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Research Article

Isolation and Characterization of Salt Tolerant Bacteria from Saline Areas of Khyber Pakhtunkhwa

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Abstract

The survival of microbes in various severe condition leads to developments in the medical, agricultural, and bioenergy sectors. In this study we isolate, identify and characterize salt tolerant bacteria from saline areas. Sample was taken from hyper saline areas of KP. Four bacterial isolates were isolated from sample and different techniques were applied. All the bacterial isolates were able to grow on agar media in the presence of salt this shows the strict halotolerant nature of isolates. Serial dilution was performed to check the salt concentration at which minimum and maximum growth occurs. The growth of isolates was inversely proportional to salt concentration in media as the concentration of salt in media was increases the growth of isolates were decreases and vice versa. Only one isolate was able to show resistance to 2.5M salt in media and 3 isolates were able to show resistance to 2M salt in media. Gram staining was performed to check that the isolates are gram positive or gram negative; all of the isolates were gram positive. For molecular analysis genomic DNA was extracted and checks the result on gel.

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Introduction

Adaption is an evolutionary process in which living organisms learn to live in new environments. Lower to higher living organisms are influenced but have evolved to cope with various abiotic stresses [1], such as changes in temperature, pH, soil and water

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salinity, atmospheric humidity, air circulation, and radiation. Among these, soil and water salinity have the greatest stress impact on life, and it is becoming more prominent over time [2,3]. Bacteria are tiny living things that can't be seen with the naked eye but are all around us. They can be found in water, soil, and the air [4]. They can be beneficial, as we can use them in the fermentation, decomposition and the process of various food products [5], or pathogenic, as when they cause infection [6].

There are two methods through which bacteria are classified phenotypic basis and genetic basis. On the basis of morphology and different staining properties bacteria can be classify as gram positive or gram-negative bacteria [7]. The difference between two groups is due to complex cell wall of gram-positive bacteria which contain large amount of peptidoglycan while gram negative has less amount [8] on the basis of 16s ribosomal RNA sequencing bacteria are also classified, 16s ribosomal RNA sequence are highly conserved, and this technique is also used to find out the similarities and differences between different organisms [9]. Salinity is one of the major problems in eco system now days. High salinity in soil inhibits some stages of plant life such seed germination to maturity and inhibit some growth stimulating factors. Halotolerant PGPB tolerate the extra salt in soil due to which plant can maintain their normal cell activities like hormonal balance and transport of food and minerals [10]. Hypersaline environments are extreme habitats where bacteria can survive and tolerate the extreme environment. Salinity of that area is more than the concentration of salt in sea water. Beside salinity the organism in that habitat have to face a lot of other challenges like high temperature, less availability of oxygen and acidic environment. That organism adopted themselves to continue their life in extreme environment and they have the ability to tolerate the extreme environment [11]. These bacteria are important in biotechnology for different purposes like for extraction of halophilic enzymes, for treatment of saline water and can be used in biofuel and bioplastic [12]. Some halotolerant species isolated from the hypersaline lakes of Vestfold Hills, Antarctica are Halomonasmeridiana, Halomonassubglaciescola, Halomonaselongata and Halomonashalmophila [13]. According to study in Pakistan a diverse group of halophilic bacteria exist in different areas of KP [14]. The aim of the work is to isolate a new strain of bacteria from saline areas of KP, and study different characteristic and techniques of microbial world. In future we will work to reduce the salinity from the environment, and also for extraction of halophilic enzymes, for treatment of saline water.

Material and methods

Sample collection

Sample was collected in 50ml sterile Falcon tubes from hypersaline environment in different areas of KP and brought in the lab for further analysis. The temperature was recorded to be 34 °C at noon time.

Preparation of media

Agar media was prepared for growth of bacteria. 1.04g of tryptone was added to 0.52g of yeast extract, 1.04g of Nacl and 1.6g

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technical agar water was added so that the total volume reached up to 100ml. Flask was labeled and put in autoclave to remove contamination.

Isolation of strains

Using 100ml nutrient agar media, four petri plates (growth media) were prepared and labeled. In each petri plate 200µl of sample was added through spread plate method. Petri plates were incubated at 37 °C for 24 hours. After incubation diverse microbial colonies were observed on all plates.

