EurAsian Journal of BioSciences
EurAsia J BioSci 4, 33-40 (2010)
DOI: 10.5053/ejobios.2010.4.0.5

Isolation and characterization of salt-tolerant nitrogen-fixing microorganisms from food

Girish Gajanan Jadhav[1], Dipti Sambhaji Salunkhe[3], Devidas Punaji Nerkar[2], Rama Kaustubh Bhadekar[3]*

[1] Department of Polysaccharide Conjugate Vaccine, Serum Institute of India, Pune, 411028 Maharashtra, India

[2] Poona College of Pharmacy, Bharati Vidyapeeth University, Pune, 411038 Maharashtra, India

[3] Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth University, Pune, 411043 Maharashtra, India

*Corresponding Author: neeta.bhadekar@gmail.com

Abstract
Halophilic microorganisms are already in use for some biotechnological processes, such as commercial production of ß-carotene, polymers (polyhydroxyalkanoates and polysaccharides), enzymes, compatible solutes etc. Considering their commercial importance, food samples (crude salt crystals and raw mango pickle) were used for isolation of halotolerant microorganisms. Two bacterial isolates obtained from food samples were examined for their ability to survive under stressed conditions and their growth response in increasing levels of NaCl (1 to 15% w/v), pH (5.0 to 10.0) and temperature (10 to 70°C). The isolates were rod shaped Gram-positive, salt-tolerant, non-halophilic, nitrogen-fixing strains. Different sugars such as glucose, fructose, maltose, sucrose, xylose and lactose were used to check for acid and gas production. The organisms were studied for their ability to hydrolyse substrates such as casein, starch, gelatin, etc. These organisms (i) grew well in SM basal salt medium and nitrogen-free semi-solid LGI medium (ii) tolerated 10-15% salt concentration (iii) produced acid from D-glucose, D-fructose and sucrose and (iv) utilized glycerol and citrate as carbon source, and v) survived acidic (pH 4-5) and alkaline (pH 9-10) conditions. The results suggested that there is potential to improve their performance as sources of industrially important enzymes. On the basis of morphological attributes and biochemical characteristics the isolates belonged to the genus *Bacillus*. The results of partial sequencing of 16S rRNA also revealed that the isolates 1 and 2 are closely related to *Bacillus subtilis* subsp. *subtilis* NCIB 3610T (97.9% pair-wise similarity) and *Bacillus sonorensis* NRRL B-23154T (99.8% pair-wise similarity) respectively.

Keywords: *Bacillus*, halotolerant, salinity, soil fertility, thermo-tolerant.

Jadhav GG, Salunkhe DS, Nerkar DP, Bhadekar RK (2010) Isolation and characterization of salt-tolerant nitrogen-fixing microorganisms from food. EurAsia J BioSci 4, 5, 33-40.
DOI:10.5053/ejobios.2010.4.0.5

INTRODUCTION

Nearly 40% of world's surface has salinity problems. Most of the salinic areas confined to the tropics and the Mediterranean region and has made the salt tolerance an urgent priority for the future of agriculture (Corodovilla et al. 1994, Gisbert et al. 2000). The productivity of crops is greatly affected by salt stress. Highly alkaline (pH greater than 8.0) soils tending to be high in sodium chloride, bicarbonate and borate, are often associated with high salinity. This reduces nitrogen fixation (Bordeleau and Prevost 1994). Saline conditions reduce the ability of plants to absorb water, induce many metabolic changes causing rapid reduction in growth rate, similar to those caused by water stress (Epstein 1980). If salt-tolerance cannot be improved, by perforce vast amounts of soils may be left uncultivated. The failure of nitrogen fixing activity of some nitrogen-fixing

Received: January 2010
Received in revised form: February 2010
Accepted: February 2010
Printed: March 2010

organisms in high salinity clearly inhibits the induction of lupines (Bordeleau and Prevost 1994). In such soils, microorganisms tolerating high concentration of salt and yet capable of fixing nitrogen are of importance in increasing its fertility. The halotolerant microorganisms are effective in the treatment of waste from tannery industry or pickle industry (Kubo et al. 2001, Sivaprakasam et al. 2008). These organisms are isolated from sources such as marine environment, soils, rhizosphere or industrial waste. They are also known to be the potential sources of extracellular enzymes with novel properties, useful for diverse industrial applications. Hence the objective of the present study is isolation and characterization of high salt-tolerant, non-halophilic microorganisms from food samples and evaluation of their characteristics under stress conditions.

