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Species Composition and Diversity Dynamics of Actinomycetes in Arid and Semi-arid Salt Basins of Rajasthan

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Abstract

Species composition and diversity dynamics of the actinomycetes was studied in five salt basins of arid and semi-arid areas of Rajasthan, India. A novel approach integrating molecular (16S rRNA gene) and diversity indices was applied to reveal species composition and diversity dynamics. Fifty-three actinomycetes isolates were isolated from five arid and semi-arid salt basins. Molecular characterization resulted in the identification of actinomycetes species belonging to three genera namely, *Streptomyces, Nocardiopsis,* and *Actinoalloteichus*. The diversity study among actinomycetes species validates their universal occurrence in arid and semi-arid regions of Rajasthan. The species *N. dassonvillei* subsp. *albirubida* was omnipresent in all the five salt basins but its relative manifestation was not static across habitats. The study revealed that three species *N. chromatogenes*, *S. durbertensis,* and *S. mangrovicola* are being reported for the first time from India. The maximum species of actinomycetes were recorded from Pachpadra (14) and the minimum from Didwana area (6). This study not only documents the hitherto wealth of actinomycetes species in arid and semi-arid salt basins of Rajasthan but also reveals the composition and diversity dynamics of actinomycetes.

Introduction

Globally, several halophilic and halotolerant microbial genera viz., *Halobacterium*, *Halomonas*, *Haloactinospora*, *Salinibacterium*, *Marinactinospora* etc. have been isolated and identified. Ma and Gong [1] reported the incidence of approximately 50% of archaeal diversity and less than 25% of bacterial diversity from the saline soil habitats. Some research studies on soil microbial biomass [2], enzymatic activities of salt tolerant microbes [3], and characterization of salt tolerant species of *Rhizobium* [4] have been carried out in Indian saline environment. Quantitative assessment of microbial diversity using 16S rRNA gene have been carried out from various salt-affected areas such as Great Salt Lake Utah, USA, [5], Sidi Ameur and Himalatt Salt Lakes of the Algerian [6], Keke Salt Lake China [7], Yuncheng Salt Lake, China [8], and Sambhar salt lake, India [9]. However, integrated scientific inventories utilizing molecular and biochemical tools with combinations of community ecology techniques are lacking.

Rajasthan lies in the north western part of the India between 23°30′ and 30°12′ North latitude and 69°30′ and 78°17′ East longitude covering Aravalli mountain range and great Indian Thar Desert [10]. Indian Thar Desert is also known for some of the saline basins such as Pachpadra salt lake (Barmer), Lunkaransar salt lake (Bikaner), Phalodi salt belt (Jodhpur), Didwana salt lake (Nagaur), and Sambhar salt lake (Jaipur). Actinomycetes are one of the most diverse groups of bacteria, exhibiting larger niche breadth by virtue of their diverse metabolic traits. These filamentous bacteria have been recognized to have unprecedented potential in production of antibiotics and biologically active natural product. The investigation of underexplored saline playas of arid and semi-arid regions of Thar desert could be considered for isolation of novel members of actinomycetes that could be a source of new bio-molecules for human welfare. Therefore, the present study was undertaken with the objectives (a) to identify actinomycetes isolates from soil of different salt basins of Rajasthan using 16S rRNA molecular markers, (b) to quantify the community structure and diversity indices, and (c) to establish relationships between diversity and soil parameters.

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Materials and Methods

Survey and Collection of Soil Samples

Five salt basins namely Pachpadra (25°55′N and 72°13′E; Barmer district), Lunkaransar (28°29′N and 73°44′E; Bikaner district), Phalodi (27°16′N and 72°23′E; Jodhpur district), Didwana (27°23′N and 74°34′E; Nagaur district), and Sambhar (26°54′N and 75°11′E; Jaipur district) were surveyed for the collection of soil samples. Location of each soil sampling site was recorded with the help of mobile Global Positioning System (GPS). A map showing the location of each site is presented in Supplementary Fig. 1. An area of 2000 m^2 was marked at each salt pan and five soil samples from each site were collected up to 5 cm depth using a sterile spatula. The collected soil samples were brought to the laboratory and were stored in airtight plastic bags at 4 °C for further study.

Physico-Chemical Analysis of Soil Samples

The air-dried soil samples were mixed thoroughly to form a composite sample. As the samples were collected from the periphery of the salt basins and it was assumed that the physico-chemical values may be *at par*, the samples were pooled for the analysis. The air-dried samples were passed through 0.5 mm sieve to remove bigger soil particles. The analysis of various physico-chemical parameters viz., soil pH, electrical conductivity, organic carbon, available nitrogen, available phosphorous, and potassium was performed by the methods described in standard soil analysis manual [11].

Isolation and Purification of Actinomycetes

Actinomycetes were isolated from the collected soil samples by serial dilution method [12]. The 0.5 ml of 10^{-6} dilution was aseptically plated on actinomycetes Isolation Agar media (AIA; sodium caseinate 2 g/l, L-Asparagine 0.01 g/l, sodium propionate 4 g/l, dipotassium phosphate 0.5 g/l, Magnesium Sulfate 0.1 g/l, ferrous sulfate 0.001 g/l, Agar 15 g/l, pH 8.5) supplemented with 0.5 M NaCl as suggested by Malviya et al. [13]. AIA media was also supplemented with nystatin (25 µg/ml) to minimize the fungal growth. After incubation at 37 °C in a BOD incubator for 10 days, the colony forming units (CFUs) were counted. Isolate codes were assigned to all distinct CFU's. Only morphologically distinct plates having chalky and powdery appearance of actinomycetes colony were picked and re-streaked on Starch Casein Agar media (SCA; Starch 10 g/l, Sodium Chloride 3.7 g/l, Casein 1 g/l, Agar 15 g/l, pH 8.5) to obtain pure cultures. Purified cultures were sub-cultured and maintained on the SCA medium at 4 °C for further characterization and were also preserved in 20% glycerol at − 20 °C.

Morphological and Cultural Characterization

The morphological characteristics of the isolated actinomycetes were recorded on AIA media using standard guidelines established by the International Streptomyces Project [14]. The preliminary characteristic such as aerial and substrate mycelium with their pigmentations facilitated the identification of actinomycetes isolates.

Genomic DNA Isolation and Quantification

Purified isolates were inoculated in Starch Casein broth media and incubated for 10 days. Extraction of genomic DNA of actinomycetes was done using the method described by Cheng and Jiang [15]. The quality of DNA was determined on agarose gel electrophoresis using Gel Doc System (BIO-RAD XR+). The yield of DNA was quantified by using DeNovix DS-11 spectrophotometer.

