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Lysinibacillus acetophenoni and *Pseudomonas stutzeri* with High Salt effect, Recovered from High Salinity Soil Area (Indo-Gangetic Plain of India)

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Authors' contributions

This work was carried out in collaboration among all authors. Author PBK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SS and SR managed the analyses of the study. Author VV managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Soil salinity has affected many soil microbial communities as well as economic value of forest ecosystem for many years. The plant growth-promoting bacteria have developed several different mechanisms that have a positive influence on plant development and growth. Designated strain L-PB424 and P-PB466 was isolated and identified from saline soil of block Ashabutter khair forest in Punjab North zone in India, were investigated for their plant growth-promoting characters such as production of indole acetic acid, phosphate solubilization, Ammonium production and fermentation of polysaccharides. Comparative analysis of 16SrRNA gene sequences revealed that L-PB424 was closely related to *Lysinibacillus manganicus* DSM 26584 strain Mn1-7 (98.76%), on the other hand strain P-PB466 was closely related to *Pseudomonas songnenensis* strain NEAUST5-5. This research paper is a study in evaluation and variety of possible halophlic/halotolerant bacterial strains in salt-affected soils of block Ashabutter khair forest in Punjab North zone in India. The use of Halophilic bacteria in saline soil is interesting for future analysis and biotechnological development.

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Keywords: Halophilic plant growth promoting bacteria; soil salinity; evolutionary tree; 16SrRNA.

1. INTRODUCTION

Acclimatization is an evolutionary process by which living creatures learn to adapt to new environments. Abiotic stressors, such as variations in soil and water salinity, temperature, pH, atmospheric humidity, air circulation, and radiation, affect lower to higher living species, yet they have evolved to cope with them [1]. In the current investigation, a new test approach was proposed to demonstrate erraticism in dangerous conditions with development and advancement of farming soil bacterial species in stepwise transformation in high salt (NaCl) conditions. Two different bacterial species were isolated from saline soil of Gangetic plain. These isolates, showed salt tolerance up to 10% NaCl concentration. Biochemical and molecular (16SrRNA sequencing) characterization revealed the strains to be *Pseudomonas stutzeri* and *Lysinibacillus acetophenone.* The halophilic bacteria keep an occasional degree of ionic fixations to include viable solutes, to regulate the diffusion level within the cytoplasm with respect to external environment. These balancing mechanisms of the internal environment and the properties of the cytoplasmic membrane facilitate them to acclimatize to changes within the saline condition as salt lakes, saline soils, and salinity mediated products [2].

Halophilic and halotorant bacteria are required in production of essential salty food e.g., pickling brines, Thai food sauces etc. [3].

Decolorizing and utilization ability of azo dyes was reported in textile industry microbial isolates [4]. Ability of production of hydrolases, lipases, cellulases, proteases, amylases and biopolymers were found in halotolerant bacteria involved in composting process [5]. The halotolerant species (*Bacillus atrophaeus, Halomonas shengliensis, Halomonas koreensis* and *Virgibacillus salarius*) showed the ability to metabolize hydrocarbons and microorganisms like *V. salarius* and *Brevibacillus* sp. show potential towards bioremediation [6]. *Corynebacterium xerosis* was reported as potential hydrocarbons degraders [7]. Halophilic and halotolerant bacteria have different immunological properties and used for the production of different enzymes [8] and also needed for maintaining the soil texture and nutrient recycling in a saline condition [9]. The genus *Lysinibacillus* belongs to the family Bacillaceae of the phylum Firmicutes, and was proposed by Ahmed et al., [10]. We studied the diversity of halotolerant and halophilic bacteria in saline soils in block Ashabutter khair forest in Punjab, North India.

2. MATERIALS AND METHODS

2.1 Bacterial Strains

Two strains named L-PB424 and P-PB466 was isolated from saline soil belong to block Ashabutter khair forest in Punjab North zone in India. Nutrient agar medium and Nutrient broth were routinely used for culturing and maintenance of the recovered bacteria. The maintenance was performed at 4°C in the glycerol stock medium. Then investigated for plant growth promoting trait.

2.2 Indole Test

Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase.

Production of indole is detected using Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

2.3 Phosphate Solubilization

Recovered strains were evaluated for the phosphate solubilization activity on Pikovskaya's agar medium. The incubation temperature 28°C was provided for 5 days, after that the clear zone around the bacterial colonies were thought to be positive for the test. The capability of the phosphate solubilization capacity was described by the phosphorous solubilization index (Edi-Premono et al. 1996).