MATERIAL AND METHODS

Sampling

Six samples each of crude salt and raw mango pickle stored at room temperature for more than eight months were collected in sterile screw capped glass bottles.

Isolation and purification of microorganisms

Individual samples were suspended in 1 mL sterile distilled water and its 0.1 mL aliquot was inoculated on SM basal medium (Composition: Per liter, Na_2HPO_4 4.5 g, KH_2PO_4 1.5 g, NH_4Cl 0.3 g, $MgSO_4.7H_2O$ 0.1 g, $Na_2S_2O_3$ solution 100 mL [Add 10 g of $Na_2S_2O_3$ to100 mL distilled water] and trace metal solution 5.0 mL (per liter composition trace metal solution: EDTA 50 g, $ZnSO_4.7H_2O$ 22 g, $CaCl_2$ 5.54 g, $MnCl_2.4H_2O$ 5.06 g, $FeSO_4.7H_2O$ 4.99 g, $CoCl_2.H_2O$ 1.61 g, $CuSO_4.5H_2O$ 1.57 g and $(NH_4)6Mo_7O_{24}.2H_2O$ 1.1 g) (Loganathan and Nair 2004) and incubated at 37°C for 48 h. The cultures obtained were transferred on nitrogen-free medium LGI medium (Composition: per liter, $CaCO_3$ 1.0g, K_2HPO_4 1.0 g, $MgSO_4.7H_2O$ 0.2 g, $FeSO_2.7H_2O$ 0.1 g, $Na_2MoO_4.2H_2O$ 5.0 mg, Sucrose 5 g and bromophenol blue solution 5mL [Add bromophenol blue 0.5 g to 100 mL of 0.2N KOH]) (Cavalcante and Dobereiner 1988), incubated at 37°C for 24 h and maintained on yeast extract mannitol agar (YEMA) medium.

Morphological and biochemical characterization

The isolates showing high salt-tolerance (10% and above) were selected for morphological and biochemical characterization, using different sugars (xylose, glucose, fructose, sucrose, lactose, and maltose). They were also studied qualitatively for their ability to secrete extracellular enzymes (amylase, urease, protease, gelatinase and ß-galactosidase) (Collee et al. 1989).

NaCl tolerance

The cultures obtained on nitrogen-free medium were further screened for their salt-tolerance. For this purpose, nutrient broth supplemented with various concentrations of NaCl (ranging from 1-25%) was used for inoculation (10^7 cells/mL), nutrient broth supplemented with 0.5% (w/v) NaCl was used as a control and incubation was carried at 37°C, 120 rpm for 24 h. Total viable count (TVC) of all cultures was determined on nutrient agar. The isolates showing high salt-tolerance were characterized as mentioned above.

16S rRNA sequencing

16S rRNA partial sequencing of the isolate was carried out and compared with those available in Gen Bank + EMBL+DDBJ+PDB databases using BLASTN 2.2.17. The genomic DNA was isolated as described by Ausubel et al. (1987). The PCR assay was performed using Applied Biosystems, model 9800 with 1.5 μL of DNA extract in a total volume of 25 μL. The PCR master mixture contained 2.5 μL of 10X PCR reaction buffer (with 1.5 M $MgCl_2$), 2.5 μL of 2 mM dNTPs, 1.25 μL of 10 pm/ μL of each oligonucleotide primers 27f (5' CCAGAGTTTGATCGTGGCT-CAG 3'), 1488R (5'CGGTTACCTTGTTACGA-CTTCACC 3'), 0.24 μL of Taq DNA polymerase and 15.76 μL of glass distilled PCR water.