16S rRNA Gene Amplification, Sequencing, and Submission to GenBank

16S rRNA gene was amplified using universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTACGACTT-3′) [16]. The amplified 16S rRNA genes were sequenced using the dideoxy chain termination method through outsourcing from Xcleris Genomics Labs Ltd., Ahmadabad (India). Sequences of 16S rRNA genes were analyzed, complied, and aligned by software Bio-Edit 7.2. These sequences were compared with the available reference sequences of GenBank using of Basic Local Alignment Search Tool (BLAST) to record the percent identities. All the gene sequences were submitted to GenBank database of NCBI. The Molecular Evolutionary Genetic Analysis (MEGA-7) [17] software was used for construction of neighbor-joining phylogeny tree, inferring distances and percent similarity.

Community Dynamics Analysis

Diversity of the actinomycetes isolated from different salt basin was estimated with the help of two data-sets (1) community dynamics using binomial data and Principal Component Analysis (PCA) and (2) community dynamics with quantitative quadrate analysis. The attributes of second data set were also utilized for Dominance Diversity (DD) Curve, alpha, beta and gamma diversities and for partial least square (PLS) regression.

The binomial datasets were recorded on the basis of absence (0) and presence (1) of a species at a particular

habitat. PCA was calculated as data reduction technique and was performed using Pearson correlation coefficient. For quadrate studies; density, frequency, abundance and Relative Importance Values (RIV) of different species isolated from five sampling sites of a particular habitat were quantified using following ecological formulae [18, 19].

$$\text{Density of a species} = \frac{\text{Total number of individuals of a species in all the sampling areas}}{\text{Total number of area studied}}$$

$$\text{Frequency of a species} = \frac{\text{Total number of sampling areas in which species occur}}{\text{Total number of area studied}} \times 100$$

$$\text{Abundance of a species} = \frac{\text{Total number of individuals of a species in all the sampling areas}}{\text{Total number of sampling areas in which the species occurs}}$$

$$\text{Relative Density of a Species} = \frac{\text{Density of a species}}{\text{Sum of all the desities}} \times 100$$

$$\text{Relative Frequency of a Species} = \frac{\text{Frequency of a species}}{\text{Sum of all the frequencies}} \times 100$$

$$\text{Relative Abundance of a Species} = \frac{\text{Abundance of a species}}{\text{Total Abundance of all the species}} \times 100$$

$$\text{Relative Importance Value(RIV)} = \frac{\text{Relative Density} + \text{Relative Freqency} + \text{Relative Abundance}}{3}$$

Dominance Diversity curve (DD curve) was prepared to evaluate the abundance trends of different species from different salt areas. The RIV of different species was converted into log transformation, arranged in descending order and presented graphically.

Diversity of actinomycetes species was assessed at three levels (a) diversity within a habitat (α, alpha diversity) (b) diversity between habitat (β, beta diversity), and (c) diversity along the habitat (γ, gamma diversity). Alpha diversity was calculated using Shannon's diversity index (H'), Simpson's diversity index (λ), Margalef's richness index ($R1$), Menhinick's richness index ($R2$), Brillouin index, and evenness ($E5$). All the alpha diversity indices are mathematically independent to each other [20]. Beta diversity was calculated with Wilson Shimda index and presented as comparative similarity among studied areas. Gamma diversity was estimated with the multiplication of average values of alpha and beta diversities and total number of zones [21]. All the diversity indices were calculated using Paleontological Statistics (PAST) version 1.92 software.

16S rRNA gene sequence accession numbers: MT669267 to MT669319.

Results and Discussion

Actinomycetes are the taxonomically diverse group of microbial community which are known for their potential to produce antibiotics, enzymes and bioactive secondary metabolites. Since the discovery of first antibiotics streptomycin from *Streptomyces*, the actinomycetes are constantly explored and isolated from diverse habitat. There are many actinomycetes species flourishing under extreme environmental conditions that need taxonomic identification and characterization for important by-products. There are few preceding studies on characterization of actinomycetes from non-saline habitat of great Indian Thar Desert [22–24].

In the present study, total 146 colonies were isolated from the five salt basins; 35 isolates (PP-1 to PP-35) from Pachpadra, 26 isolates (LK-1 to LK-26) from Lunkaransar, 33 isolates (PH-1 to PH-33) from Phalodi, 18 isolates (DW-1 to DW-18) from Didwana and 34 isolates (SL-1 to SL-34) from Sambhar Lake (Table 1). Out of the 146 colonies, fifty-three isolates purified on SCA media which exhibited the

Table 1 Details of sampling sites, colony forming units (CFUs) and actinomycetes isolates

S. no.	Salt Lake basin	Bacterial colony forming units (CFUs)	Number of bacterial colony isolated	Actinomycetes colony forming units (CFUs)	Colony showing typical characteristics of actinomycetes and percentage	Isolate code of colony showing typical characteristics of actinomycetes
1	Pachpadra (Barmer) Arid region	3.8×10^6	35 (PP1 to PP35)	1.78×10^6	16 (10.96%)	PP1, PP2, PP3, PP4, PP6, PP7, PP8, PP9, PP10, PP11, PP13, PP15, PP16, PP17, PP21, P23
2	Lunkaransar (Bikaner) Arid region	2.8×10^6	26 (LK1 to LK26)	0.78×10^6	7 (4.79%)	LK1, LK2, LK7, LK8, LK13, LK16, LK18
3	Phalodi (Jodhpur) Arid region	3.6×10^6	33 (PH1 to PH33)	1.38×10^6	12 (8.22%)	PH1, PH5, PH6, PH8, PH10, PH15, PH23, PH25, PH26, PH29, PH31, PH32
4	Didwana (Nagaur) Semi-arid region	2.0×10^6	18 (DW1 to DW18)	0.88×10^6	8 (5.48%)	DW1, DW3, DW5, DW9, DW10, DW11, DW13, DW15
5	Sambhar (Jaipur) Semi-arid region	3.7×10^6	34 (SL1 to SL34)	1.18×10^6	10 (6.85%)	SL2, SL4, SL10, SL12, SL13, SL14, SL16, SL19, SL25, SL26

typical characteristics of actinomycetes (chalky and powdery appearance of colony, colony texture, color of aerial and substrate mycelium and pigmentation). In all, the maximum (16) isolates were from Pachpadra salt basin followed by Phalodi (12 isolates), Sambhar (10 isolates), Didwana (8 isolates), and Lunkaransar (7 isolates) salt basin (Table 1). The findings indicate that there are variations in distribution dynamics of actinomycetes communities among the selected sites. Similarly, Kumar et al. [23, 24] also reported prevalence of actinomycetes in the Indian Thar Desert, Rajasthan.