Purification of strains

From diverse microbial colonies on each petri plate random selection of single colony was done and cultured on freshly prepared nutrient agar media plates through streak plate method. Streak plate method is used to isolate a specific bacterial colony from mixed bacterial population and can be cultured on separate petri plate. Similarly, 3 more colonies were picked and cultured. Petri plates were incubated at 37 °C for 24 hours and checked for results.

Screening of strains in different salt concentration

Salts solutions of 1M, 2M, 2.5M, 3M and 5M were prepared. For 1M salt solution 5.844g of salt was added to flask and water was added until total volume of salt and water reached up to 100ml. For 2M salt solution 11.688g of salt was added to water and total volume of solution was 100ml. For 2.5M salt solution 14.61g of salt was added to water and total volume of solution was brought to 100ml mark. For 3M salt solution 17.532g of NaCl was added to water and total volume of solution was made to be 100ml.

For 5M salt solution 29.22g of salt was added to water and total volume of solution was made to be 100ml. NaCl concentration was further increased to 2.5M then 3M and 5M. (Used 2.5M, 3M and 5M salt solution in media respectively instead of water) at each stage growth was observed.

Morphological identification

For morphological identification gram staining was performed. For gram staining fresh culture was prepared in LB media. A drop of water was added to the slide and bacteria were transfer from fresh culture. Water drop was heat dried and the bacteria were attached to the slide. After that crystal violet was added for one minute. The slide was then gently washed with an indirect stream of water. Next, the slides were left to be dry. Afterwards, ethanol was added for one minute and allowed to air-dry. After ethanol, Safranin was added for a minute and then the slide was washed gently and allowed to dry. Finally, the slides were observed under the microscope.

Molecular analysis and polymerase chain reaction

For molecular analysis genomic DNA was extracted through C-TAB method and the results were verified on agarose gel. To amplify and get many copies of DNA, polymerase chain reaction PCR were performed. In PCR for 50 µl master mix solution we needed 5 µl of 10x taq buffers, 1µl forward primer, 1 µl reverse primer, 0.5 µl DNTPs, 0.25 µl taq polymerase and 42.25 µl of

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water, and checked the result through gel electrophoresis technique.

Results and discussion

Halotolerant nature of isolates

The bacterial isolates were cultured at different salt concentration and the results were checked. The isolates showed growth in the presence and in the absence of salt in the media which shows that the isolates are halotolerant in nature can grow in the presence and in the absence of salt Figure 1 (a and b).

Halotolerant bacteria don't need salt to survive but if salt is present in media, they can tolerate it. The growth of bacteria was observed to decreases as concentration of salt increased and vice versa.

Isolation of Strains

200µl from sample was added to each plate and spread through spread plate method. After incubation diverse microbial colonies were observed which are shown in Figure 2.

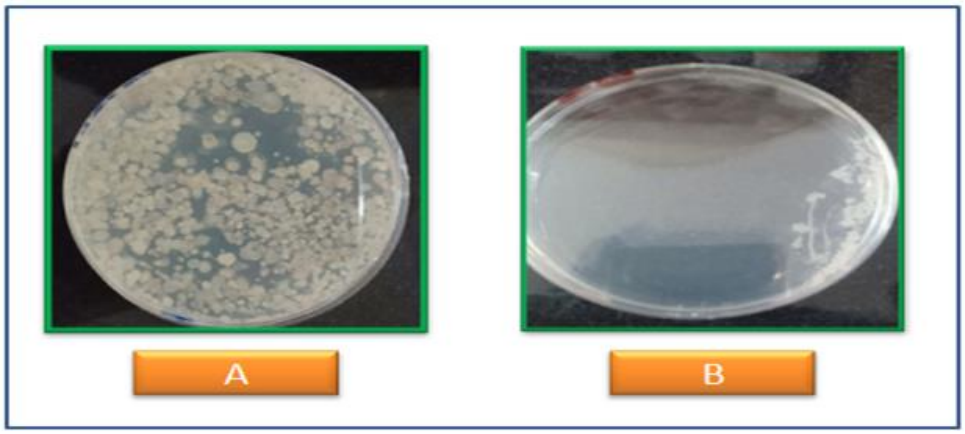

Figure 1: Growth of bacteria in the presence and absence of salt.