Initially denaturation accomplished at 94°C for 3 min. Thirty-five cycles of amplification consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. A final extension phase at 72°C for 10 min was performed.The PCR product was purified by PEG-NaCl method. The sample was mixed with 0.6 times volume PEG-NaCl [20% PEG (MW 6000), 2.5M NaCl] and incubated for 40 min at 37°C. The precipitate was collected by centrifugation at 3,800 rpm for 28 min. The pellet was washed with 70% ethanol, air dried.

The sample was sequenced using 96 well Applied Biosystems sequencing plate as per manufacturer's instructions.

The thermocycling for the sequencing reactions was performed with a 9800 PCR model (Applied Biosystems). It began with an initial denaturation at 94°C for 2 min, followed by 35 cycles of PCR consisting of denaturation at 94°C for 10 sec, annealing at 50°C for 10 sec and extension at 68°C for 4 min.

The samples were purified using standard protocols described by Applied Biosystems, Foster City, USA. To this, 10 μL of Hi-Di formamide was added and vortexed briefly. The DNA was denatured by incubating at 95°C for 3 min, kept on ice for 5-10 min and was sequenced in a 3730 DNA analyzer (Applied Biosystems) following manufacturer's instructions.

pH tolerance

High salt-tolerant, nitrogen-fixing cultures were further screened for pH tolerance in nutrient broth adjusted to pH 5.0-10.0. The media at different pH were inoculated with overnight grown inoculum (10^7 cells/mL), incubated at 37°C, 120 rpm for 24 h and cell growth determined by measuring absorbance at 660 nm.

Temperature tolerance

Nitrogen-fixing, high salt-tolerant cultures were used to examine their temperature tolerance by inoculating equal volume of overnight grown culture (10^7 cells/mL) in nutrient broth and incubating at temperatures ranging from 10°C to 70°C for 24 h and TVC of all the cultures determined on nutrient agar plate.

Growth curve studies

Growth curve characteristics of cultures in the presence and absence of high salt concentration were compared by inoculating equal volume (10^7 cells/mL) of overnight grown cultures in nutrient broth, containing 10% and 15% NaCl. The nutrient broth containing 0.5% NaCl was used as a control. Growth determined at intervals of 2 h for 30 h by measuring absorbance at 660 nm.

RESULTS AND DISCUSSION

Isolation and purification

Six samples each; from mango pickle and crude salt were used to isolate salt tolerant, nitrogen-fixing single colonies of microorganisms. From 100 isolates obtained on SM basal medium, only 10 isolates were found to grow on nitrogen-free LGI medium (*Azospirillum amazonense* medium) indicating that they could fix nitrogen for survival and growth. However, it is difficult to rationalize their presence in crude salt or pickle with their ability to fix nitrogen.

Morphological and biochemical characterization

Both the isolates characterized morphologically and biochemically (Table 1 and 2) were found to be (a) Gram positive (b) spore-forming rods and (c) secreted extracellular amylase, gelatinase and protease. Though the isolate II is able to grow even at 60°C its amylase activity could be observed only upto 50°C, indicating its thermostability. Both isolates (a) produced acid from D-glucose, D-fructose and sucrose (b) utilized citrate and glycerol as carbon source. However only isolate II could produce acid from D-maltose.

NaCl tolerance

Out of 10 isolates screened for salt-tolerance (i) only 5 isolates tolerated salt concentration above 1%, (ii) 2 isolates tolerated 10% (1.75 M) and 15% NaCl (2.75 M) respectively and characterized as mentioned above (iii) NaCl concentration beyond 15% inhibited their growth (Fig. 1).

On the basis of morphological and biochemical characteristics isolates I and II belonged to the genus *Bacillus*. The partial 16S rRNA sequencing was carried out and the sequences were deposited in GeneBank. Their accession numbers are GU593321 and GU593322 respectively. Further comparative analysis of the partial 16S rRNA sequences showed that they were closely related to *Bacillus subtilis* subsp. *subtilis* NCIB 3610T (97.9% pair-wise simiarity) and *Bacillus sonorensis* NRRL B-23154T (99.8% pair-wise similarity).

