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Bacterial community metagenomic and variation of some medicinal plant rhizosphere collected form Sinai

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Abstract:

The rhizosphere microbes play an important role in improving medicinal values of medicinal plants. The role of microbes in plant growth, nutrient availability, disease resistance, yield and quality of medicinal compounds is demonstrated in medicinal plants. There are increasing interests in the research of the interaction between medicinal plant and their rhizosphere microbes for the improvement of medicinal plants. Dry Rhizosphere of medicinal plants collected form Sinai, Egypt and water treated represents a common physiological stress for the microbial communities residing in surface of these medicinal plants. A dry and wetting by water induce lysis in a significant proportion of the microbial biomass and, for a number of reasons, Effect directly or indirectly on microbial community composition. In this study Dry sand and water treated as common stress in the laboratory by exposing three different rhizosphere immersed in water to 10 day and 20 day period.

The three rhizospheric medicinal plants were collected from Saint Katherine Mountain, Sinai, Egypt. Bacterial community of dry and immersed with distilled water were evaluated every 10 day of incubation. Total DNA was extracted from sand samples and characterized its bacterial communities using the terminal restriction fragment length polymorphism method and degrading gradient Gel electrophoresis. This work revealed that water changed bacterial community in all samples compared with dry sand due to water may induced shifts in bacterial community. Bacterial community clone library of *Capparis spinosa* rhizosphere were studied and phylogenetic tree of bacterial community were evaluated

Key wards: Microbial fingerprint, bacterial community, TRFLP, medicinal plants, soil DNA extraction, 16S ribosomal RNA

Introduction:

Microorganisms has fundamental role in all ecological processes that occure in soil such as structure of soil formation, decomposition of pestcides and organic matter and xenobiotics, and recycling of essential elements as carbon and phosphorous and nutrients. Thus, microbes play a unique role in all biogeochemical cycles and its effect on all lives on Earth. In addition, all organisms either directly or indirectly depend on microbial activities in soil ecosystems, as it promote changes in vegetation microorganisms, promoting plant growth and suppressing soil-borne plant diseases (Garbeva et al. 2004).

Standard methods of culture techniques used to characterize microbial diversity involve isolation and characterization of bacteria using slandered growth media such as has lot of Limitations (Kirk *et al*. 2004). Several improved cultivation procedures and culture media have been devised that mimic natural environments in terms of nutrients (composition and concentration), oxygen gradient, pH, etc. to maximize the cultivable fraction of microbial communities. For example, a technique has been devised for the cultivation of uncultured microorganisms from different environments including seawater and soil that involved encapsulation of cells in gel microdroplets for large-scale microbial cultivation under low nutrient flux conditions (Zengler *et al*. 2005).

The diversity and functions of microbes in the rhizosphere, a narrow region around the root, are related to the root exudates (proteins and sugars), biogeochemical reactions and respiration

(Narula *et al*. 2009). A wide range of organic compounds secreted by plant roots in the rhizosphere provide a food source for microorganisms increasing microbial density and activity in the rhizosphere than in the soil away from the rhizosphere. Most of the microorganisms in the rhizosphere are related to plant species that can efficiently solubilize poorly soluble inorganic P and mineralize organic phosphorous sources and markedly increase plant growth in soils with low phosphorous availability. Medicinal plants are a rich source of bioactive compounds (Toussaint *et al*. 2007), and these are thought to be safe to human beings and the environment compared to the synthetic medicines for the treatment of cancer and many other diseases (Nema *et al*. 2013)

The endophytic strain of Bacillus pumilus isolated from tissues of the medicinal plant Ocimum sanctum can be used as a bioinoculant to enhance plant growth and also as a probiotic (Murugappan *et al*., 2013). The potential of phosphate-solubilizing bacteria were evaluated as Burkholderia gladioli, *Enterobacter aerogenes* and *Serratia marcescens*, for utilizing Mussoorie phosphate to enhance the medicinal plant growth as biofertiliser because some medicinal plants are less dependent on chemical fertilisers. The strains differed in the extent of rhizosphere colonisation, carbon source utilisation pattern and whole cell fatty acid methyl esters composition (Gupta *et al*., 2011)

In this Study the bacterial community indicate what type of microorganisms are present and also enzyme profile and activity of these bacterial community finally find the relationship between activities of all bacterial community with ecosystem functions. In this study deals also with study effect of water addition in bacterial community and their interactions with each other from three sample of rhizospher of medicinal plants collected from Sinai Egypt.