2.4 Ammonium Production Test

Bacteria, particularly those growing naturally in an environment exposed to urine may decompose urea by means of the enzyme urease. The occurrence of this enzyme can be tested by growing the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator. An alternative method is to test for the production of ammonia from urea by means of Nessler's reagent and to detect NH_4^+ production due to L-arginine breakdown. A positive reaction for presence of ammonia is a colour ranging from a pale yellow to a dark brown precipitate [11].

2.5 Carbohydrate Fermentation Test

The principle of carbohydrate fermentation states that the action of organism on a carbohydrate substrate results in acidification of the medium, detected by a pH indicator dye. Carbohydrate fermentation is the process bacteria use to produce energy. Most of the bacteria convert glucose to pyruvate during glycolysis; however, some organisms use alternate pathways. A fermentation medium consists of a basal medium containing a single carbohydrate (glucose, lactose, sucrose, mannitol etc.) for fermentation. However, the medium also contains various pH indicators. In addition to a pH indicator to detect the production of acid from fermentation, a Durham tube is placed in each tube to capture gas produced by metabolism. The carbohydrate fermentation patterns shown by different organisms are useful in differentiating among bacterial groups or species [12].

2.6 Molecular Identification

DNA was isolated from the culture with the help of HiMedia Bacterial DNA isolation kit. Its quality was evaluated on 1.0% agarose gel, a single band of high-molecular weight DNA has been observed. Fragment of 16SrRNA gene was amplified by 16SrRNA-F and 16SrRNA-R primers. A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel. The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 16SrRNA-F and 16SrRNA-R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer.

Consensus sequence of 16SrRNA gene was generated from forward and reverse sequence data using aligner software. The 16SrRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA6 [13].

2.7 Phylogenetic Analysis of Strains

The 16SrRNA gene sequence was used to carry out BLAST with the 'nr' database of NCBI GenBank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix and phylogenetic tree was constructed was constructed using MEGA 10.

3. RESULTS

The recovered bacterial isolates were evaluated on the bases of morphology followed by biochemical, physiological and molecular identification (16SrRNA sequence). The morphological, physiological and biochemical characteristics of bacterial strains are given in Table 1. They have a very strict aerobic respiratory metabolism with oxygen but in some cases, nitrate has been used as an alternative that allows anaerobic growth.

In the study we have observed that the strain P-PB466 & L-PB424 were positive for P-solubilization and ammonium extraction while L-PB424 was found positive for carbohydrate fermentation and negative for PB-466.

The halophilic strain was tested for salinity tolerance. Both the strain showed growth in sodium chloride medium. One strain L-PB424 performed best at 10% NaCl concentration indicating they are highly salt tolerant. Another strain P-PB466 performed moderate growth at 2% and 5% thus, indicating they are moderately halophilic. Both strains show no growth at 5°C but show little growth 15°C and 50°C whereas both strains show highest growth at 32°C. At 32°C the highest growth rate was found (OD=0.98) after 24 hours of incubation to verify the effects of pH and temperature of medium on the growth rate of isolates, a number of experiments were carried out which were presented in Fig. 1 respectively. The optimal pH for the growth of the isolates was 7.0 while extreme pH was 5.0 which shows no growth and 9.0 which restricted the growth of strain P-PB466 but shows growth of strain L-PB424.

3.1 PCR Amplification of 16S rRNA Gene

Amplification of the 16SrRNA gene was examined in isolated DNA strains (L-PB424 and P-PB466). On a 1.0 percent agarose gel, it was found to contain a single band of high-molecular-weight DNA, as shown in Fig. 2. 16SrRNA-F and 16SrRNA-R primers were used to amplify a fragment of the 16SrRNA gene. When resolved on an agarose gel, a single distinct 1500bp PCR amplicon band was identified. To eliminate impurities, the PCR amplicon was purified. On an

ABI 3730xl Genetic Analyzer, forward and reverse DNA sequencing reactions of PCR amplicons were performed with 16SrRNA-F and 16SrRNA-R primers using BDT v3.1 Cycle sequencing kit. Using aligner software, a consensus sequence of the 16S rRNA gene was produced from forward and reverse sequencing data.