Table 1 and 2 show characteristics differentiating our isolates from other closely related *Bacillus* sp. *Bacillus subtilis* subsp. *subtilis* NCIB 3610T and *Bacillus sonorensis* NRRL B-23154T isolated in this work, tolerated very high salt concentration (10% and 15% respectively) as compared to other *Bacillus* sp. These characters confirmed that these isolates with significantly high salt tolerance are distinctly different from the known *Bacillus* sp. High salt-tolerant organisms are reported earlier from different sources; for example (i) *Bacillus clausii* isolated from the east coast of India (Asha Devi et al. 2008) (ii) different sp. of *Bacillus* isolated from the soil in Sonoran Desert, Arizona and Dead Sea, etc. (Arahal et al. 1999, Palmisano et al. 2001) (iii) *Bacillus cereus* isolated from wastewater of plum pickle plant tolerated only 10% NaCl (Kubo et al.2001) and (iv) *Lactococcus lactis* (subsp. *lacti*) isolated from intestinal tract of coastal fish tolerated 6% NaCl (Rasa et al. 2001). However none was able to fix nitrogen indicating that the halotolerant *Bacillus* sp. with nitrogen fixing ability are the novel isolates.The conventional nitrogen-fixing *Rhizobia* isolated from *Acacia, Prosopis*, and

Table 1. Characteristics differentiating isolate I from closely related *Bacillus* species.1) Isolate I (this work, Genebank accession no. GU593321); 2) *Bacillus subtilis* NRRL-NRS 744 (Robert et al. 1996); 3) *Bacillus velezensis* (Ruiz-García et al. 2005); 4) *Bacillus subtilis* subsp. *subtilis* (Nakamura et al. 1999); 5) *Bacillus marismortui* (Arahal et al. 1999).
All *Bacillus* sp. 1) form spores 2) ferment maltose and 3) show optimum pH between 7.0 to 8.0.

Characteristic	1	2	3	4	5
Catalase	+	+	+	+	+
B-galactosidase	-	N.A.	+	N.A.	-
Hemolysis	+	N.A.	+	N.A.	NA
Acid production from:					
D-Xylose	-	+	+.	+	N.A.
D-Glucose	+	+	+	+	+
Fructose	+	N.A.	+	N.A.	+
Sucrose	+	N.A.	+	N.A.	-
Lactose	-	-	+	N.A.	-
Hydrolysis of:					
Casein	+	+	+	-	+
Gelatin	+	-	+	N.A.	+
Starch	+	+	+	+	-
Urea	-	N.A.	N.A.	N.A.	+
Utilization of:					
Glycerol	+	N.A.	+	N.A.	+
Citrate	+	+	+	+	+
NaCl Tolerance (%, w/v):	10	10	12	7	25

+ = Positive; - = negative; N.A. = Not Available

Table 2. Characteristics differentiating isolate II from closely related *Bacillus* species. 1) Isolate II (this work, GeneBank accession no. GU593322); 2) *Bacillus sonorensis* (Palmisano et al. 2001); 3) *Bacillus licheniformis* NRRL NRS 1264 (Palmisano et al. 2001); 4) *Bacillus licheniformis* SB3086 (Drahos and West 2003).
All *Bacillus* sp. 1) form spores 2) ferment glucose and 3) show optimum pH between 7.0 to 8.0.

Characteristic	1	2	3	4
Catalase	+	+	+	+
B-galactosidase	-	N.A.	+	+
Hemolysis	+	N.A.	N.A.	-
Acid production from:				
D-Xylose	-	+	+	N.A.
Fructose	+	NA	N.A.	NA
Lactose	-	NA	+	NA
Hydrolysis of:				
Casein	+	+	+	+
Gelatin	+	N.A.	N.A.	+
Starch	+	+	+	NA
Urea	-	N.A.	N.A.	NA
Utilization of:				
Glycerol	+	N.A.	+	+
Citrate	+	+	+	+/ -
NaCl Tolerance(%, w/v):	15	3	10	7

