DNA ladder.

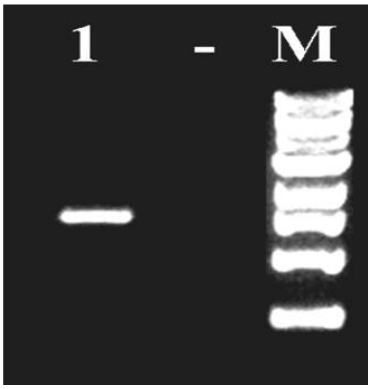

Figure 3. Agarose gel electrophoresis of amplified *sacB* gene from the DNA extracted from *Streptomyces* species Si-1 (lane 1). -: Negative control (PCR mixture with no template). M: 1 Kb DNA ladder.

One can recommend the use of the halotolerant *Streptomyces* strains, based on their antimicrobial activities for biologically controlling the phyto-pathogenic fungi in saline soils. In addition, the isolated salt tolerance genes require more confirmation *via* some molecular studies, i.e., nucleotide sequencing aligning with those previously isolated genes. Furthermore, these genes can be constructed and used in the production of transgenic plants conferring tolerance to salt stress in the future. Ronde *et al.* (2001) showed that the L-DELTA1-pyrroline-5-carboxylate reductase (*P5CR*) gene controls the common step in the both pathways governing the biosynthesis of proline from ornithine and glutamic acid. Abebe *et al.* (2003) showed that ectopic expression of the *mtlD* gene of *Escherichia coli* for the biosynthesis of mannitol in wheat (*Triticum aestivum* L. cv Bobwhite) improved tolerance to water stress and salinity. They concluded that the improved growth performance of mannitol-accumulating calluses and mature leaves was due to other stress-protective functions of mannitol. Also, Wang-HuiZhong *et al.* (2003) integrated *mtlD* gene into the rice genome mediated by *Agro-bacterium tumefaciens* LBA4404 (pBIM). The damage to the membrane structure of the transgenic plant was lower than in the control. Some transgenic plant lines could grow normally under 1.0% NaCl stress, whereas the controls could not grow and died after 2 weeks under the same environment.

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REFERENCES:

Abdel-Fattah,H.I., Cultural, morphological, physiological and molecular studies on some streptomycete isolates. Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo **13**(2): 249-268 (2005).

Abebe, T.; A.C. Guenzi; B. Martin and J.C.Cushman, Tolerance of mannitol accumulating transgenic wheat to water stress and salinity. Plant Physiology **131**(4): 1748-1755 (2003).

Antón,J.; R. Rosselló-Mora; F. Rodríguez- Valera and R. Amann, Extremely Halophilic Bacteria in Crystallizer Ponds from Solar Salterns. Appl. Environ. Microbiol. **66**: 3052-3057 (2000).

Bellier, A. and P. Mazodier. ClgR, a Novel Regulator of clp and lon Expression in *Streptomyces*. J. Bacteriol. ***186****: 3238-3248 (2004)*.

Diab, A. and M.Y.Al-Gounaim, *Streptomyces ahmadiensis*, a new species from the soil of Kuwait. Egypt. J. Microbiol. 21: 239-250 (1986).

Ebskamp, M.J.M.; I.M. Van-der-Meer; B.A Spronk; P.J. Weisbeek; S.C.M. Smeekens and I.M. Van-der-Meer, Accumulation of fructose polymers in transgenic tobacco. Bio/Technology **12**(3): 272-275 (1994).

Gadda, G. and E.E. McAllister-Wilkins, Cloning, Expression, and Purification of Choline Dehydrogenase from the Moderate Halophile *Halomonas elongata*. Appl. Environ. Microbiol. **69**: 2126-2132 (2003).

Gochnauer, M.B.; G.G. Leppard; P. Komaratat; M. Kates; T. Novitsky and D.J.Kushner, Isolation and characterization of *Actinopolyspora halophila*, gen. et sp. nov., an extremely halophilic actinomycete. Can. J. Microbiol. **21**: 1500-1511 (1975).