Molecular Characterization and Identification of Actinomycetes

The 16S rRNA gene sequences of 53 actinomycetes isolates were subjected to BLAST search and were aligned with that of available reference sequences of NCBI databases for molecular identification on the basis of the maximum similarities. The molecular characterization of all the 53 isolates eventually delineated into species belonging to three genera viz., *Streptomyces, Nocardiopsis,* and *Actinoalloteichus*. The gene sequences were submitted to NCBI Genbank (accession numbers MT669267 to MT669319). The nucleotide length, GC content, similarity percentage, and GenBank accession numbers along with their reference sequences of all the actinomycetes isolates are presented in Table 2. Out of 53 isolates, 34 isolates belonged to *Streptomyces* species followed by 18 isolates of *Nocardiopsis* species and one isolate of *Actinoalloteichus* species indicating the presence of diversity of actinomycetes in the salt basin of Rajasthan (Table 2). Similarly, Kumar et al. [24] reported four actinomycetes genera viz., *Streptomyces, Nocardiopsis, Saccharomonospora,* and *Actinoalloteichus* from arid zone of Indian Thar desert, Rajasthan while Tiwari et al. [22] reported five actinomycetes genera *Streptomyces*, *Actinomadura*, *Nocardia*, *Nonomuraea*, and *Spirillispora* from Indian Thar Desert. Kumar et al. [23] also demonstrated density and diversity of *Streptomyces* from the soils of semi-arid regions of Rajasthan.

The maximum six isolates were identified as *N. dassonvillei* subsp. *albirubida* while five and four isolates as *S. erythrogriseus*, and *N. synnemataformans*, respectively. Three isolates of *N. dassonvillei* subsp. *dassonvillei*, three isolates of *S.mangrovicola*, *S. radiopugnans*, and *S. rochei*, two isolates of *S. durbertensis*, *S. speibonae*, and *S. vinaceusdrappusi* have been reported from the salt basin. *A. cyanogriseus*, *N. alba*, *N. chromatogenes*, *N. lucentensis*, *N. potens*, *N. prasina*, *S. albogriseolus*, *S. alkaliphilus*, *S. althioticus*, *S. carpaticus*, *S. ardesiacus*, *S. fenghuangensis*, *S. gelaticus*, *S. griseoloalbus*, *S. griseomycini*, *S. mutabilis*, *S. paradoxus*, *S. tendae*, *S. thermolilacinus*, and *S. thermospinosisporus* represented with single isolate revealing the dominance of *Streptomyces* species in the region. Present findings corroborate with that of earlier reports underlining that actinomycetes population were dominated by the *Streptomyces* species in arid and semi-arid zone [22–25]. Out of these species composition, three species namely *N. chromatogenes*, *S. durbertensis,* and *S. mangrovicola* are being reported for the first time from India.

Table 2 Molecular characterization of actinomycetes isolates using 16S rRNA gene sequencing

S. no.	Isolate code	Nucleotide length	G+C (%)	Reference sequence	Identity (%)	Actinomycetes species	GenBank accession number
1	PP1	1427	59.70	*S. erythrogriseus*(NR42294)	99.86	*S. erythrogriseus* PP1	MT669294
2	PP2	1056	59.37	*S. speibonae* (NR025212)	98.68	*S. speibonae* PP2	MT669295
3	PP3	1029	58.89	*S. thermolilacinus* (NR125444)	99.90	*S. thermolilacinus* PP3	MT669296
4	PP4	1388	59.65	*S. erythrogriseus* (NR112438)	100	*S. erythrogriseus* PP4	MT669297
5	PP6	1421	58.55	*N. dassonvillei* (NR074635)	100	*N. dassonvillei* sub sp. *dassonvillei* PP6	MT669298
6	PP7	1423	59.45	*S. rochei* (NR116078)	100	*S. rochei* PP7	MT669299
7	PP8	1403	59.03	*S. rochei* (NR116078)	99.93	*S. rochei* PP8	MT669300
8	PP9	1004	59.06	*S. carpaticus* (NR112450)	99.70	*S. carpaticus* PP9	MT669301
9	PP10	1395	59.12	*S. ardesiacus* (NR112454)	99.71	*S. ardesiacus* PP10	MT669302
10	PP11	1416	59.25	*S. paradoxus* (MK424310)	99.58	*S. paradoxus* PP11	MT669303
11	PP13	1236	59.38	*S. mutabilis* (NR044139)	100	*S. mutabilis* PP13	MT669304
12	PP15	1410	59.43	*S. vinaceusdrappus* (NR112368)	100	*S. vinaceusdrappus* PP15	MT669305
13	PP16	1040	59.71	*S. tendae* (NR025871)	99.42	*S. tendae* PP16	MT669306
14	PP17	1418	59.16	*N. dassonvillei* subsp. *albirubida* (NR112743)	98.87	*N. dassonvillei sub sp. albirubida* PP17	MT669307
15	PP21	1449	59.69	*Streptomyces sp.* (KR827683)	99.03	*S. griseoloalbus* PP21	MT669308
16	PP23	883	60.02	*S. griseostramineus* (NR041067)	99.43	*S. griseomycini* PP23	MT669309
17	LK1	1385	58.41	*N. synnemataformans* (NR112742)	99.86	*N. synnemataformans* LK1	MT669275
18	LK2	1408	58.73	*N. dassonvillei* subsp. *albirubida* (NR112743)	99.79	*N. dassonvillei sub sp. albirubida* LK2	MT669276
19	LK7	1339	59.74	*N. chromatogenes* (NR043032)	98.81	*N. chromatogenes* LK7	MT669277
20	LK8	1337	60.20	*S. mangrovicola* (NR148322)	99.55	*S. mangrovicola* LK8	MT669278
21	LK13	1422	59.21	*N. lucentensis* (NR026342)	99.02	*N. lucentensis* LK13	MT669279
22	LK16	1396	59.38	*A. cyanogriseus* (NR024650)	99.79	*A. cyanogriseus* LK16	MT669280
23	LK18	1406	58.67	*N. alba* (NR026340)	99.79	*N. alba* LK18	MT669281
24	PH1	1398	59.29	*S. rochei* (NR116078)	100	*S.rochei* PH1	MT669282
25	PH5	934	59.52	*S. speibonae* (NR025212)	99.36	*S. speibonae* PH5	MT669283
26	PH6	1340	60.07	*S. mangrovicola* (NR148322)	99.63	*S. mangrovicola* PH6	MT669284
27	PH8	902	59.86	*S. albogriseolus* (NR112487)	100	*S. albogriseolus* PH8	MT669285
28	PH10	1390	58.41	*N. synnemataformans* (NR112742)	99.78	*N. synnemataformans* PH10	MT669286
29	PH15	1422	59.63	*S. erythrogriseus* (NR112438)	100	*S. erythrogriseus* PH15	MT669287
30	PH23	1403	60.29	*S. mangrovicola* (NR148322)	99.36	*S. mangrovicola* PH23	MT669288
31	PH25	1404	59.68	*S. radiopugnans* (NR044013)	99.57	*S. radiopugnans* PH25	MT669289
32	PH26	1400	59.85	*S. radiopugnans* (NR044013)	99.64	*S. radiopugnans* PH26	MT669290
33	PH29	1402	58.84	*N. dassonvillei* subsp. *albirubida* (NR112743)	99.00	*N. dassonvillei sub sp. albirubida* PH29	MT669291
34	PH31	1396	59.16	*N. dassonvillei* subsp. *albirubida* (NR112743)	99.00	*N. dassonvillei sub sp. albirubida* PH31	MT669292
35	PH32	1395	60.28	*S. fenghuangensis* (NR117502)	99.28	*S. fenghuangensis* PH32	MT669293
36	DW1	1389	58.31	*N. synnemataformans* (NR112742)	99.78	*N. synnemataformans* DW1	MT669267
37	DW3	1385	59.35	*S. thermospinosisporus* (KU141346)	99.93	*S. thermospinosisporus* DW3	MT669268
38	DW5	1413	60.15	*N. potens* (NR116914)	99.86	*N. potens* DW5	MT669269
39	DW9	1419	58.35	*N. prasina* (NR044906)	99.30	*N. prasina* DW9	MT669270
40	DW10	1399	58.68	*S. durbertensis* (KY318506)	99.36	*S. durbertensis* DW10	MT669271
41	DW11	1433	59.24	*N. dassonvillei* subsp. *albirubida* (NR112743)	98.95	*N. dassonvillei sub sp. albirubida* DW11	MT669272
42	DW 13	1394	58.46	*N. synnemataformans* (NR112742)	99.86	*N. synnemataformans* DW13	MT669273

