ISOLATION AND CHARACTERIZATION OF AN *AERIBACILLUS COMPOSTI* SJP40 STRAIN, FROM THE ARID ZONE OF RAJASTHAN

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ABSTRACT

The strain SJP40 was identified from soil samples collected in desert and arid zones of Rajasthan. SJP40 was found to be a gram-positive, aerobic, rod-shaped, motile, and endospore-forming bacteria in an experiment. The best growth was seen at 55°C and an alkaline pH. The strain was able to survive in the medium supplemented with 5% NaCl. Agarose (1.2 %) was used to examine the DNA extracted from the culture. On the basis of nucleotide homology, phylogenetic analysis, and Bayesian inference, the strain was identified as Aeribacillus composti after sequence analysis by 16S rRNA. The strain SJP40 has been assigned the accession number MZ596340 by the NCBI.

Keywords: *Polyextremophiles, Extremozymes, Thermophile, Halotolerant, Aeribacillus composti.*

1. Introduction

The majority of the microbial life on Earth is mesophilic. Surprisingly, certain bacteria grow at high temperatures, salt concentrations, pH, pressure, water and nutrient availability, and so on, and are categorised as thermophiles, halophiles, barophiles, and xerophiles (Cowan et al., 2015). Thermophiles are the most common type of extremophile, and their members can survive high temperatures without losing activity (Satyanarayana et al., 2013) (DeCastro et al., 2016). Because of their excellent stability at severe temperatures, these microorganisms are popular for a variety of industrial applications (Adrio & Demain, 2014; Parihar & Bagaria, 2019; Raddadi et al., 2015). The majority of these thermophiles live in hydrothermal vents, deserts, deep ocean floors and volcanoes. Microorganisms of the other group of halophiles thrive in saltwater or hypersaline environment. They were widely studied for their salt-resistant genes and carotenoid synthesis, which have important uses in sectors such as cosmetics, optoelectronic devices, treatment of saltwater and hypersaline waste streams, bioplastics and biofuel, and so on (Margesin & Schinner, 2001; Oren, 2010). The desert regions are dry, with little available water. The soil's alkalinity and high salt content make it suitable for thermophiles, halophiles, and alkalophiles to thrive (Poli et al., 2010; Yasawong et al., 2011) (Zheng et al., 2012) (Brocchieri, 2013; Gugliandolo et al., 2014; Radchenkova et al., 2013) (Oikonomou et al., 2014). The adaption and sustainability of extremophilic bacteria (a thermophile in this study) are related to proteins with stable structures, changed metal ions, and complex membrane lipids that improve thermophilic protein stability (Huiyan & Jingyu, 2000; Shen & Shen, 2010). The other kind of extremophilic bacteria is halophiles, which can live at high salt concentrations. It has been discovered that they collect KCl ions in their cells to maintain the osmotic stability established by the bacteriorhodopsin surface-bound proton-pump activity. In hypersaline circumstances, the cellular architecture is subsequently stabilised by an increase in water potential inside the cell, which shields the protein molecules from denaturation. The protein produced by halophiles is adapted to a high salt environment, which promotes riparophilic polypeptide chain folding and results in a functional protein that modulates enzyme activity (Kong & Wang, 2017; Mao & Guo, 2018). Enzymes isolated from these extremophiles, including lipase, xylanase, pectinase, protease, amylase, and catalase, are in great demand for a variety of applications in biotechnological functions and industries. The current research includes the identification and characterisation of thermophilic-halotolerant isolated strains isolated from soil samples in Rajasthan's desert areas.

2. Materials and Methods

2.1 Sample Collection

In June 2017, soil specimens were collected from 11 designated locations in the region of

Desert National Park, Jaisalmer (Latitude: 26° 13' 40.8" N; Longitude: 70° 38' 24.6" E), Rajasthan, India (Figure 1). The samples were kept in sterile zip lock envelopes before being sent to the research lab for more analysis.