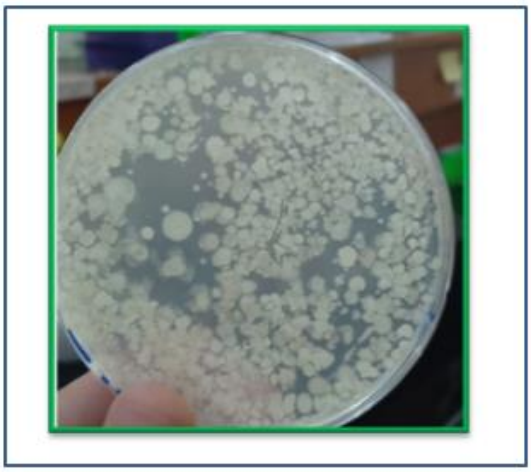

Figure 2: Bacterial growth on agar media.

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Purification of strains

From each plate one specific bacterial colony was transferred to freshly prepared agar media through streak plate method. 4 colonies were transferred to new plates and after incubation each plate contained one pure bacterial colony (Figure 3).

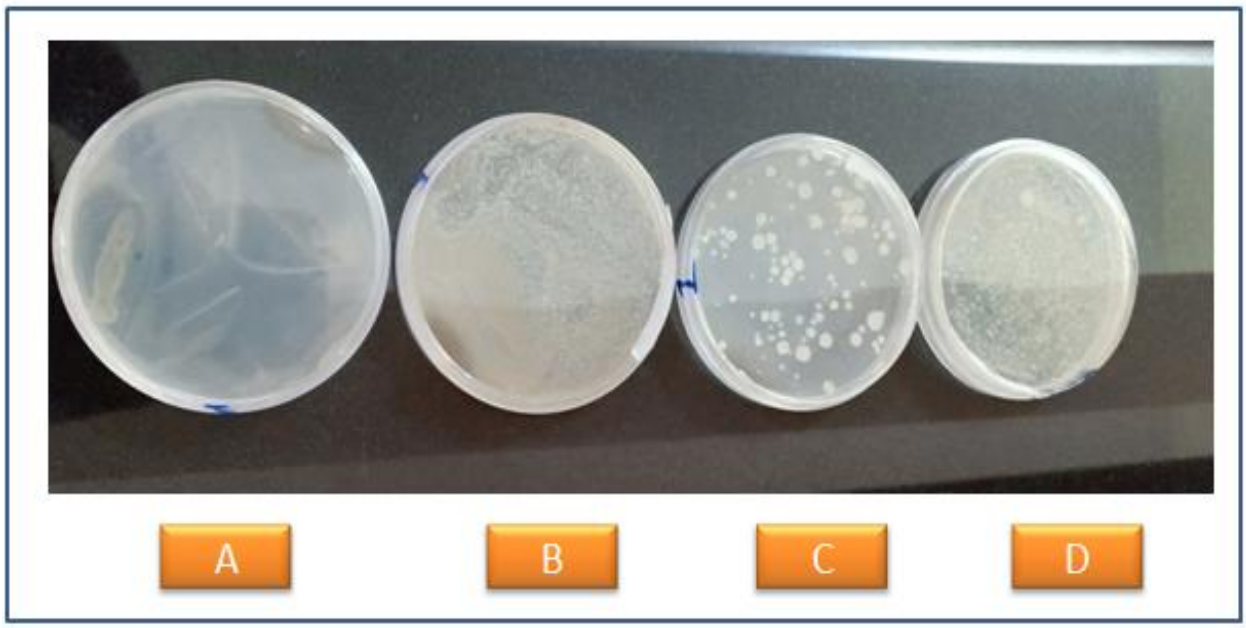

Figure 3: Plates containing pure bacterial culture.

Screening of strains in different salt concentration

Serial dilution was performed to check the minimum and maximum growth of bacterial isolates at different salt concentration. The growth of bacterial isolates S1, S2, S3 and S4 were observed at 1M and 2M salt supplemented media, the growth of S4 was also observed at 2.5M salt supplemented media (Table 1).

	1M	2M	2.5M	3M	5M
S1	+ +	+	-	-	-
S2	+ +	+	-	-	-
S3	+ +	+	-	-	-
S4	+ +	+	+	-	-

Table 1: Bacterial growth at different salt concentration.