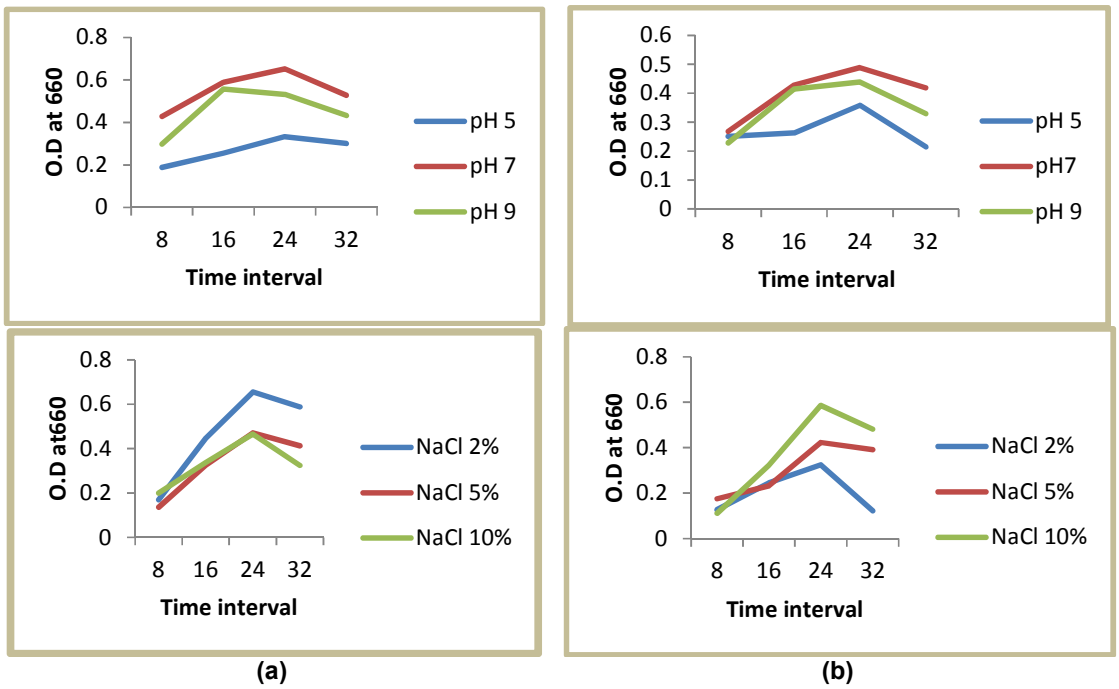

Fig. 1 Different pH and NaCl concentration for growth of bacterial isolates: (a) L-PB424 and (b) P-PB466

16SrRNA Gene sequence for L-PB424:

TTGGGATAACTCCGTGAAACCGGGGCTATACCGGATAATACAATCTAGCTCCTGCTAATGTTGAA
AGATGGTTTCGGCTATCGCTATGGATGGGCCCGCGGCGCATTACTAGTTGGTAGGGTAACGGCC
TACCAAGGGACGATGCGTAGCCGACCTGAGAGGGTGAGGCCACACTGGGACTGAGACACGGCC
CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAAC
GCCGCGTGAGTGAAGAAGGTTTTCGG
ATCGTAAAACTCTGTTGTAAGGGAAGAACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACC
TTATTAGAAAGCCACGGCTAACTAGTGCCAGCAGCCGCGGTAATACGTGGTGGCAAGCGTTGTC
CGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCTCGGCT
CAACCGAGGAGGGTCATTGGAAACTGGAGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAA
GTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTG
TAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACG
CCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTA
AGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCC
CGCTGACCGCTATGGAGACATAGCCTTCCCTTCGGGGACAGCGGTGACAGGTGGTGCATGGTT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTCTTAG
TTGCCATCATTTAGTTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAC
GGTCGCGAAGTCGCGAGACGAAGCCAATCCGATAAAACCGTTCTCAGTTCGGATTGTAGGCTGC
AACTCGCCTACATGAAGCCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTT
CCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGG
TAACCCTTACGGGAGCCAGCCGCCGAAGGTGGGACAGATGATTGGGGTGAAGTCGTACAAGGG
GGTTAACCGAAAA

16SrRNA Gene sequence for P-PB466:

GCGGCAGGCCTAACACATGCAAGTCGAGCGGATGAGTGGAGCTTGCTCCATGATTCAGCGGCG
GACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTA
ATACCGCATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAGATGAGCCT
AGGTCGGATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAG
AGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGAT
TGTAAAGCACTTTAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAAC
AGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGTAATACGAAGGGTGCAAGCGTTAATCG
GAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCA
ACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTATGGCAGAGGGTGGTGGAATTTCCTGTG
TAGCGGTGAAATGCGTAGAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGGCTAATACTG
ACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAA
CGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCATTAAGTCGACC
GCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACT
TTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTC
GTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACG
TTAAGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAA
GTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCA
AGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGAC
TGCGTGAAGTCGGAATCGCTAGTAATCGTGAATCAGAATGTCACGGTGAATACGTTCCCGGGCC
TTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCTCCAGAAATAGCTAGTCTAACCTTCG
GGGGGACGGTTACCACGGAGTGATTC

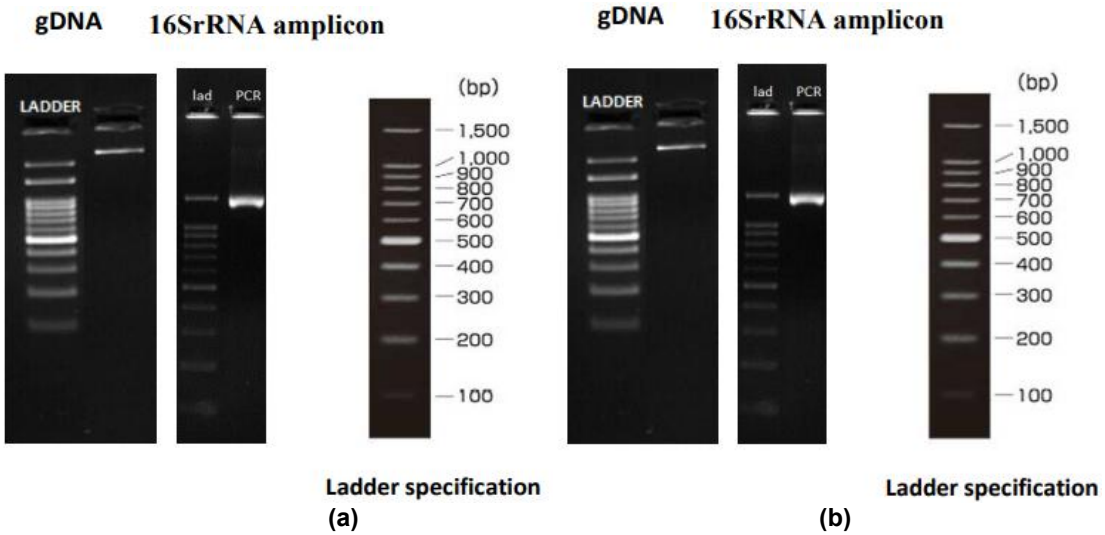

Fig. 2. 16SrRNA gene (1500bp) amplification of bacterial strain (a) L-PB424 (b) P-PB466

3.2 BLAST Result of L-PB424 & P-PB466

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. Fig. 4 depicts the tree with the highest log likelihood (-2380.84). The initial tree(s) for the heuristic search were automatically generated by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances calculated using the Maximum Composite Likelihood (MCL) technique, and then picking the topology with the best log likelihood value. The branch lengths are measured in the number of substitutions per site, and the tree is depicted to scale. There were 11 nucleotide sequences in this study. 1st+2nd+3rd+Noncoding codon locations were included. The total number of locations in the final dataset was 1440. MEGAX was used to perform evolutionary analysis [14].