Material and methods:

Site and soil sampling

The Saint Katherine Mountain has an extremely arid climate with long, hot and rainless summers and cool winters. The mean annual precipitation in Saint Katherine area over 25 years is 45 mm per year, although the high mountains receive more precipitation (100 mm/year) as rain and snow. In some parts of this area, floods resulting from continual rain may occur during winter and spring. The mean maximum air temperature ranged from 15.1°C to 32.7°C and the mean

minimum temperature ranges from 1.9°C to 20.2°C, with the lowest temperature in December and January and the highest temperature in July and August (Mosallam 2007).

Sampling was performed from the rhizosphere by collecting the soil near the roots (a maximum of 5 cm from the root) and 10 cm from the soil surface. Rhizospheric soil samples were randomly taken from beneath three medicinal plants species common in Saint Cathren mountain *Capparis spinosa*, *Chiliadenus iphionoides* and *Artemisia herba-alba.* All the samples were sealed in plastic bags and transported on ice. In the laboratory, samples were stored at - 80 °C until use.

Microcosms design:

Four microcosms set up for each treatment (seeded from the same sand). Each microcosm contains 10 gram sand plus 3 ml sterile distilled water. Bacterial community will be characterized every week by extracting DNA from one microcosm. Bacterial community was compared between the dry sand and the sand treated for distilled water for 20 day

DNA isolation

Total DNA isolation for each treatment were collected in duplicate from dry sand and sand treated with water every 10 day extraction method was performed with the Power Soil extraction kit (MoBio Laboratories Inc., CA, USA)

Total DNA isolated was checked by electrophoresis in agarose (1% w/v). The electrophoresis was performed at 120 V for 20 min in 1X TAE running buffer. The concentration of DNA in each sample was measured directly in a spectrophotometer at 260 nm.

Polymerase Chain Reaction (PCR).

The quality of the DNA obtained was tested by its ability to produce polymerase chain reactions with primers for the 16S of bacterial ribosomal RNA (rRNA). The 16S rRNA was amplified using the primers 16S 27F forward primer Sequence **5-AGAGTTTGATCATGGCTCAG 3** and 16S 1100R reverse primer Sequence: **5- GGG TTG CGC TCG TTG 3-**. The following standard conditions were used for bacterial 16S rRNA gene amplification: initial denaturation at 95°C for 5 min; 35 cycles of denaturation (30 s at 94°C), annealing (30 s at 55°C), and extension (1.5 min at 72°C); and a final extension at 72°C for 5 min. The PCR products were purified with a The UltraClean® PCR Clean-Up Kit (MO BIO laboratories, Inc) according to the manufacturer's suggested protocol

DNA digestion from PCR product with Restriction Enzymes for TRFLP profiles:

Hae III restriction Enzyme was used in this work for DNA digestion to digest 15to 20 ng per reaction. Hae III Restriction Enzyme 0.4 µL, Cut smart buffers 1.6 µL, water PCR grade10 µL and template DNA 8 µ to total volume 20 µL this mix was incubated for 3 h at 37º C. after digestion the DNA precipitated using sodium acetate and pellet paint. After centrifugation 37 µL of 95 % ethanol mix and allow to sit on bench at room temperature for 10 min. remove all liquid after centrifuge for 15 min at 4º C. Another purification of DNA digestion using 125 µL of 70 % ethanol to remove excess salt. Remove all liquid after centrifuge for 4 min at 4º C and allow the DNA digestion to dry completely for 25 min at 30 º C. The pellet was resuspended in formamide solution and Rox standard ABI. This mixture was denaturant at 94º C for 2.5 min and then plunge into ice untile introduced to ABI PRISM 310 Genetic Analyzer for terminal restriction fragment length polymorphism (TRFLP).

Resulting TRFLP profiles were standardized for analysis using procedures similar to those described by Dunbar *et al*. (2001) and Fierer *et al*. (2003). First, data were normalized to account for any potential variability in total DNA quantity among samples on the capillary gel. Fragments were therefore rescaled to reflect their proportional abundance in each sample, and fragments representing less than 0.5% of the total TRFLP profiles were then manually aligned to prevent similar peaks from being grouped separately. While time-consuming, this procedure was necessary to avoid errors associated with automated rounding algorithms (Gene Mapper software calculates DNA fragment sizes to 0.01 bp, but sequencing errors can be up to 0.5 bp.