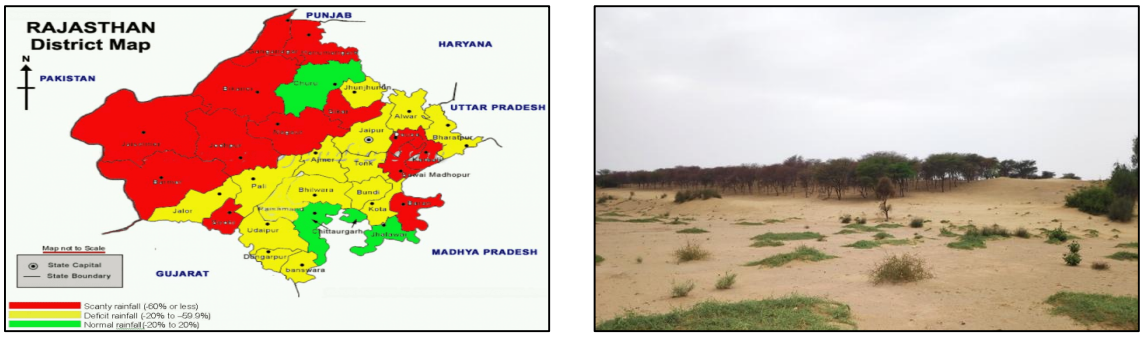

Figure 1: This map shows a) arid and semi-arid regions of Rajasthan. b) Sampling site.

2.2.Physicochemical Profiling of Soil Samples

The soil samples are pooled and filtered across a 2mm mesh before being tested for physical and chemical characteristics such as pH, EC, temperature, macronutrients (carbon, phosphorous, and potassium), and micronutrients (iron, copper, zinc, and manganese) using standard techniques (Lindsay & Norvell, 1978). The chemical properties, such as total organic carbon (TOC), were determined using a fast titration technique. Phosphorus, potassium, and micronutrient content (iron, copper, zinc, and manganese) were determined using a spectrophotometer, a flame photometer, and atomic absorption spectroscopy (Agilent Technologies). The physical properties of the samples, such as pH and EC, were assessed. the means of a multi-parameter device (Bandyopadhyay et al., 2013; Cota-Ruiz et al., 2018; Yadav et al., 2018). Statistical errors for all components examined in this study are 5%.

2.3.*Isolation of Extremophilic Bacteria*

To culture, the bacteria, Nutrient agar, and broth medium (Himedia) were utilized. The nutrient agar media was made at neutral pH and autoclaved. As a dilution medium, phosphate buffer saline (PBS) with a pH of 7 was used. The experiments were performed in replicates of three, samples were combined and serially diluted up to 10^{-8} in dilution media. Each 10^{-8} diluted replication tube's inoculum (0.1mL) was distributed on an agar medium and incubated overnight at 37°C. Following growth observation, a single colony was selected based on colony shape and inoculation in nutrient broth medium before incubating for 24 hours at 37°C (Baltaci et al., 2017).

2.4. *Growth on Different Temperature*

To test the viability of pure isolates at extremely high temperatures, bacteria are cultured at various temperatures such as 37°C, 45°C, 50°C, and 60°C. The growth was seen after 24 hours, and the optical density (OD) was measured at 600nm with a spectrophotometer (Shimadzu) (Alrumman et al., 2018).

2.5. *Morphological and Biochemical Characterization*

Gram's staining, Endospore staining (Holt et al., 1994), Indole test, Citrate test, Starch hydrolysis, Deaminase, Motility, and Catalase were all used to screen the obtained pure cultures for different biochemical assays (Timilsina et al., 2020).

Salt Tolerance Test

The pure culture was examined for tolerance to various concentrations of NaCl salt ranging from 1 to 8% (w/v). The isolated culture was inoculated in a nutrient broth medium with concentrations of NaCl salt. The optical density (OD) at 600nm was used to measure the growth.

2.6. *pH Sensitivity Test*

For the pH sensitivity test, medium with pH ranging from 4 to 9 were prepared, and the pure isolates were inoculated in media with varying pH. After overnight incubation at 60°C, the capacity of bacteria to withstand different pH ranges was determined by

detecting growth or turbidity in nutrient broth at 600nm.

2.7. *Molecular identification*

The genomic DNA was isolated from SJP40 pure cultures for molecular characterization using the QIA amp DNA Mini Kit (Qiagen). The DNA quality was measured using an agarose gel (0.8 per cent) and the amount using a nanodrop (Thermo Scientific, Germany). The isolated bacterial DNA was kept at -80°C until it was processed.

2.8. *16S rDNA Gene Amplification*

DNA was extracted from bacterial isolate SJP40 but also amplified with 8Fand 1492R universal primers of 16S rDNA gene by using Thermal Cycler (Veriti® 96 well). The PCR reaction products PCR buffer (10X), forward and reverse primers (10M), dNTP mix (10mM), Dream Taq DNA polymerase (1.5U), and up to 100ng DNA template was mixed gently, and the reaction was carried out for 35 cycles under standard PCR cycling protocols. The amplified product was purified on the gel using the Gel extraction kit and submitted to 16S rDNA region sequencing (Eurofins Genomics, India, Pvt. Ltd.). Editseq software was used to verify and align the obtained sequences. After that, the aligned sequences were examined and were compared to other gene sequences using the BLAST tool, and then uploaded to the NCBI GenBank.