Table 1. Morphological and biochemical characteristics of isolated bacterial strains

S.NO	Bacterial strain	Colony morphology				IAA	P- solubilization	NH_3 excretion	Carbohydrate fermentation	Gram reaction	Bacterial species
1.	L-PB424	Cream	Irregular	Raised	Undulated	+	+	+	+	Gram positive(rod)	*Lysinibacillus acetophenoni*
2.	P-PB466	White	circular	Raised	Undulated	+	+	+	-	Gram negative(rod)	*Pseudomonas stutzeri*

Table 2. BLAST result of L-PB424

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Lysinibacillus manganicus DSM 26584 strain Mn1-7	2569	2569	99%	0	98.76%	NR_118533.1
Lysinibacillus acetophenoni strain JC23	2549	2549	98%	0	98.81%	NR_135864.1
Lysinibacillus massiliensis 4400831 = CIP 108448 = CCUG 49529	2429	2429	96%	0	97.80%	NR_043092.1
Lysinibacillus chungkukjangi strain 2RL3-2	2412	2412	98%	0	97.00%	NR_109669.1
Lysinibacillus endophyticus strain C9	2401	2401	98%	0	96.75%	NR_146821.1
Lysinibacillus halotolerans strain LAM612	2350	2350	96%	0	96.94%	NR_134073.1
Lysinibacillus alkaliphilus strain OMN17	2344	2344	96%	0	96.67%	NR_136779.1
Ureibacillus defluvii strain DX-1	2342	2342	97%	0	96.30%	NR_133885.1
Lysinibacillus odysseyi 34hs-1 = NBRC 100172 strain 34hs1	2335	2335	97%	0	96.28%	NR_025258.1
Lysinibacillus telephonicus strain S5H2222	2333	2333	96%	0	96.47%	NR_157637.1

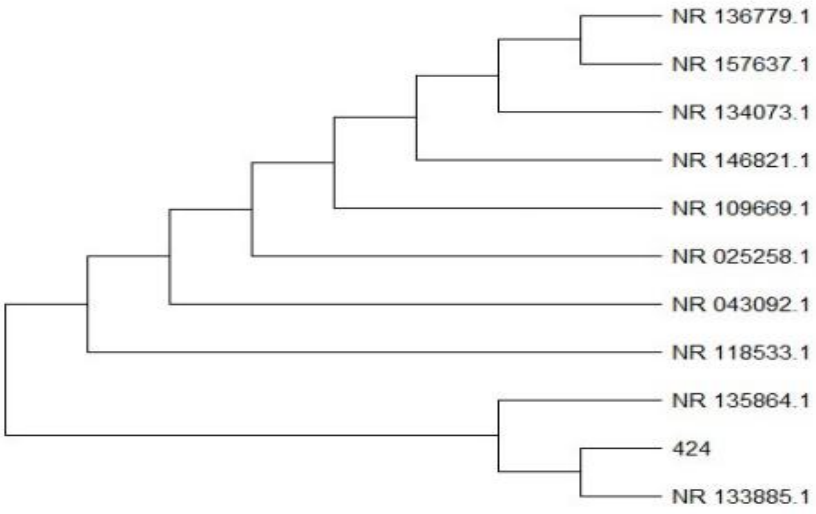

Fig. 3 Evolutionary relationship of taxa with reference to L-PB424

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The tree with the highest log likelihood (-3419.39) is shown in Fig. 3. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum

Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the variety of substitution per website this analysis is concerned for the 11 sequences and sequence clusters with respect to position and there is 1464 sequence position within the final dataset and MEGAX was used to analyze the evolutionary relationship between the sequences [14].

Table 3. BLAST result of P-PB466

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas stutzeri ATCC 17588 = LMG 11199	2647	2647	100%	0	99.93%	NR_041715.1
Pseudomonas stutzeri strain CCUG 11256	2647	2647	100%	0	99.93%	NR_118798.1
Pseudomonas stutzeri strain NBRC 14165	2643	2643	100%	0	99.86%	NR_113652.1
Pseudomonas stutzeri ATCC 17588 = LMG 11199	2641	2641	100%	0	99.86%	NR_103934.2
Pseudomonas stutzeri strain VKM B-975	2636	2636	100%	0	99.79%	NR_116489.1
Pseudomonas songnenensis strain NEAU-ST5-5	2556	2556	100%	0	98.82%	NR_148295.1
Pseudomonas chloritidismutans strain AW-1	2516	2516	100%	0	98.26%	NR_115115.1
Pseudomonas kunmingensis strain HL22-2	2497	2497	100%	0	98.05%	NR_133828.1
Pseudomonas guariconensis strain PCAVU11	2488	2488	100%	0	97.92%	NR_135703.1
Pseudomonas knackmussii B13	2486	2486	99%	0	98.04%	NR_117756.1