Ligation, Cone library and sequencing:

Genomic DNA in rhizosphere of *c. spinosa* as sand represent the bacterial community in the area of Saint Catherine mountain environment was extracted and purified. The PCR conditions were the same as mentioned above.

The PCR product was ligated into the pGEM-T vector (Promega Inc., Madison, WI) and transformed into *Escherichia coli* DH5_ competent cells. The transformed cells were plated on Luria-Bertani plates containing 100 _g/ml of ampicillin, 80 _g/ml of X-Gal (5-bromo-4-chloro-3 indolyl-_-D-galactopyranoside), and 0.5 mM IPTG (isopropyl-_-D-thiogalactopyranoside) and incubated overnight at 37°C. Gene clone libraries of 16S rRNA were constructed, and 40 to 50 randomly chosen colonies per sample were analyzed for insert 16S rRNA gene sequences.

Results and discussion:

Bacterial community of *Capparis spinosa* rhizosphere

Total DNA that characterize bacterial community of *C. spinosa* rhizosphere digest with Hae III restriction Enzyme for analysis fingerprint of bacterial community by TRFLP and

Bacterial community in *C. spinosa* rhizosphere in all dry sand and microcosms with distilled sterile water for 10 and 20 days of incubation in room temperature was **172** different 16S rRNA fragments. Only 16 fragment of 16S r RNA were presence in all condition with percentage **9.3 %** of total bacterial community all Experiment was done duplicate. That revealed that large changes in bacterial community occurred during 20 day in both dry and water immersed sand, only 9.3 % of bacterial community was present in all contion during 20 day of experiment.

Six unique 60S rRNA length of different bacterial strain appeared in case of microcosms experiment only for 10 day and 20 day and disappear in Dry rhizosphere of *C. spinosa* for 10 and 20 day. These results indicate that 6 bacterial strains appear only in case of presence of water due to vegetation of these bacterial spores only when water was found

Also, one DNA length appears only in 20 day of experiment in both dry sand and microcosms of water treated sand experiment

In addition, 230, 253 and 290 bp of rRNA appears only in dry sand of *C. spinosa* rhizosphere for 10 and 20 day only and disappear in water treated sand experiment

In the other hand 315 DNA length appears only after 20 day in dry sand and also after 20 day of microcosms of *C. spinosa* rhizosphere

Two (187 and 203) DNA length disappeared only after 20 day of water treated experiment, in contrast Two (204 and 405) DNA length appeared only after 20 day of water treated experiment

Also, Cont. 20 day of *C.spinosa* was richness community with different 44 bacterial strains

Nearly no vast changes occurred in dry rhizosphere of *C. iphionoides* and immersed in water for 10 and 20 day (Table No 1, Fig. No 1 and 2)

Table No 1: Richness microbial community of TRFLP for *C. spinosa* microcosms experiment sand rhizosphere of Saint Catherine Mountain

Samples	Total bacterial community	Percentage with all communities	Percentage of shared DNA	Persentage of diff. DNA
C. spinosa cont. 10	40	23.25	40	60

C. spinosa cont 20	45	26.16	35.55	64.44
C. spinosa M10	43	25	37.20	62.79
C. spinosa M20	44	25.58	36.36	63.63
Sum of total community	172	100	9.30	