+ = Positive; - = negative; N.A. = Not Available

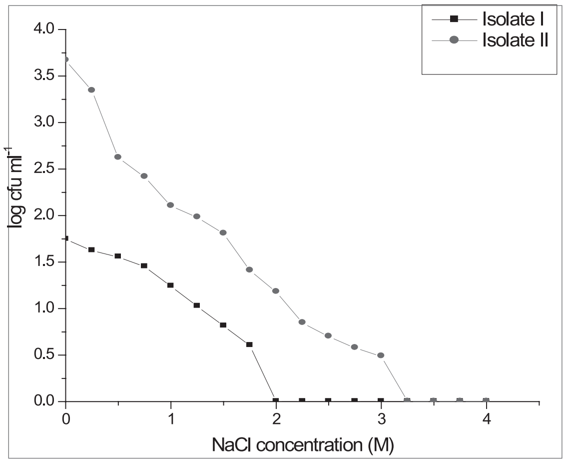

Fig 1. Salt-tolerance of isolates I and II. The cultures were inoculated in nutrient broth - NaCl medium for 24 h, plated on to nutrient agar medium, incubated for 24 h at 37°C and CFUs expressed as relative values in relation to the number of colonies on nutrient agar medium.

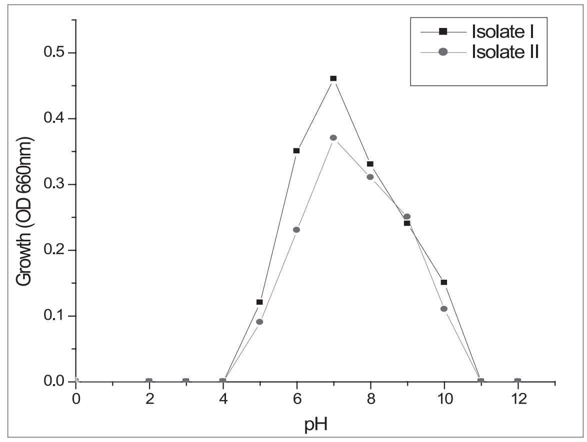

Fig 2. pH tolerance of isolates I and II. The cultures were inoculated in modified nutrient broth with pH ranging from 2.0 to 12.0 and incubated for 24 h at 37°C. Cell growth was determined by measuring the absorbance at 660 nm.

Leucaena tolerated 4.9% NaCl (Lal and Khanna 1993), while that from Egyptian lupines were inhibited by 8% NaCl (Itoi et al. 2008). Loganathan and Nair (2004) isolated a salt -tolerant, nitrogen-fixing and phosphate-solubilizing *Swaminathania salitolerans* (Acetobacteraceae), which grew well at 3% NaCl concentration. Compared to all these species, nitrogen-fixing *Bacillus* sp. isolated in the present study have 15% salt-tolerance, indicating they are new novel strains qualified by adaptation to environment and thereby acquiring additional traits.

pH and temperature tolerance

Both the isolates showed optimum pH for the growth in 7.0 to 7.5 ranges (Fig. 2), but were tolerant to wide pH range of 5.0-10.0. *Azotobacter* strains isolated from oat and wheat rhizosphere grew between pH 6.0 to 8.0 and at temperature of 25°C to 40°C. For most *Rhizobia*, the optimum range for growth was 28°C to 31°C, unable to grow at 37°C (Graham 1992). However our isolates tolerated temperature upto 60°C, neither of them could grow below 10°C (Table 1 and 2). These characteristics indicated their utility in wide spectrum of pH and temperature, which is a major prerequisite as soil inoculant. The pH and temperature profile of these isolates confirmed that they were neither *Azotobacter* nor *Rhizobium* but the novel sp. with desirable characteristics.