Hamdi, Y.A.; Al-Tai M. Amira and A. Dewedar. A novel *Micromonospora lutea* nov. Sp. isolated from Iraqi soils. Egypt. J. Microbiol. **15**: 1-6 (1980).

Hussein, A.M. and H.A. Abbas. *Actinopolyspora jlexuosa* and *A. fusca* two halophilic new species of genus *Actinopolyspora*. Egyptian Society of Applied Microbiology. Proc. VI. Conf. Microbiol. Cairo, May 1986. Vol. I-Part II. Soil Microbiol., Pp. 363-378 (1986).

Johnson, K. G.; P. H. Lanthier and M. B. Gochnauer, Studies of two strains of *Actinopolyspora halophila* an extremely halophilic actinomycete. Arch. Microbiol. **143**: 370-378 (1986).

Kushner, D.J., Life in high salt and solute concentrations: *halophilic bacteria. In*: D.J. Kushner (ed.), Microbial life in extreme environments. Academic Press, Ltd. London, United Kingdom. P*p. 317-368* (1978).

Kushner, D.J. Halophilic bacteria: life in and out of salt. In: *Recent advances in Microbial Ecology*. T. Hattori, Y. Ishida, Y. Maruyama, R.Y. Morita, and A. Uchida (ed.), Japan Scientific Societies Press, Tokyo, Japan Pp. 60-64 (1989)

Kuznetssov, V.D.; T.A. Zajtseva; L.V. Vakulenko and S.N. Flippova, *Streptomyces albiaxialis* sp. nov.-a new oil hydrocarbon degrading species of thermo- and halotolerant *Streptomyces*. Int. J. Syst. Bacteriol, **43**: 398-399 (1993).

Kwak, J. and K.E. Kendrick. Bald mutants of *Streptomyces griseus* that

prematurely undergo key events of sporulation. J. Bacteriol. 178: 4643-4650 (1996)

LeFevre, E. and L. A. Round, A preliminary report upon some halophilic bacteria. J. Bacteriol. **4**:177-182 (1919).

Van-der-Meer-IM- M.J.M. Ebskamp, R.G.F.Visser, P.J. Weisbeek, S.C.M. Smeekens, I. M. Van-der-Meer, Fructan as a new carbohydrate sink in transgenic potato plants. Plant Cell **6**(4): 561-570 (1994).

Mahfouz, H.T. and Mohamed H. Sonya, Physiological, antagonistic and fingerprinting studies on some halotolerant *Streptomyces* strains. Arab Biotechnol 5: 103-120 (2002).

Mohamed H. Sonya; Sh. M. Selim and E.A. Saleh, Taxonomical and biochemical studies on some halotolerant actinomycetes isolated from sandy soil in Egypt. Arab Univ. J. Agric. Sci. Ain Shams Univ. Cairo **8**(1): 41-61 (2000).

Mohamed H. Sonya, H.I. Abdel-Fattah; Sh.M. Selim and M.S. Sharaf, Identification and molecular studies on some halotolerant actinomycetes isolated from Sinai sandy soil. Arab Biotechnol **4**: 179-196. (2001)

Prior, C.; S. Potier; J.L. Souciet and H. Sychrova. Characterization of the *NHA1* gene encoding a Na^+/H^+-antiporter of the yeast *Saccharomyces cerevisiae*. FEBS Lett. **387**: 89–93 (1996).

Qiu-DongLiang, Lin-Peng; D.L. Qiu, and P. Lin, Advances in molecular mechanisms of salt tolerance in plants. Journal of Tropical and-Subtropical Botany **10** (3): 281-292 (2002).

Ramos-Cormenzana, A., Ecological distribution and biotechnological potential of halophilic micro-organisms *In*: *Microbiology of extreme environments and its potential for biotechnology* M.S. Da Costa, J.C. Duarte, and R.A.D. Williams (eds.) Elsevier Applied Science, London, United Kingdom. Pp. 289-309 (1989).

Roessler, M. and V. Muller, Chloride, a New Environmental Signal Molecule Involved in Gene Regulation in a Moderately Halophilic Bacterium, Halobacillus halophilus. J. Bacteriol. **184**: 6207-6215 (2002).