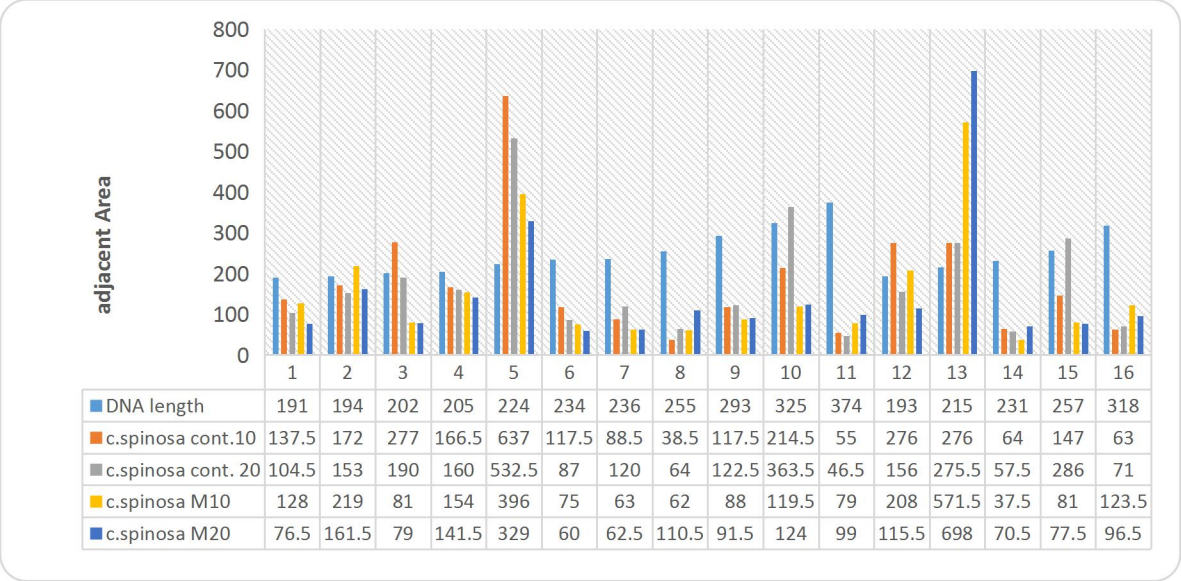


Fig No 1: *C. spinosa* dry sand and microcosms Fragments shared by its DNA length and adjacent area for 10 and 20 day

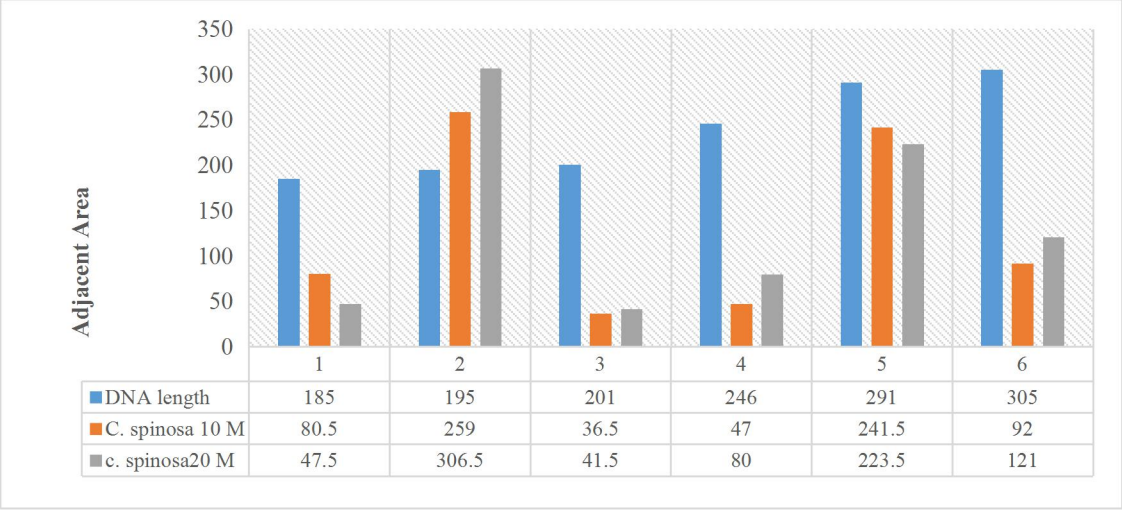


Fig No 2: Unique DNA fragment length in case of microcosms experiment only for 10 day and 20 day

Bacterial community of *Chiliadenus iphionoides* rhizosphere:

Total DNA that characterize bacterial community of *Chiliadenus iphionoides* rhizosphere digest with Hae III restriction Enzyme for analysis fingerprint of bacterial community by TRFLP