Table 2 (continued)

S. no.	Isolate code	Nucleotide length	G+C (%)	Reference sequence	Identity (%)	Actinomycetes species	GenBank accession number
43	DW15	1396	58.81	*S. durbertensis* (KY318506)	99.36	*S. durbertensis* DW15	MT669274
44	SL2	1399	59.32	*S. althioticus* (NR043359)	99.64	*S. althioticus* SL2	MT669310
45	SL4	1407	59.34	*S. erythrogriseus* (NR112438)	99.86	*S. erythrogriseus* SL4	MT669311
46	SL10	1400	59.50	*S. erythrogriseus* (NR112438)	99.93	*S. erythrogriseus* SL10	MT669312
47	SL12	1395	58.78	*N. dassonvillei* subsp. *albirubida* (NR112743)	99.86	*N. dassonvillei sub sp. albirubida* SL12	MT669313
48	SL13	1408	58.59	*N. dassonvillei* (NR074635)	100	*N. dassonvillei*s ub sp. *dassonvillei* SL13	MT669314
49	SL14	1399	59.32	*S. vinaceusdrappus* (NR112368)	99.93	*S. vinaceusdrappus* SL14	MT669315
50	SL16	1356	59.07	*S. gelaticus* (NR043488)	98.60	*S. gelaticus* SL16	MT669316
51	SL19	1416	58.75	*N. dassonvillei* (NR074635)	99.79	*N. dassonvillei* sub sp. *dassonvillei* SL19	MT669317
52	SL25	1369	59.82	*S. radiopugnans*(NR044013)	99.93	*S. radiopugnans* SL25	MT669318
53	SL26	932	59.97	*S. alkaliphilus* (NR136864)	99.57	*S. alkaliphilus* SL26	MT669319

A Actinoalloteichus, *N Nocardiopsis*, *S Streptomyces*

The phylogram of 16S rRNA gene of all the actinomycetes isolates along with type strains was generated by neighbor-joining method (Fig. 1). Phylogenetic analysis of *Nocardiopsis* isolates revealed that all the isolates delineated into two major clades of two distinct groups (Fig. 1A). Phylogenetic tree of *Streptomyces* revealed the wide range of diversity prevailing in salt lake basins of Thar desert having monophyletic association (Fig. 1B). Phylogram also showed that different isolates of same species had their affinities with other species indicating the presence of genetic variability between isolates. This may be attributed to the single nucleotide polymorphisms (SNPs) in 16S gene region during the process of evolution by the way of insertion, deletion or substitution (INDELS) at several sites of the genome [26].

Community Dynamics with Binomial Data-Set

A total 30 species were isolated and identified from the soil samples collected from all the five salt basin habitats under the study. The maximum number of species were recorded from Pachpadra (14) followed by Phalodi (9), Sambhar salt basin (8), and Lunkarasar (7) while the least numbers (6) of species were isolated from Didwana area. Species *N. dassonvillei* subsp. *albirubida* was widespread and common species recorded from all the habitats studied. *N. synnemataformans* was recorded from Lunkarasar, Phalodi, and Didwana and *S. erythrogriseus* was recorded from Pachpadra, Phalodi, and Sambhar. *N. dassonvillei* subsp. *dassonvillei*, *S. mangrovicola*, *S. radiopugnans*, *S. rochei*, *S. speibonae*, and *S. vinaceusdrappus* were recorded from more than two habitats. Species *A. cyanogriseus*, *N. alba*, *N. chromatogenes*, *N. lucentensis*, *N. potens*, *N. prasina*, *S. albogriseolus*, *S. alkaliphilus*, *S. althioticus*, *S. carpaticus*, *S. ardesiacus*, *S. durbertensis*, *S. fenghuangensis*, *S. gelaticus*, *S. griseoloalbus*, *S. griseomycini*, *S. mutabilis*, *S. paradoxus*, *S. tendae*, *S. thermolilacinus*, and *S. thermospinosisporus* were found to be habitat specific.