Growth curve studies

Comparative studies of growth characteristics in the presence of NaCl revealed different growth pattern for both the isolates. In presence of 0.5% NaCl, both organisms entered the stationary phase after 10 h growth (Fig. 3a). However in presence of 10% and 15% NaCl (for isolate I and isolate II respectively), longer exponential phase was observed. Isolate I entered into stationary phase after 20 h while isolate II required 17 h (Fig. 3b). Halophilic bacteria isolated from Dead Sea coast also exhibited longer exponential period in the presence of higher NaCl concentration (Arahal et al. 1999). In high salinity (salt concentration above 8%) soils, nitrogen-fixing non-symbiotic (e.g. *Azotobacter*) and symbiotic (e.g. *Rhizobium*) microorganisms cannot survive. The legume-*Rhizobium* symbiosis and nodule formation on legumes are quite sensitive to salt or osmotic stress due to inhibition of the initial steps of Rhizobium-legume symbiosis (El-Shinnawi et al.1989). The reduction of N_2-fixing activity and photosynthetic activity by salt stress is usually attributed to reduction in (i) respiration of nodules (Delgado et al. 1994) (ii) cytosolic

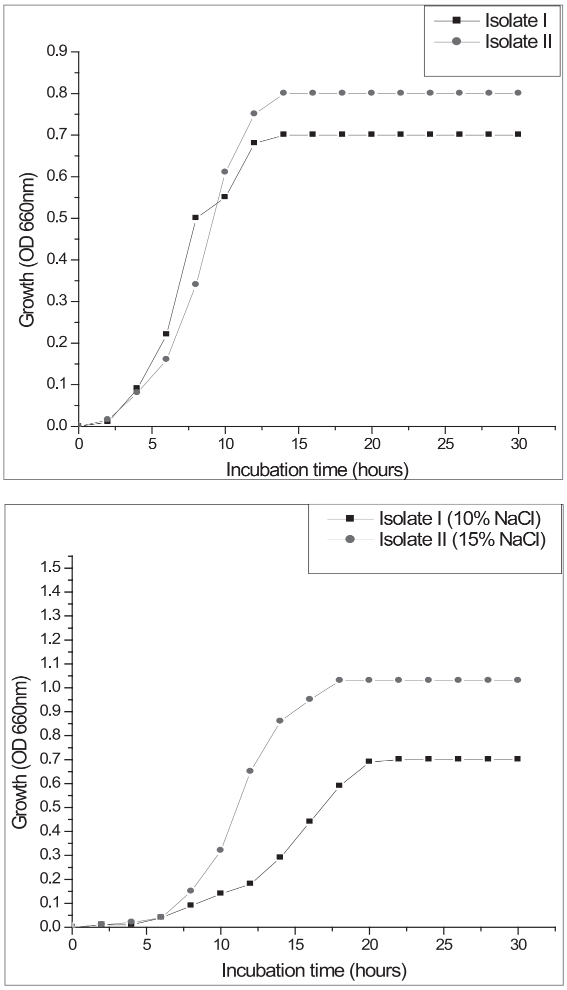

Fig 3. Growth curves of isolates I and II. The cells were inoculated in the broth, incubated at 37°C, 120 rpm and their growth was determined by measuring the absorbance at 660 nm.a) In nutrient broth, b) In nutrient broth containing 10% and 15% NaCl, respectively.

protein production (Delgado et al. 1993) (iii) dry weight and (iv) nitrogen content in the shoot (Cordovilla et al. 1995, Georgiev and Atkias 1993). Thus, considering the fertility problems of saline soils, the halotolerant novel *Bacillus* sp. prove promising and can be further exploited as soil inoculants. The enzymes produced by *Bacillus subtilis* and *Bacillus licheniformis* are widely used as additives in laundry detergents. The *Bacillus* strains isolated in this work with tolerance to high salt concentration, high pH, high temperature and ability to secrete extracellular enzymes at higher temperature, can be used as potential sources of industrially useful enzymes.

ACKNOWLEDGMENTS

The authors are indebted to Dr. S. S. Kadam, Vice Chancellor, Bharati Vidyapeeth University, Pune, Maharashtra, India for allowing to conduct this work at Rajiv Gandhi Institute of IT and Biotechnology, Bharati Vidyapeeth University and Mr. Sandip Walunjkar from National Center of Cell Sciences, Pune for sequencing experiments. The authors are gratefully thankful to Dr. R. M. Kothari, Principal, Rajiv Gandhi Institute of IT and Biotechnology, for critically reviewing the manuscript.