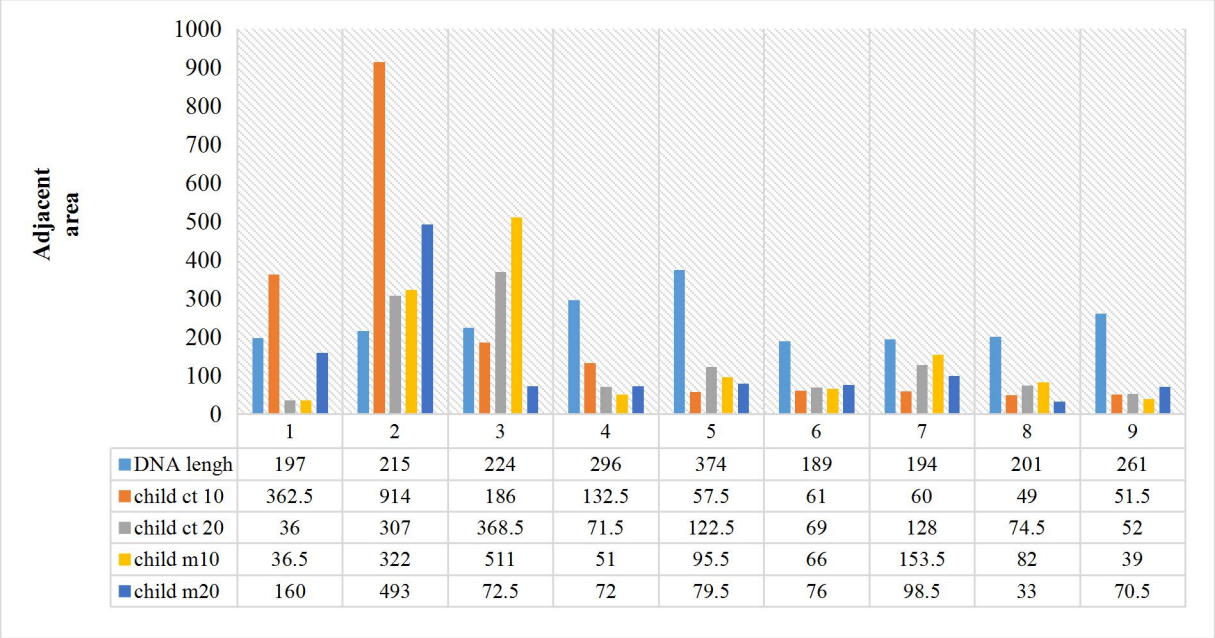
Bacterial community in *Chiliadenus iphionoides* rhizosphere in all dry and water treated sand with distilled sterile water for 10 and 20 days of incubation in room temperature was 170 different bacterial 16 S r RNA of different bacterial species. Only 9 different species were presence in all condition with percentage 5.2 % of total bacterial community

Four unique bacteria strains DNA fragment length (195, 218, 235 and 259) appeared after 10 day and predominant in all experiment in both microcosms and dry sand

Also, rhizosphere of *Ch. iphionoides* immersed in water for 20 day was richness community with different 46 bacterial strains as shown in table No 2 and fig. No 3 and 4

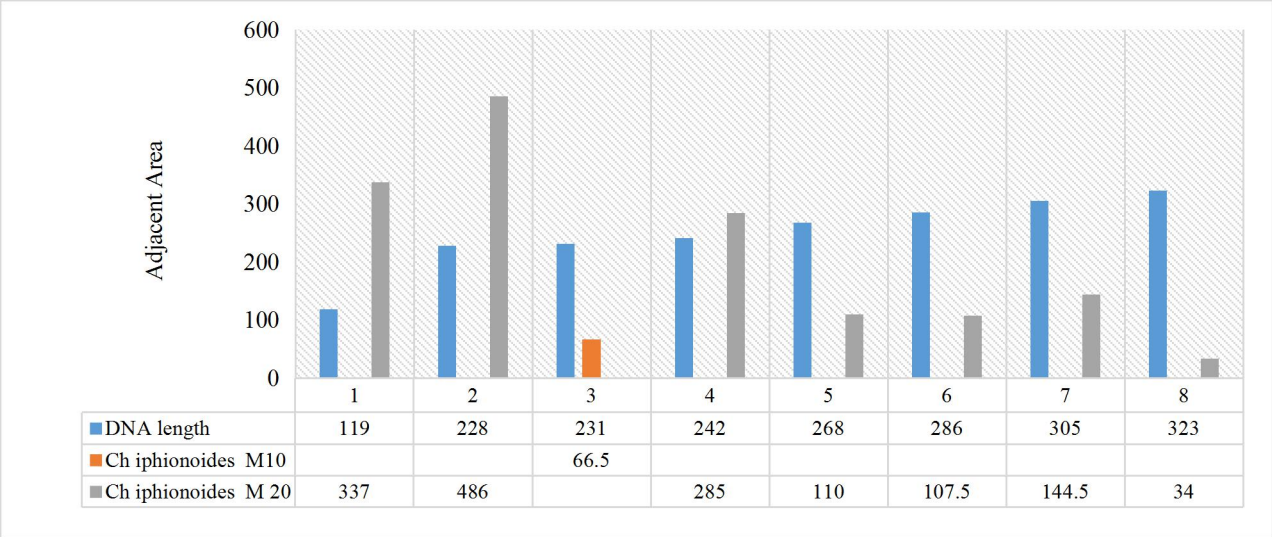
Table No 2: Richness microbial community of TRFLP for *Chiliadenus iphionoides* microcosms experiment sand rhizosphere of Saint Catherine Mountain