2.9. *Phylogenetic Analysis*

For implying evolutionary history, the Neighbour-joining approach was utilized (Saitou & Nei, 1987). Using a bootstrap consensus tree, the lineage of all species studied was reconstructed from 1000 replicates (Felsenstein, 1985). Branches were fragmented when compared to parts regenerated in less than 50% bootstrap repetitions. The evolutionary distances were estimated using the Maximum Composite Likelihood method (Kimura, 1980) but were in base replacements per site unit. For the study, a total of 16 nucleotide sequences were analysed. It contains the first, second, third, and noncoding codon locations. The data that was missing or incomplete was deleted. A total of 1410 locations were contained in the final data set. The lineage history was examined using the MEGA X software (Kumar et al., 2018).

3 Results and Discussions

3.1. Physicochemical Profiling of Soil Samples

Soil samples are collected near the Desert National Park in Jaisalmer and their physical parameters, such as pH, EC and temperature, as well as chemical properties, such as micro and macronutrient concentrations, were measured. Table 1 shows the concentrations of different nutrients such as carbon, phosphorus, potassium, zinc, copper, iron, and manganese found in the soil. The reliability of the results was verified by repeating the tests three times.

Table 1. Soil profiling

Sr. No.	Properties	Soil Parameters	Observations
1.	Physical Properties	a) pH	8.25
		b) EC	0.09 (dSm^{-1})
2.	Chemical properties		
	A. Macronutrients	a) C	0.24 ($g\ kg^{-1}$)
		b) P	42.0 ($g\ kg^{-1}$)
		c) K	293 ($g\ kg^{-1}$)
	B. Micronutrients	a) Zi	0.51 ppm
		b) Cu	4.17 ppm
		c) Fe	0.28 ppm
		d) Mn	2.65 ppm

3.2. *Isolation of Extremophilic Bacteria*

Thermophilic bacteria are robust enough to survive in harsh environments. Several researchers have confirmed that such capability may be due to modifications at the cell and subcellular levels. In our current investigation, thermophilic bacteria were found in soil samples taken from dry regions of Rajasthan, India. In this work, we isolated 40 bacteria at 37°C and labelled them SJP1 through SJP40.

3.3. Growth on Different Temperature

All 40 bacterial isolates were cultured at various temperatures, however, all bacterial isolate failed to grow above 37°C except isolate SJP40. Only one isolate, SJP40, was able to develop at 37°C, 45°C, 55°C, and 60°C. Bacterial culture was inoculated and incubated overnight at various temperatures, and growth was observed. A spectrophotometer was used to measure the optical density (OD) at 600nm. Figure 2 depicts the growth curves of bacterial isolates at various temperatures. It demonstrates that the optimal temperature for growth is 55°C. The bacteria's ability to survive at 55°C temperature revealed that it was a moderate thermophile. Finore et al. (2017) described thermophilic bacteria that grow best at 60°C (Finore et al., 2017). Several investigations found thermophilic bacteria from various regions that could withstand temperatures of up to 60°C (Harnvoravongchai et al., 2020; Naresh et al., 2019; Pawar & Borkar, 2018).

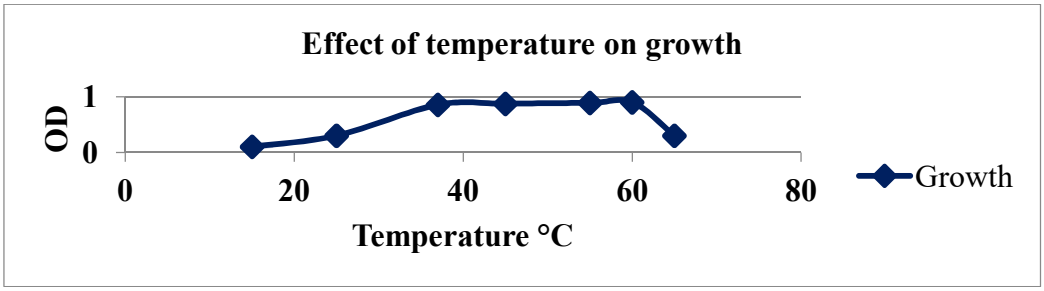

Figure 2. Effect of temperature on bacterial growth.