Morphological identification

Gram staining were performed for all bacterial isolates i.e., S1, S2, S3 and S4. All isolates were gram positive. Gram positive bacteria showed blue color bacterial colonies under microscope and gram-negative show pink or red color colonies.

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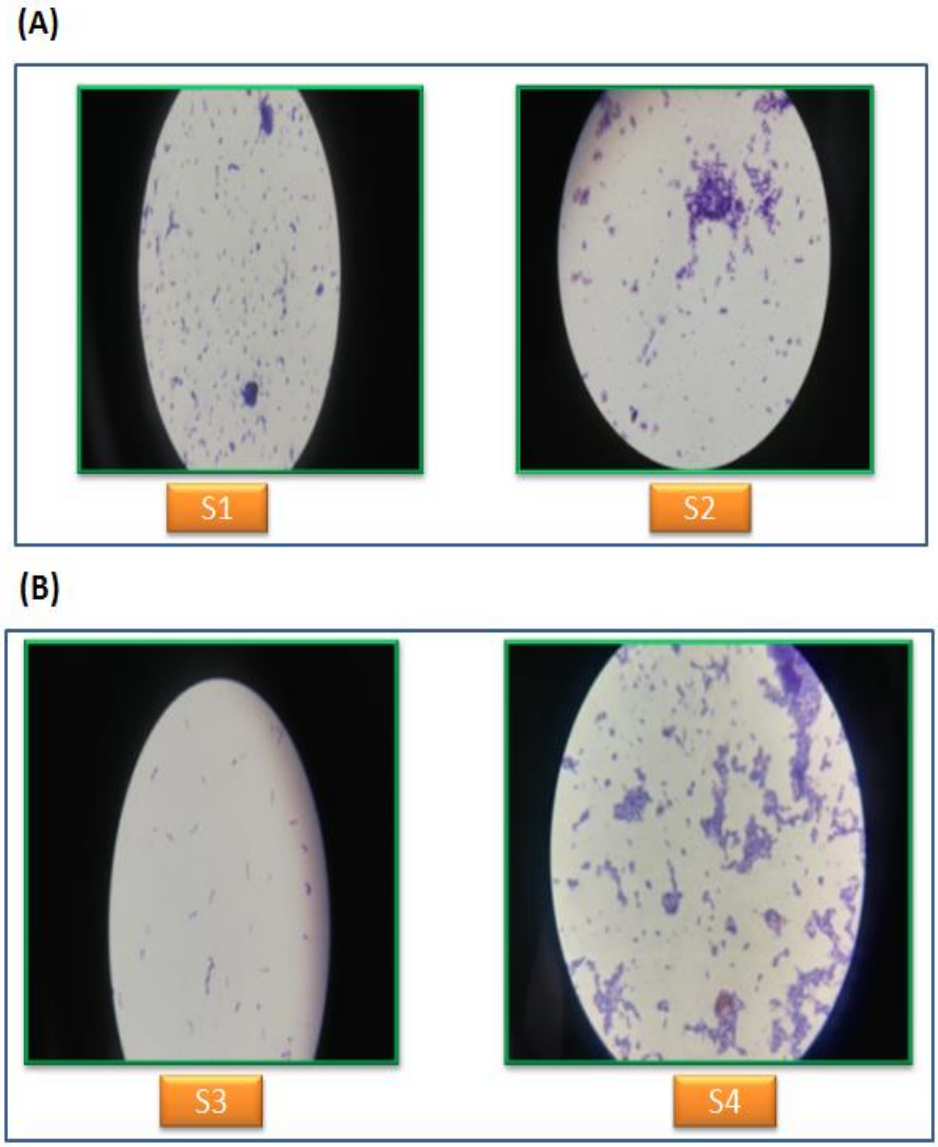

Figure 4: (A) and (B) Shows the strain Morphology.

Molecular analysis and polymerase chain reaction

For molecular analysis genomic DNA was successfully extracted through C-TAB method and the results were checked on agarose gel. To amplify and get many copies of genomic DNA for sequencing PCR was performed and checked the results on agarose gel.