REFERENCES

Arahal DR, Carmen Marquez M, Volcani BE, Schleifer KH, Ventosa A (1999) *Bacillup*. nov. a new moderately halophilic species from the Dead Sea. International Journal of Systematic Bacteriology 49, 521-530.

Asha Devi NK, Balakrishnan K, Gopal R, Padmavathy S (2008) *Bacillus clausii* MB9 from the east coast regions of India: Isolation, biochemical characterization and antimicrobial activity. Current Science 95, 627-636.

Ausubel FM, Rrent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1987) Current Protocaol in Molecular Biology. Wiley, New York.

Bordeleau LM, Prevost D (1994) Nodulation and nitrogen fixation in extreme environments. Plant and Soil 161, 115-125.

Cavalcante VA, Dobereiner J (1988) A new acid-tolerant nitrogen fixing bacterium associated with sugar cane. Plant Soil 108, 23-31.

Collee JG, Duguid JP, Fraser AG, Marmion BP (1989) Practical Medical Microbiology. Churchill Livingstone, Medical Division of Longman Group UK Ltd., New York.

Cordovilla MP, Ocana A, Ligero F, Lluch C (1995) Salinity effects on growth analysis and nutrient composition in four grain legumes-*Rhizobium symbiosis*. Journal of Plant Nutrition 18, 1595 - 1609.

Corodovilla MP, Ligero P, Lluch C (1994) The effect of salinity on nitorgen fixation and assimilatin in Vicia faba. Journal of Experimental Botany 45, 1483-1488.

Delgado MJ, Garrido JM, Ligero F, Lluch C (1993) Nitrogen fixation and carbon metabolism by nodules and bacteroids of pea plants under sodium chloride stress. Plant Physiology 89, 824 -829.

Delgado MJ, Ligero F, Lluch C (1994) Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean and soybean plants. Soil Biology and Biochemistry 26, 371 - 376.

Drahos DJ, West L (2003) Bacillus licheniformis biofungicide. United States Patent 6569425.

El-Shinnawi MM, El-Saify NA, Waly TM (1989) Influence of the ionic form of mineral salts on growth of faba bean and Rhizobium leguminosarum. World Journal of Microbiology and Biotechnology 5, 247-254.

Epstein E (1980) Response of plants to saline environments. In: Rains DW, Valeintine RC, Hollaender A (eds), Genetic Engineering of Osmoregulation, Plenum Press, New York, 7-21.

Georgiev GI, Atkias CA (1993) Effects of salinity on N_2 fixation, nitrogen metabolism and export and diffusive conductance of cowpea root nodules. Symbiosis 15, 239 -255.

Gisbert C, Rus AM, Carmen Bolarin M, Isabel Arrillaga JM, Montensinos C, Caro M, Moreno R (2000) The Yeast HAL1 gene improves salt tolerance of Tra Tomato. Plant Physiology 123, 393-402.

Graham PH (1992) Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Canadian Journal of Microbiology 38, 475 -484.

Itoi S, Abe T, Washio S, Lkuno E, Kanomata Y, Sugita H (2008) Isolation of halotolerant *Lactococcus lactis* subsp. *lactis* from intestinal tract of coastal fish. International Journal of Food Microbiology 12, 116-121.

Kubo M, Hiroe J, Murakami M, Fukami H, Tachiki (2001) Treatment of Hypersaline-Containing Wastewater with Salt-Tolerant Microorganisms. Journal of Biosciences and Bioengineering 91, 222-224.

Lal B, Khanna S (1995) Selection of salt tolerant *Rhizobium* isolates of *Acacia nilotica.* World Journal of Microbiology and Biotechnology 10, 637 -639.

Loganathan P, Nair S (2004) *Swaminathania salitolerants* gen. nov., sp. nov., a salt-tolerant, nitrogen-fixing and phosphate-solubilizing bacterium from wild rice (*Proteresia corctata* Tateoka). International Journal of Systematic and Evolutionary Microbiology 54, 1185-1190.

Nakamura LK, Roberts MS, Cohan FM (1999) Relationship of *Bacillus subtilis* clades associated with strains 168 and W23: A proposal for *Bacillus subtilis* subsp. *subtilis* subsp. nov. and *Bacillus subtilis* subsp. *spizizenii* subsp. nov. International Journal of Systematic Bacteriology 49, 1211-1215.