3.4. Morphological and Biochemical Characterization

The extracted bacterial strain's morphology shows that SJP40 is a gram - positive, motile, as well as endospore-forming. Finore et al. (2017) discovered similar microorganisms in olive mill pomace compost samples (Finore et al., 2017). On nutrient agar plates, the strain produced opaque and circular colonies with uniform borders and tested positive for enzymes like deaminase, catalase, and tryptophanase but negative for caseinase and amylase.

Table 2. Morphological and Biochemical Characterization

S.No	Characteristics	SJP40
1	Shape	Rod
2	Colour	Light Cream
3	Opacity	+
4	Gram stain	+
5	Endospore stain	+
6	O_2	Aerobe
7	Motility	+
8	G+C content (%)	56.8

3.5. Salt Tolerance Test

At 55°C, bacterial cultures' salt tolerance was tested in nutrient broth with NaCl concentrations ranging from 0 to 8% (w/v). The observation reveals that the highest growth occurs at 5% NaCl and that growth decreases beyond this concentration. The optimal salt content is 4% NaCl (Table 3). Finore et al. (2017) described a bacterial strain that has a 6 per cent optimal salt tolerance (Finore et al., 2017).

Table 3. Salt Tolerance Test

S.No	Salt Conc. (w/v)	SJP40
1	0%	+++
2	1%	+++
3	2%	+++
4	3%	+++
5	4%	++++
6	5%	+++
7	6%	++
8	7%	+
9	8%	-

3.6. pH Sensitivity Test

pH was shown to influence the growth of bacterial isolates. The results revealed that maximal growth was attained at pH 8.25, while optimal pH was at 8, and that pH values above these levels were declining. Bacterial growth was considerably reduced at acidic pH. This indicates that bacterial isolates are more active in alkaline pH environments (Table 4).

Table 4. pH Sensitivity Test

S.No	pH	SJP40
1	4.0	-
2	4.5	-
3	5.0	-
4	5.5	+
5	6.0	+
6	6.5	++
7	7.0	++++
8	7.5	++++
9	8.0	++++
10	8.5	++++
11	9.0	++
12	9.5	+
13	10.0	-

3.7. Molecular Identification

The bacterial isolate SJP40 was molecularly characterised using the 16S rDNA molecular method. On 1.2 per cent agarose gel, amplification of extracted DNA using 16S rDNA Specific Primer (8Fand 1492R) revealed a single distinct PCR amplicon band of about 1500 bp (Figure 3). The amplified PCR 16s rDNA sequence was sequenced and the aligned sequence was submitted to the NCBI gene database (accession number MZ596340).

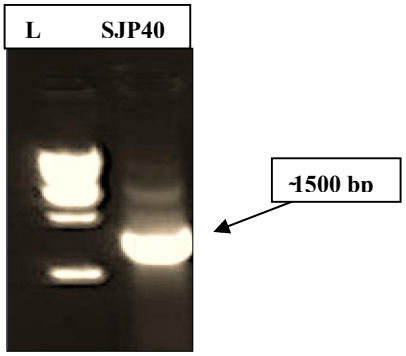

Figure 3. A single 1500 bp of 16S rDNA amplicon was showing on agarose gel (1.2%). Lane1(L): 1Kb DNA ladder; Lane 2 (SJP40): 16S rDNA amplicon

3.8. Phylogenetic Analysis

The neighbour-joining technique was applied for phylogenetic analysis, and SJP40 exhibited 99 per cent similarity to *Aeribacillus composti*, strain N.8, based on nucleotide homology, and was closely linked to *Aeribacillus pallidus* (Figure 4). Extremophilic bacteria Aeribacillus pallidus have been identified and described from beet vinasse (Harirchi et al., 2020), petroleum polluted soil (Tao et al., 2020), and oily wastewater (Ktata, Krayem, et al., 2020).