Ordination analysis of different species with repsect to their presence and or absence at different habitats was carried out using PCA. The results of PCA revealed that first four axes of bi-plot (Supplementary Table 1) together accounted more than 80 per cent variablities with individual contribution of 41.12% (F1), 22.26% (F2), 19.63% (F3), and 16.97% (F4). Present results indicate the appropriate use of the ordination tools and corroborte with findings of previous workers [27, 28]. Factor loading scores of different species and different habitats are depicted in Supplementary Tables 2 and 3, respectively. Species *N. dassonvillei* subsp. *dassonvillei*, *S. carpaticus*, *S. ardesiacus*, *S. erythrogriseus*, *S. griseoloalbus*, *S. griseomycini*, *S. mutabilis*, *S. paradoxus*, *S. rochei*, *S. speibonae*, *S. tendae*, *S. thermolilacinus*, and *S. vinaceusdrappus* were better linked with F1 axis. While four different species namely *S. alkaliphilus*, *S. althioticus*, *S. gelaticus*, *S. radiopugnans* and *N. synnemataformans*, *S. albogriseolus*, *S. fenghuangensis*, *S. mangrovicola* were linked with axes F2 and F4, respectively. The remaining species viz., *A. cyanogriseus*, *N. alba*, *N. chromatogenes*, *N. lucentensis*, *N. potens*, *N. prasina*, *S. durbertensis*, and *S. thermospinosisporus* were located on F3 axis. The indicator and transit species with respect to habitats can be envisaged through PCA biplots. Species *S. alkaliphilus*, *S. althioticus*, and *S. gelaticus* were the indicator species for Sambhar habitats. *S. albogriseolus* and *S. fenghuangensis* were indicator species for Phalodi habitats. Whereas *S. erythrogriseus*

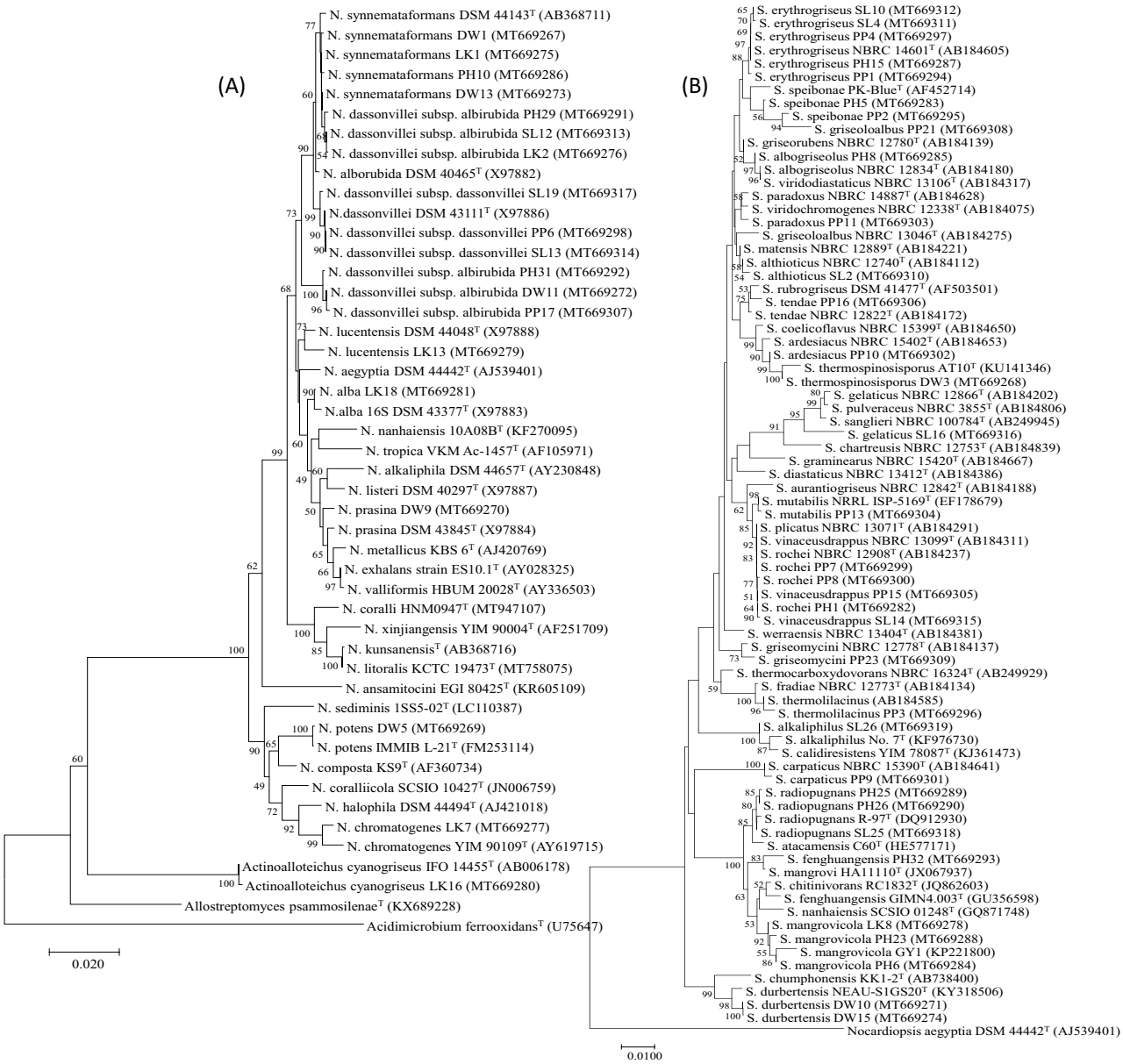

Fig. 1 Neighbor-joining phylogenetic tree of 16S rRNA gene of **A** *Nocardiopsis* and *Actinoalloteichus* species, and **B** *Streptomyces* species isolated from salt basin of Rajasthan

and *S. radiopugnans* were found as transit species between Sambhar and Phalodi habitats. Similarly, *A. cyanogriseus*, *N. alba*, *N. chromatogenes*, *N. lucentensis*, and *S. mangrovicola* were the indicator species of Lunkaransar habitats. *N. potens*, *N. prasina*, *S. durbertensis*, and *S. thermospinosisporus* were recorded only at Didwana area and thus can be regarded as indicator species for this habitat. *N. synnemataformans* was the transit species between Lunkaransar and Didwana habitats. Eight species namely, *S. carpaticus*, *S. ardesiacus*, *S. griseoloalbus*, *S. griseomycini*, *S. mutabilis*, *S. paradoxus*, *S. tendae*, and *S. thermolilacinus* were the indicator species for Pachpadra habitat (Fig. 2). *S. rochei* and *S. speibonae* were transit species between Pachpadra and Phalodi habitats, while *N. dassonvillei* subsp. *dassonvillei* and *S. vinaceusdrappus* were the transit species between Pachpadra and Sambhar habitats.