Ronde, J.A.; W.A. Cress; J. van Staden, J.A. de-Ronde and J. van-Staden, Interaction of osmotic and temperature stress on transgenic soybean. South African Journal of Botany **67** (4): 655-660 (2001).

Saito, K.; Y. Oda; F. Tomita and A. Yokota, Molecular cloning of the gene for 2,6-beta-D-fructan 6-levanbiohydrolase from *Streptomyces exfoliatus* F3-2. FEMS Microbiology Letters **218**(2): 265-270 (2003).

Saleh, E.A.; M.M. Zaki; M.E. El-Demerdash and Mohamed H. Sonya, Identification of some halotolerant streptomycetes isolated from marine ecosystems in Egypt. Annals of Agric. Sci. Ain Shams Univ., Cairo Special Issue 409-425 (1990).

Sambrook, J.; E.F. Fritsch and T. Maniatis, Gel electophoresis of DNA. In: *Molecular Cloning:A Laboratory Mannual*, Part 6, 2nd Ed . Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York *Pp. 1-15* (1989).

Tarczysnki, M.C.; R.G. Jensen and H.J. Bohnert, Expression of a bacterial *mtlD* gene in transgenic tobacco

leads to production and accumulation of mannitol. Proceed. National Acad. Sci. USA. **89** (7): 2600-2604 (1992).

Tokunaga, H., K. Mitsuo, S. Ichinose, A. Omori, A. Ventosa, T.Nakae and M. Tokunaga. Salt-Inducible Multidrug Efflux Pump Protein in the Moderately Halophilic Bacterium *Chromohalobacter* Sp. Appl. Environ. Microbiol. **70**: 4424-4431 (2004).

Trüper, H.G.; J. Severin; A. Wohlfarth; E. Müller and E.A. Galinski, Halophily, taxonomy, phylogeny and nomenclature In: *General and applied aspects of halophilic microorganisms* F.Rodriguez-Valera (eds.) Plenum Press, New York, P*p. 3-7 (1991)*.

Varela, C.A.; M.E. Baez and E. Agosin, Osmotic Stress Response: Quantification of Cell Maintenance and Metabolic Fluxes in a Lysine-Overproducing Strain of *Coryne bacterium glutamicum*. Appl. Environ. Microbiol. **70**: 4222-4229 (2004).

Vreeland, R.H., Mechanisms of halotolerance in microorganisms. Crit. Rev. Microbiol. **14**: 311-356 (1987).

Waksman, S.A. and H.A. Lechevalier, Classification, identification and description of genera and species In: *The Actinomycete.* The Williams and Wilkins, Co., Baltimore, USA. Vol. II Pp. 340 (1961).

Wang-HuiZhong; Liu-JunJun; Lu-DeZhao; Zhao-Yan; Yan-MeiXian; Qian-Qian; Peng-XueXian; Chen-ShouYi; Huang-DaNian; H.Z.Wang; J.J.Liu; D.Z.Lu; Y.Zhao; M.X.Yan; Q. Qian; X.X. Peng; S.Y. Chen and D.N. Huang, Transformation of rice with *mtlD* gene. Chinese Journal of Rice Science **17** (1): 6-10 (2003).

Zaki, M.M.; E.A. Saleh; M.E. El-Demerdash and Mohamed H. Sonya, Antimicrobial activity of the halotolerant *Streptomyces albidoflavus* strain Qa-53 as affected by different concentrations of raw materials and pH. Annals Agric. Sci, Ain Shams Univ., Cairo **36**:355-361 (1991).

Zaki, M.M.; E.A. Saleh; M.E. El-Demerdash and Mohamed H. Sonya, Antimicrobial activities of some halotolerant streptomycete strains as affected by incubation period and medium composition. *4th Conf. Agric. Dev. Res., Ain Shams Univ., Cairo, Feb. 13-18, 1993.* Annals Agric. Sci., Sp. Issue, **2:** 519-529. (1993).