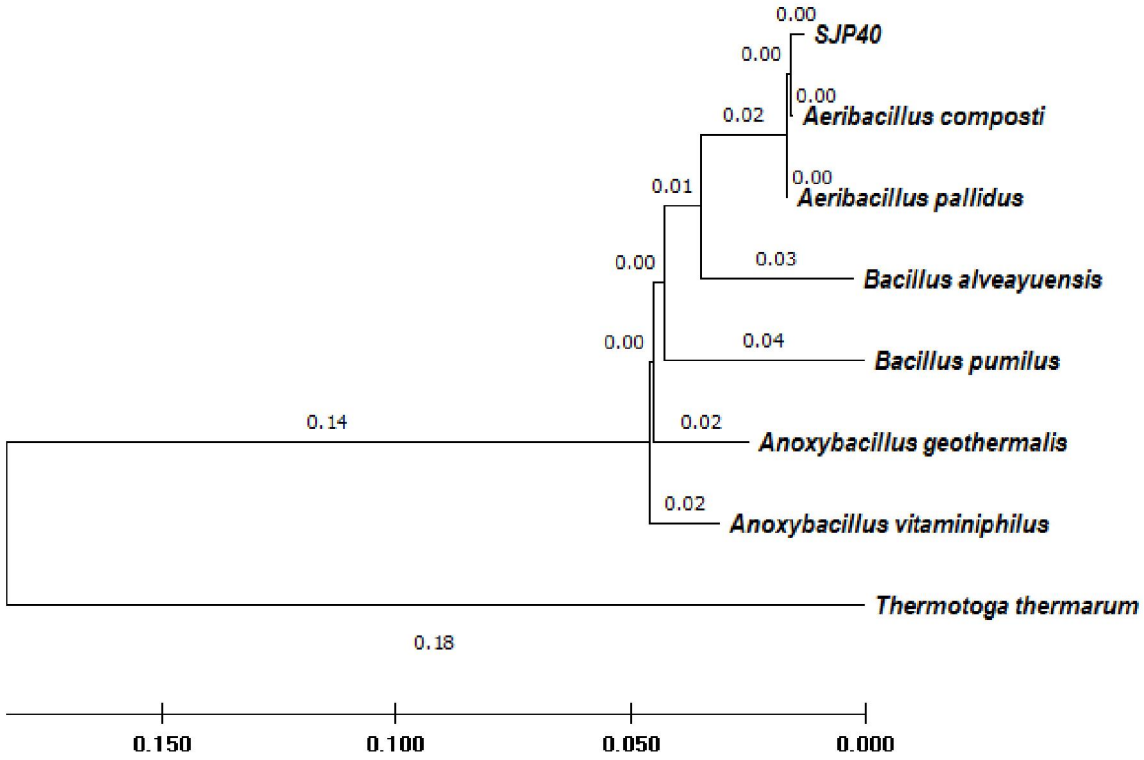

Figure 4. Evolutionary Relationship based on 16S rDNA gene amplicon sequences of strain SJP40 (MZ596340).

4. Conclusion

The current study shows that soil samples from Rajasthan's desert climates are a rich source of thermo-halotolerant microorganisms, and to the best of our knowledge, this is the first of its type to be reported from this location. The strain SJP40 has an enzymatic activity like deaminase, tryptophanase, and catalase. This was discovered through the biochemical study described in the paper. Deaminase enzymes are engaged in the deamination process, which involves the removal of an amine group by hydrolysis. Deamination can be harmful if it inhibits protein synthesis in any way. Several structural investigations on deaminase have been conducted (Kamat et al., 2011). This opens the door to future investigation of the strain's 3-D structural characteristics, which would contribute to the process of adaptation to severe biomes. *Aeribacillus composti* were identified as thermophiles from olive mill pomace compost in 2017 (Finore et al., 2017). In similar research, the prokaryotic community in compost produced from waste biomass, such as ground coffee from an improper roasting procedure, was studied using culture-dependent and culture-independent approaches (Papale et al., 2021). They have presented different chances for the synthesis of non-aqueous peptides in another research (Mechri et al., 2017). *Aeribacillus composti* is most closely related to the *Aeribacillus pallidus* strain which is similar biochemically as well as molecularly. Contributing to studies on *Aeribacillus composti*, which is isolated mostly from deserts, marshlands, and industrial wastewater contaminated areas (Bose & Satyanarayana, 2016; Mechri et al., 2017; Muhammad & Ahmed, 2015; Radchenkova et al., 2015). Another study demonstrated the lipase catalytic activity utilised in detergent compositions for the treatment of oily wastewater (Ktata, Karray, et al., 2020; Ktata, Krayem, et al., 2020). According to certain research, thermophilic bacteria were utilised to synthesise nanoparticles for antibacterial activity (Jagdish et al., 2021). Furthermore, the thermostability of the enzymes produced by these isolated bacteria reveals that their biotechnological potential has been widely explored (Harirchi et al., 2020; Kita et al., 2020) (Miyazawa et al., 2020; Rassa, 2020; Tao et al., 2020; Timilsina et al., 2020). The intriguing findings can be used to select the optimum conditions for large-scale development and characterisation of the thermostable enzymes produced by SJP40.

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Conflict of Interest

The authors declare no conflict of interest.

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