Community Dynamics with Quantiative Ecological Attributes

The observations of ecological parameters are presented in Supplementary Table 4. A total of 14 different species were

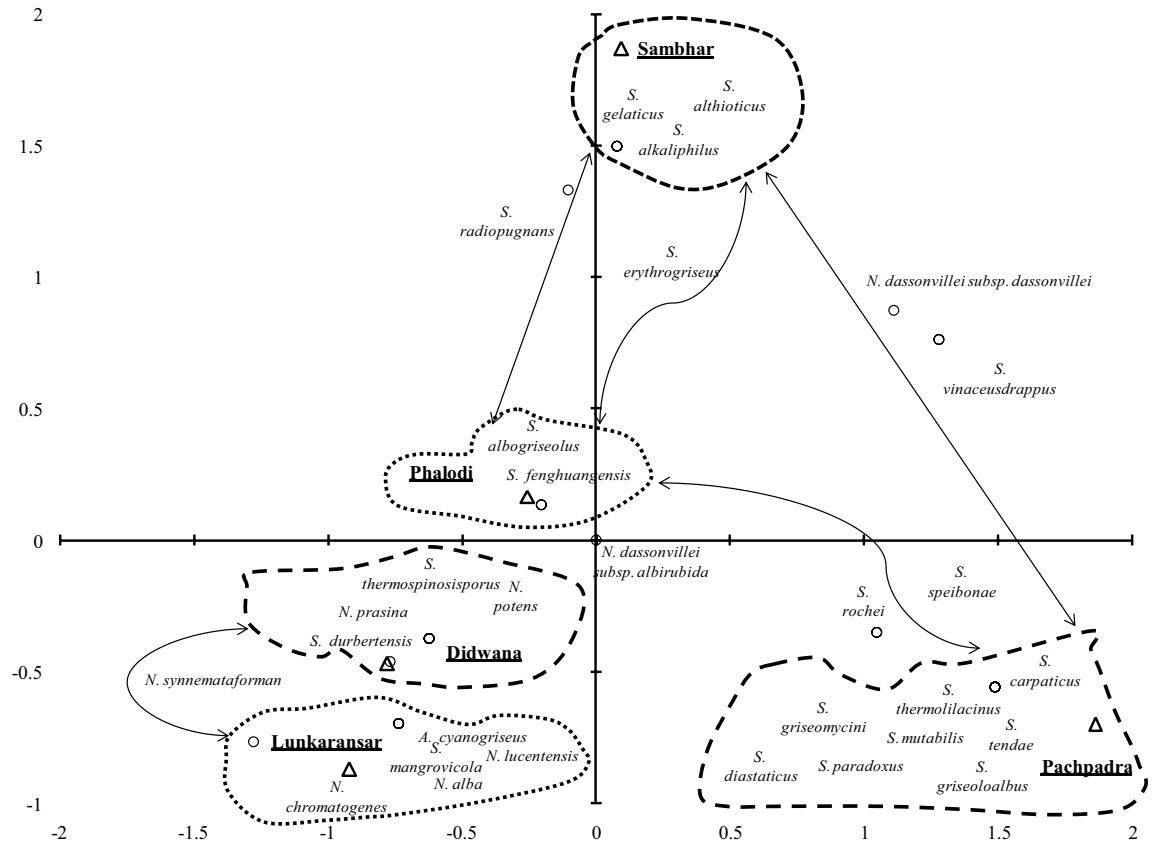

Fig. 2 Principal component analysis bi-plot of species recorded from different studied sites

recorded from Pachpadra salt basin. Among these species, *S. erythrogriseus*, *S. griseomycini*, *S. mutabilis*, and *S. rochei* were isolated twice with RIV of 9.79, while the remaining species were isolated only from one site with RIV of 6.08 (Supplementary Table 4). Seven different species having equal abundance with RIV of 14.29 were isolated from Lunkaransar salt basin area. Phalodi salt basin area had nine species, out of which three species namely, *S. mangrovicola*, *N. dassonvillei subsp. Albirubida*, and *S. radiopugnans* has RIV of 14.81 while remaining species has RIV of 9.26. Didwana salt basin area was represented by six species and out of them *N. synnemataformans* and *S. hermospinosisporus* were isolated twice with RIV of 25 and remaining species has RIV of 12.5. Sambhar salt basin area possessed eight different species and out of them *N. dassonvillei* subsp. *dassonvillei* and *S. erythrogriseus* recorded twice with RIV of 17.50 while remaining species has RIV of 10.83 (Supplementary Table 4). The species exhibiting higher RIV may be the best predictor of the area.

The DD curve for various saline habitats is depicted in Fig. 3. Log-normal distribution types were found at all the habitats studied. In this model, occupation of niche space is basically governed by a number of interacting factors that affect the outcome of inter-specific competition and abundance is governed by many independent factors and resource utilization is characterized as multidimensional. Present results are in conformity with the studies of Lussenhop [29] and Zak and Willing [30] in which it was emphasized that if fungi are collected from many micro-habitats and mixed during sampling, the resultant distribution is a relic and such result indicated a number of independent environmental factors controlling species abundance. Log-normal distributions could arise simply as the result of the multiplicative interaction of many normal random processes affecting the growth of population or could arise by combining unrelated samples. This tool was utilized by many workers for the study of fungal communities specifically for arbuscular-mycorrhizal fungus in tropical forest and pastures [31], tropical timber trees [32], endophytic fungi at *Bauhinia brevipes* leaves, Brazil [33], and dry deciduous forest of Western Southern India [34]. We found a flatten line for Lunkaransar saline habitat area under the present study may be due to the equal RIV for all the species.

Diversity Analysis (*α*, *β*, and *γ*)

Values of alpha diversity indices for different habitats are presented in Table 3. In this study, the species richness (species count) was recorded the maximum (14) at Pachpadra area and minimum (6) at Didwana area. The species richness was studied using Margalef and Menhinick diversity indices. Margalef Richness Index ($R1$) is the proportion of number of species and log number of individual, while Menchinick ($R2$) is the proportion of number of species and square root of number of individual [35]. The Margalef index measures species richness and is highly sensitive to sample size [36]. It is a very simple index to apply that can be used in conjunction with indices sensitive to evenness or changes in dominant species, such as the dominance Berger–Parker index [37]. Both Margalef and Menchinick are insensitive to changes in community structure and change to sample size, respectively [38]. Results of both indices are presented in Table 3. Based on Margalef Richness Index ($R1$) the richness trends were in order of Lunkaransar > Didwana > Pachpadra > Sambhar > Phalodi. However, results of Menhinick Richness Index ($R2$) showed a different trend viz., Pachpadra > Lunkaransar > Phalodi > Sambhar > Didwana. These indices were quantified by previous researchers [18, 39–41]. Simpson index of dominance (λ) was recorded the maximum (0.92) at Pachpadra habitat area. The values of index were almost similar at Phalodi, Lunkranasar, and Sambhar habitats while it was the minimum (0.81) at Didwana area. Results of Shannon and Weaver index revealed the similar trend as of species richness, recorded the maximum (2.58) at Pachpadra habitat and the minimum (1.73) at Didwana habitat.