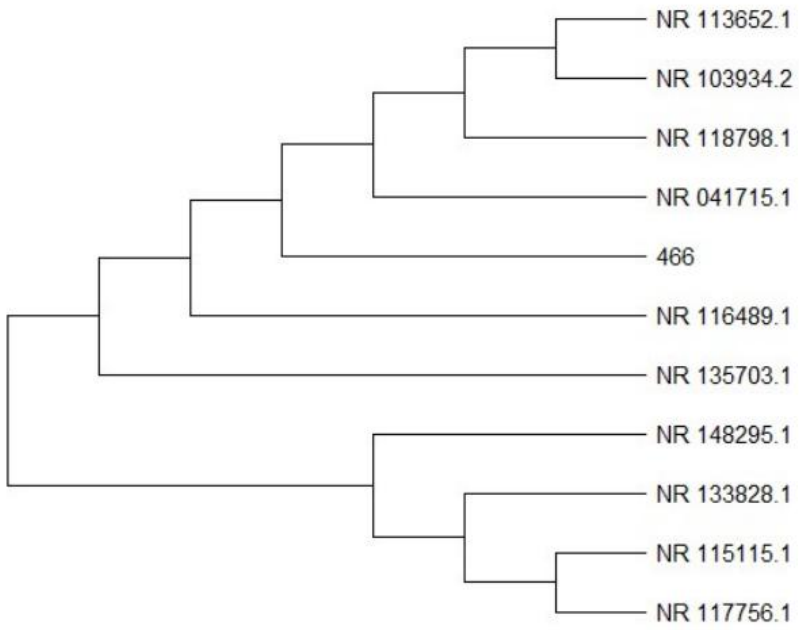

Fig. 4. Evolutionary relationship of taxa with reference to P-PB466

The evolutionary tree of L-PB424 showed that the strain shows close resemblance with *Lysinibacillus manganicus* 98.76%, *Lysinibacillus massiliensis* 97.80% and *Lysinibacillus chungkukjangi* 97.00% with this strain. It indicates that strain L-PB424 belongs to genus Bacillus. Hence, it is designated as *Lysinibacillus acetophenoni*. While the evolutionary tree of strain L-PB424 showed its close resemblance with *Lysinibacillus manganicus* with maximum 98% homology on the other hand P-PB466 showed that the strain shows close resemblance with *Pseudomonas stutzeri* 99.93%, *Pseudomonas songnenensis* 98.82% and *Pseudomonas chloritidismutans* 98.26% with this strain. It indicates that strain P-PB466 belongs to genus Pseudomonas. Hence, it is designated as *Pseudomonas stutzeri*. While the evolutionary tree of strain P-PB466 showed its close resemblance with *Pseudomonas stutzeri* with maximum 99% homology. The submission of 16SrRNA sequence to the NCBI GenBank and find the accession number of both sequences are MW362768 and MW44489. These two strains are halotolerant and possess adaptive mechanism to tolerate hypersaline condition.

4. DISCUSSION

The aim of our current inspection was to isolate plant growth-promoting *Lysinibacillus acetophenoni* and *Pseudomonas stutzeri* isolates and test them for physiological stress tolerance, such as pH and NaCl concentrations. Bacterial isolates were obtained from saline-rich soil in the block Ashabutter khair forest in Punjab, North India, in order to carry out this research. The PGP bacterial strains are beneficial to plant growth and defend plants from diseases and abiotic stress through a range of mechanisms. Those that form strong associations with plants, such as endophytes, may be more effective in promoting plant growth and increasing resistance to osmotic stress. Since The Gram-positive bacteria develop endospore, which have the ability to survive in harsh environmental conditions, the P-PB466 strain showed more resistance to high salinity (10 percent NaCl) than the P-PB 424 strain at pH 9. Our findings matched those of Damodarachari K., et al. [15], who tested bacteria for high temperatures (50°C), salinity (7%) and drought (-1.2MPa), finding that Pseudomonas isolates were less resistant than Bacillus isolates. Youssef et al. found that bacterial strains derived from salty soil grew at a comparable rate [16]. As L-PB424 was grown on nutrient broth with NaCl (2%, 5%, and 10%) and pH (5.0, 7.0, and 9.0) for two days at an 8-hour period, the maximum growth curve was obtained, which is consistent with Omotoyinbo [17]. Salinity tolerating bacteria, according to DasSarma [18], exhibit optimum growth in various saline environments, which are further classified into three classes: low salinities of 20-50 ppt, moderate salinities of 50-200 ppt, and high salinities > 200 ppt NaCl tolerating halopjiles. The 16SrRNA gene sequences of potent bacterial strains were compared with available sequences in Gene Bank to identify bacterial strains. Damodarachari K., et al. [15] recorded similar findings with Bacillus spp. BLAST search results from NCBI revealed 98.76 percent similarity of L-PB424 with *Lysinibacillus acetophenoni* and 99.93 percent similarity of P-PB466 with *Pseudomonas stutzeri.*