Conclusion

Population growth has also increased food consumption and, as a result, agriculture output. One of the biggest obstacles to improving agricultural output and quality has been salty soil. The application of microorganisms with PGPR activity, for example, can promote seedling emergence, hasten seed germination, and protect plants

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while also promoting plant growth. One of the most frequent environmental hazards to crop yield and quality is the rise in soil salinization. One of the main obstacles to crop yield in arid and semiarid regions has been identified as this issue. Every year, saline soil reduces by 1-2 percent the amount of land that can be used for agriculture, which lowers food output. In present study four bacterial isolates were isolated from and different techniques were applied.

All the bacterial isolates were able to grow on agar media in the presence of salt and in the absence of salt this result shows the strict halotolerant nature of isolates. Serial dilution was performed to check the salt concentration at which minimum and maximum growth occurs. The growth of isolates was inversely proportional to salt concentration in media as the concentration of salt in media was increases the growth of isolates were decreases and vice versa. Only one isolate was able to show resistance to 2.5M salt in media and 3 isolates were able to show resistance to 2M salt in media.

Declaration

Conflict of Interest

None of the authors of this study have disclosed any conflicts of interest.

Authors contribution

Dr. Sulaiman Faisal, Dr Muhammad Arshad Malik supervised the project. Tariq Hussain, Muhammad Ijaz, Shumaila Ubaid, Gulzar Ahmad, Sidra Usman, Abdul Aziz, Abrar Hussain, Azaz Ullah Khan, Faraz Ahmad khan perform and analyze the experiments, all the authors have written the manuscript, reviewed the manuscript and agreed to submit it.

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References

1. Khan AU, Ahmad G, Ijaz M, Ahsan K, Akhtar H, Khan FA, Ullah S. Prevalence and Antibiotic Resistance of Salmonella Species Isolated from Chicken Liver in Peshawar. J Clin Med Res. 2021;3(3):1-1. CrossRef
2. 2. Mendpara J, Parekh V, Vaghela S, Makasana A, Kunjadia PD, Sanghvi G, et al. Isolation and characterization of high salt tolerant bacteria from agricultural soil. Eur J Exp Biol. 2013;3(6):351-8.
3. 3. Rothschild LJ, Mancinelli RL. Life in extreme environments. Nature. 2001;409(6823):1092-101. PubMed | CrossRef
4. 4. West SA, Diggle SP, Buckling A, Gardner A, Griffin AS. The social lives of microbes. Annu Rev Ecol Evol Syst.2007;53-77. CrossRef
5. Rahim AH, Ahmad G, Ijaz M, Akhtar H, Ahsan K, Rizwan MM. Isolation and Identification of Antibiotics Susceptible Staph aureus from Unprocessed Milk. J Clin Med Res. 2021;3(2):1-1. CrossRef
6. 6. Sharma R, Garg P, Kumar P, Bhatia SK, Kulshrestha S. Microbial fermentation and its role in quality improvement of fermented foods. Fermentation. 2020;6(4):106. CrossRef
7. Lowy F. Bacterial classification, structure and function. New York, USA: Columbia University. 2009;1-6.

8. Beveridge TJ. Structures of gram-negative cell walls and their derived membrane vesicles. J Bacteriol. 1999;181(16):4725-33. PubMed | CrossRef
9. Frank L. Bacterial Classification, Structure and Function. 2009.
10. Nabti E, Schmid M, Hartmann A. Application of halotolerant bacteria to restore plant growth under salt stress. InHalophiles 2015. CrossRef
11. Ventosa A. Unusual micro-organisms from unusual habitats: hypersaline environments. InSymposia- Soc. for Gen. l Microbiol. Cambridge; Cambridge University Press; 1999. 2006;66:223.
12. Gupta S, Sharma P, Dev K, Srivastava M, Sourirajan A. A diverse group of halophilic bacteria exist in Lunsu, a natural salt water body of Himachal Pradesh, India. Springerplus. 2015;4:274. PubMed | CrossRef
13. James SR, Dobson SJ, Franzmann PD, McMeekin TA. Halomonas meridiana, a new species of extremely halotolerant bacteria isolated from Antarctic saline lakes. Syst Appl Microbiol. 1990;13(3):270-8. CrossRef
14. Roohi A, Ahmed I, Khalid N, Iqbal M, Jamil M. Isolation and phylogenetic identification of halotolerant/halophilic bacteria from the salt mines of Karak, Pakistan. Int J Agric Biol. 2014;16(3):564-70.

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