Both the Shannon and Brillouin indices gave similar estimates of the diversity; however, the values of Brillouin index were lower than Shannon index (Table 3). This is because the Brillouin index describes a known collection about which there is no uncertainty. While the Shannon index estimates the diversity of the un-sampled as well as the sampled portion of the community [20]. Didwana habitat was recorded with the maximum value (0.25) of Berger–Parker index and with the lowest value (0.81) of Simpson index of dominance. While the lower value (0.11) of Berger–Parker was recorded at Pachpadra habitat that showed the maximum (0.92) values of Simpson index of dominance. Evenness analysis exhibited alike trends at Pachpadra, Phalodi, Didwana, and Sambhar areas and was recorded the maximum (1.0) at Lunkaransar habitat.

The beta diversity allows the comparison of habitat diversity of two different study areas, provides information about the degree of partitioning of habitats by species, and together with alpha diversity, it provides the information about the overall diversity and biotic heterogeneity within an area [42]. In this study, Pachpadra area was more similar with Lunkaransar (Beta diversity = 9.5) and Didwana (9.0) area. Among habitats Lunkaransar and Didwana areas were the most dissimilar (4.5) with each other (Table 4). Gamma diversity at 2.40 revealed the presence of actinomycetes in the saline habitats of the arid and semi-arid geographical region of the Rajasthan, India.

Physico-Chemical Characteristics of Soil Samples

The physico-chemical parameters viz., soil pH, electric conductivity, organic carbon, available nitrogen, phosphorus, and potassium of the soil samples demonstrated the alkaline and poor nutritional status of soils (Table 5). Khandan and Janardhana [43] studied the correlation of the diversity of actinomycetes with the edaphic factor such as soil pH and minerals elements (NPK) and observed that the richness of actinomycetes increased with the increase of organic carbon, copper and moisture content of soil. Kumar et al. [24] observed a positive correlation between diversity of actinomycetes and available nitrogen of soils in arid soil of Indian Thar desert.

Higher soil pH (11.1), electric conductivity (0.97 d/Sm), and available potassium (476.8 kg/ha) were recorded at Sambhar lake basin, while higher available nitrogen (0.53%) and available phosphorus (54.25 kg/ha) were recorded at Pachpadra lake basin. The maximum organic carbon (0.94%) was recorded at Didwana lake basin area. These soil parameters were utilized as exploratory variables to identify their role in diversity dynamics of studied actinomycetes isolates using two steps PLS regression. Variable importance for the projection (VIPs) for different exploratory variable along with all the axes assessed using PLS-1 is presented in Supplementary Table 5. This method allowed identification of the exploratory variable that contributed the maximum to the model. Any independent variable with a VIP value greater than one was considered as a highly important predictor [44]. Among the studied parameter, available P and N were identified as significant predictors (VIPs > 1.0). The Q^2 cumulated index measures the global goodness of fit and in the present study, with significant VIPs the Q^2 for studied parameter approached to ideal value that is one. It implies that quality of fitness is fairly distributed among dependent and predictor variables. The cumulated R^2Y and R^2X corresponds to the correlation between the exploratory (X) and dependent (Y) variables with the component close to one

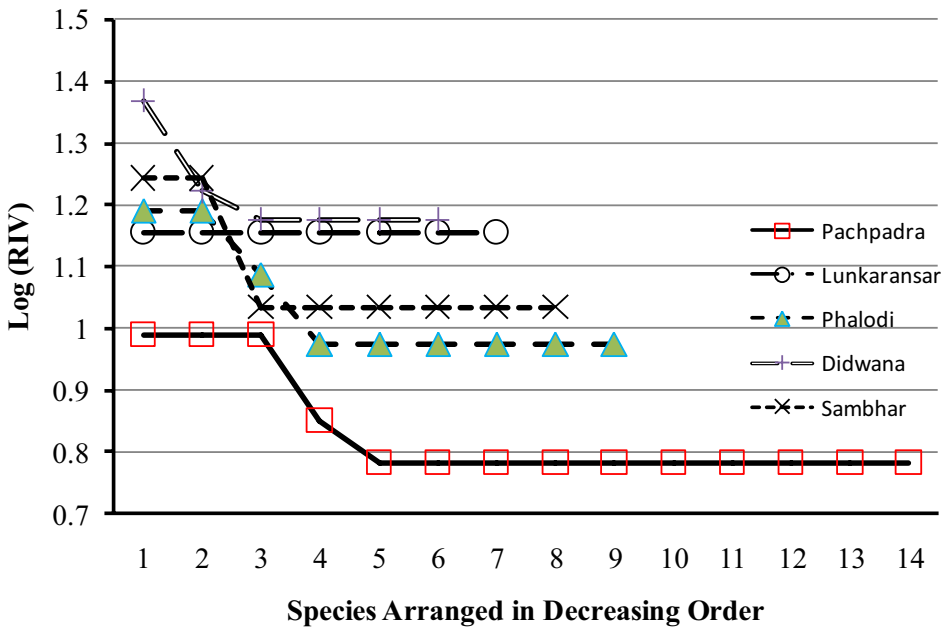

Fig. 3 Dominance diversity (DD) curve at the studied salt basin habitat

Table 3 Alpha diversity parameters of different salt basins

Diversity parameters	Pachpadra	Lunkaransar	Phalodi	Didwana	Sambhar
Species richness	14.00	7.00	9.00	6.00	8.00
Margalef richness index (*R*1)	10.15	17.83	9.14	10.64	10.10
Menhinick richness index (*R*2)	7.38	5.92	5.81	4.74	5.66
Simpson index of dominance	0.92	0.86	0.88	0.81	0.86
Shannon and Weaver diversity index	2.58	1.95	2.14	1.73	2.03
Brillouin index	1.09	0.58	0.82	0.59	0.72
Berger–Parker	0.11	0.14	0.17	0.25	0.20
Evenness *e*^H/S	0.94	1.00	0.94	0.94	0.95

Table 4 Beta diversity (Cody) among different salt basins

Sampling sites	Pachpadra	Lunkaransar	Phalodi	Didwana
Lunkaransar	9.5	–	–	–
Phalodi	7.5	5.0	–	–
Didwana	9.0	4.5	5.5	–
Sambhar	8.0	6.5	5.5	6.0

with 4th component generated by PLS summarize well, both by XS (0.99) and the YS (0.99) for the studied parameters (Fig. 4). PLS bi-plots (Fig. 5) and VIP's values indicated the significant impact of available nitrogen and phosphorus on diversity dynamics.