5. CONCLUSION

It is evident from the current sample that the sampling area contains a diverse range of bacterial species. In tryptophan broth, they react to IAA development in a significant way. IAA answer is good across the board in all isolates. Apart from that, isolates have demonstrated a significant role in phosphate solubilization, carbon source which have high impact on growth of the plant. Generation of IAA and Phosphorous Solubilizing Index has the highest response. Finally, it is clear that bacteria retrieved from saline were not only halotolerant, but also had traits that promoted plant growth. These bacteria can be used as biofertilizers to combat abiotic stress and are a blessing for sustainable agriculture in stressful situations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lynn JR, Rocco LM. Nature. 2001;409:1101.
2. Roberts MF. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems. 2005;1(1):1-30.
3. Margesin R, Schinner F. Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles. 2001;5(2):73.
4. Asad S, Amoozegar MA, Pourbabaee A, Sarbolouki MN, Dastgheib SM.

Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour Technol. 2007;98(11): 2082–8.
5. Oliveira LC, Ramos PL, Marem A, Kondo MY, Rocha R, Bertolini T, Et Al. Halotolerant bacteria in the São Paulo Zoo composting process and their hydrolases and bioproducts. Braz J Microbiol. 2015; 46(2):347 54.
6. Kothari V, Panchal M, Srivastava N. Hydrocarbon degradation potential of halotolerant bacteria. In: Scientific study. 2014. Lavik G, Stührmann T, Brüchert V, Van der Plas A, Mohrholz V, Lam P, Mußmann M, Fuchs BM, Amann R, Lass U, Kuypers MM. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. Nature. 2009;457(7229): 581–4.
7. Jyothi K, Babu KS, Clara NK, Kumar A. Identification and isolation of hydrocarbon degrading bacteria by molecular characterization. Helix. 2012;2:105–11.
8. Shirazian P, Asad S, Amoozegar MA. The potential of halophilic and halotolerant bacteria for the production of antineoplastic enzymes: L asparaginase and L glutaminase. EXCLI J. 2016;15:268.
9. Lavik G, Stührmann T, Brüchert V, Van der Plas A, Mohrholz V, Lam P, et al. Detoxification of sulphidic African shelf waters by blooming chemolithotrophs. Nature. 2009;457(7229): 581-584.
10. Ahmad F, Ahmad I, Khan MS. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol Res. 2008;163:173–181.
11. Yuen SH, Pollard AG. The determination of nitrogen in agricultural materials by the nessler reagent. I.—preparation of the reagent. Journal of the Science of Food and Agriculture. 1952;3(10):441-447.
12. Kang SM, Radhakrishnan R, You YH, Joo GJ, Lee IJ, Lee KE, et al. Phosphate solubilizing Bacillus megaterium mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. Indian Journal of Microbiology. 2014;54(4):427-433.
13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 2013;30(12):2725-2729.
14. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X. Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution. 2018;35(6):1547-1549.
15. Damodarachari K, Reddy RS, Triveni S, Rani CH, Sreedhar M. Isolation and Molecular Characterization of Abiotic Stress Tolerant Plant Growth Promoting Pseudomonas spp. from Different Rhizospheric Soils of Telangana State, India. International Journal of Bio-resource and Stress Management. 2018;9(5):611-619.
16. Youssef MA, Agrawala A, Shankar AU. WLAN location determination via clustering and probability distributions. In Proceedings of the First IEEE International Conference on Pervasive Computing and Communications, 2003;143-150.
17. Omotoyinbo OV, Omotoyinbo BI. Effect of varying NaCl concentrations on the growth curve of Escherichia coli and Staphylococcus aureus. Cell Biol. 2016;4(5):31-34.
18. DasSarma S. Extreme halophiles are models for astrobiology. Microbe-American Society for Microbiology. 2006;1(3):120.

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