Palmisano MM, Nakamura LK, Duncan KE, Istock CA, Cohan F (2001) *Bacillus sonorensis* sp. nov., a close relative of *Bacillus licheniformis*, isolated from soil in the Sonoran Desert, Arizona. International Journal of Systematic and Evolutionary Microbiology 51, 1671-1679.

Rasa S, Jornsgard B, Abou-Taleb H, Christiansen (2001) Tolerance of *Bradyrhizobium* sp. (Lupini) strains to salinity, pH, $CaCO_3$ and antibiotics. Letters in Applied Microbiology 32, 379-383.

Roberts MS, Nakamura LK, Cohan FM (1996) *Bacillus vallismortis* sp. nov., a Close Relative of *Bacillus subtilis*, Isolated from Soil in Death Valley, California. International Journal of Systematic Bacteriology 46, 470-475

Ruiz-García C, Béjar V, Martínez-Checa F, Llamas I, Quesada E (2005) *Bacillus velezensis* sp. nov., a surfactant-producing bacterium isolated from the river Vélez in Málaga, southern Spain. International Journal of Systematic and Evolutionary Microbiology 55, 191-195.

Sivaprakasam S, Mahadevan S, Sekar S, Rajakumar S (2008) Biological treatment of tannery wastewater by using salt-tolerant bacterial strains. Microbial Cell Factories 7, 15.

Azot Tutucu Tuza Dayanikli Mikroorganizmalarin Yiyeceklerden Izolasyonu ve Karakterizasyonu

Özet

Halofilik mikroorganizmalar halihazirda; ß-karoten, polimerler (polihidroksialkanoatlar ve polisakkaritler), enzimler ve uygun çözgenler gibi bazi biyoteknolojik islemlerde kullanilmaktadir. Ticari önemi göz önünde bulundurularak, gida örnekleri (kaba tuz kristalleri ve ham mango tursusu) halotolerant mikroorganizmalarin izolasyonunda kullanildi. Gida örneklerinden elde edilen iki bakteriyel izolat; stres sartlari altinda hayatta kalma ve artan NaCl (%1 ila %15 w/v), pH (5.0 ila 10.0) ve sicaklik (10 ila 70°C) seviyelerindeki büyüme tepkileri açisindan incelendi. Izolatlar, çubuk seklinde gram-pozitif, tuza dayanikli, halofitik olmayan azot tutucu suslardi. Glukoz, fruktoz, maltoz, sukroz, ksiloz and laktoz gibi farkli sekerler, asit ve gaz üretimini kontrol etmek için kullanildi. Organizmalar, kasein, nisasta ve jelatin gibi substratlari hidrolize etme yetenekleri açisindan incelendi. Bu organizmalar, (i) SM bazal tuz ortaminda ve azotsuz yari-kati LGI ortaminda iyi büyüdüler, (ii) %10-15 tuz konsantrasyonunu tolere ettiler, (iii) D-glukoz, D-fruktoz ve sukrozdan asit ürettiler, (iv) karbon kaynagi olarak gliserol ve sitrik asiti kullandilar ve (v) asidik (pH 4-5) ve alkalin (pH 9-10) sartlarda hayatta kaldilar. Bu sonuçlar; endüstriyel açidan önemli enzimlerin kaynagi olarak performanslarini gelistirme potansiyeli oldugunu gösterdi. Morfolojik ve biyokimyasal özelliklerine dayali olarak, bu izolatlarin *Bacillus* cinsine ait oldugu belirlenmistir. Ayrica 16S rRNA'nin kismi dizininden elde edilen sonuçlar, izolat 1 ve 2'nin sirasiyla *Bacillus subtilis* subsp. *subtilis* NCIB 3610T (%97,9) ve *Bacillus sonorensis* NRRL B-23154T (%99,8) ile yakin iliskili oldugunu ortaya çikarmistir.

Anahtar Kelimeler: *Bacillus*, halotolerant, termo-tolerant, toprak verimliligi, tuzluluk.