Table 5 Physico-chemical characteristics of soil samples of different salt basins

Lake basin	Soil pH	EC (dS/m)	OC (%)	Available N (%)	Available P (kg/ha)	Available K (kg/ha)
Pachpadra	8.8	0.54	0.4	0.53	54.25	307.8
Lunkaransar	8.2	0.5	0.35	0.22	35.4	424.2
Phalodi	9	0.65	0.32	0.29	39.4	438.5
Didwana	9.8	0.68	0.94	0.29	39.65	277.6
Sambhar	11.1	0.97	0.82	0.39	43.24	476.8

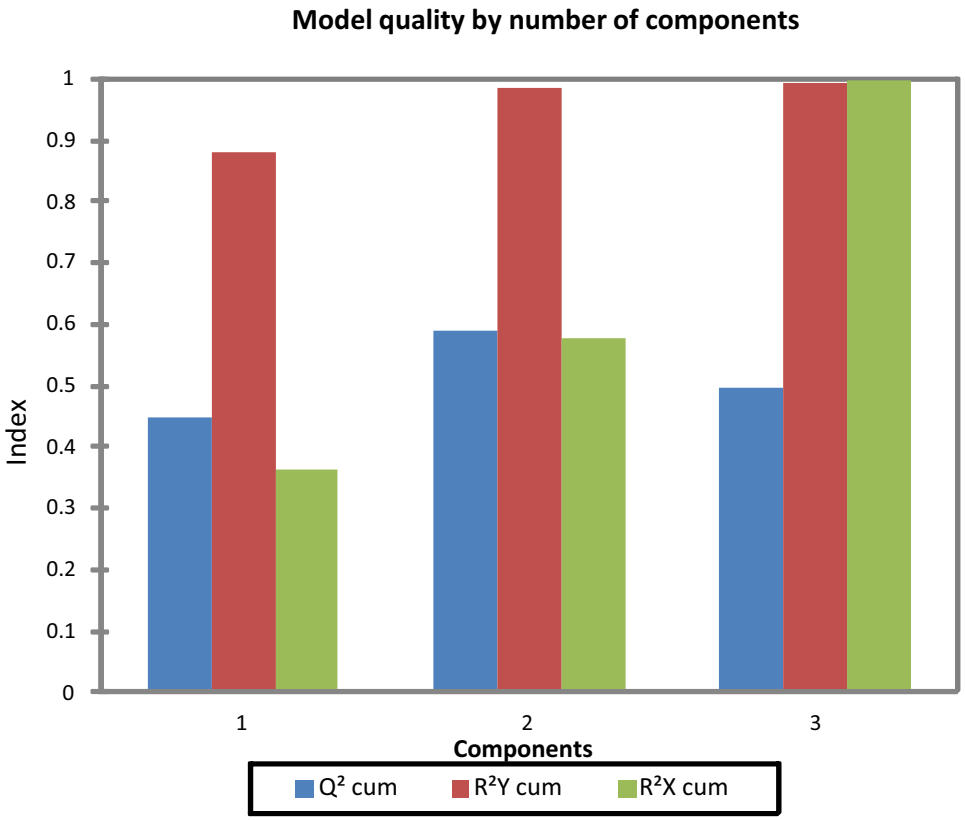

Fig. 4 Model quality for partial least square

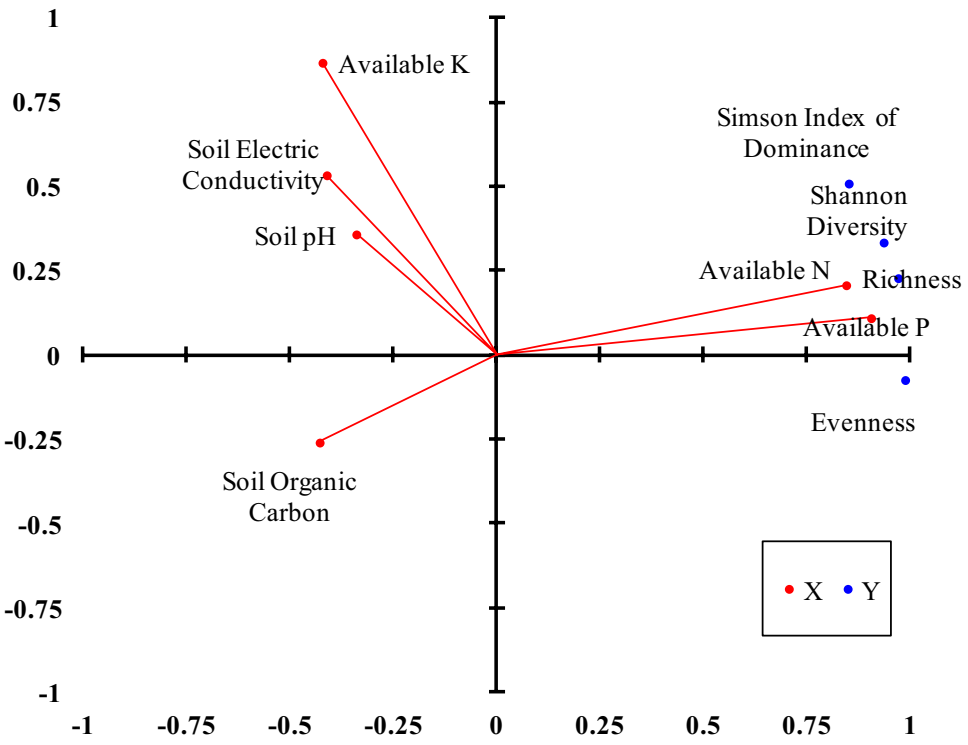

Fig. 5 Partial least square regression

Conclusion

The salt basin of Rajasthan, India harbors novel species of actinomycetes and their diversity is widespread throughout the arid and semi-arid regions of Rajasthan. Fifty-three actinomycetes isolates were identified from five arid and semi-arid salt basins in which the maximum (16) isolates were from Pachpadra salt basin followed by Phalodi (12 isolates), Sambhar (10 isolates), Didwana (8 isolates), and Lunkaransar (7 isolates) salt basin. Three species of actinomycetes viz., *N. chromatogenes*, *S. durbertensis,* and *S. mangrovicola* are being reported for the first time from India. The community dynamics suggested some indicator species of the salt basin areas, while *S. rochei* and *S. speibonae* were found as the transit species between Pachpadra and Phalodi habitats, whereas, *N. dassonvillei* subsp. *dassonvillei* and *S. vinaceusdrappus* were the transit species between Pachpadra and Sambhar habitats. The identified species may be exploited to obtain bioactive compounds.

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Author Contributions PG, MM, RP and SKS: Conceptualization. KP: Methodology investigation and validation. KP, AT, PG: Analysis of gene sequence and submission. PG, KP, MM: Writing: original draft. RP and SKS: Writing: review and editing. AT, MM: Visualization. PG and SKS: Supervision.

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Data Availability All data generated or analyzed during this study are included in this published article.

Code Availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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