Sample	Total bacterial community	Percentage with all communities	Percentage of shared DNA	Persentage of diff. DNA
Ch. iphionoides Cont. 10	37	21.76	24.32	75.67
Ch. iphionoides cont. 20	45	26.47	20	80
Ch. iphionoides M10	42	24.70	21.42	78.57
Ch. iphionoides M 20	46	27.05	19.56	80.43
Sum of total community	170	100	5.2	



	1	2	3	4	5	6	7	8	9
DNA lengh	197	215	224	296	374	189	194	201	261
child ct 10	362.5	914	186	132.5	57.5	61	60	49	51.5
child ct 20	36	307	368.5	71.5	122.5	69	128	74.5	52
child m10	36.5	322	511	51	95.5	66	153.5	82	39
child m20	160	493	72.5	72	79.5	76	98.5	33	70.5

Fig No 3: *Chiliadenus iphionides* dry sand and microcosms Fragments shared by its DNA length and adjacent area for 10 and 20 day



	1	2	3	4	5	6	7	8
DNA length	119	228	231	242	268	286	305	323
Ch iphionoides M10			66.5					
Ch iphionoides M 20	337	486		285	110	107.5	144.5	34

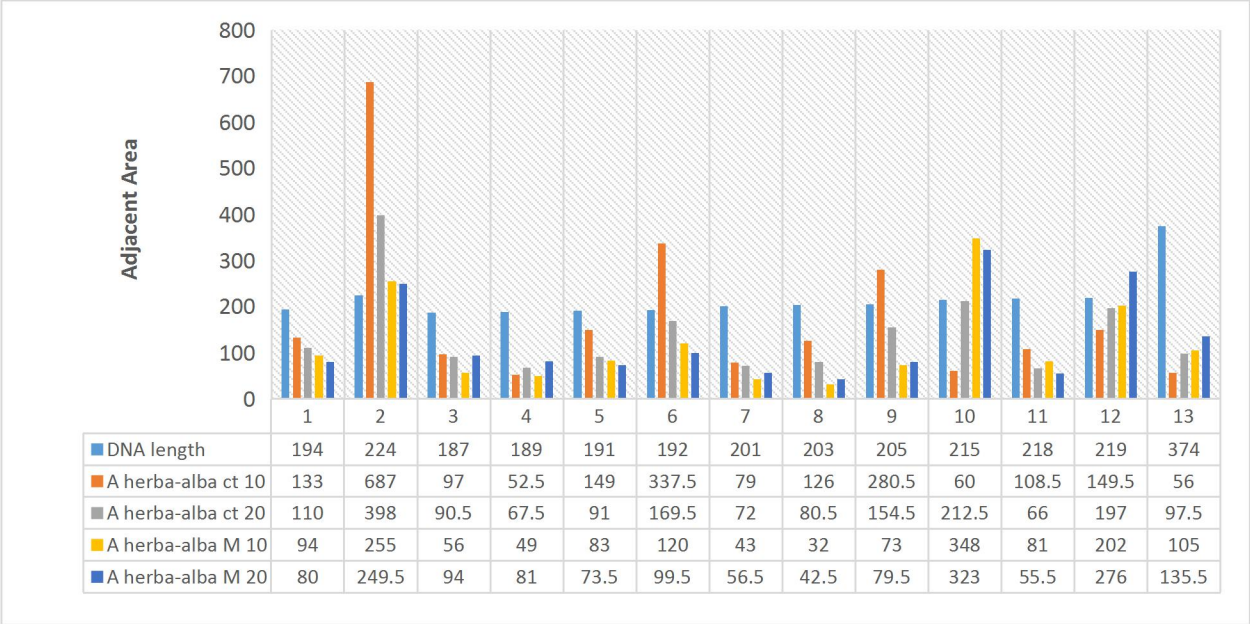
Fig No 4: *Chiliadenus iphionoides* unique bacteria strains in case of microcosms experiment only for 10 day and or 20 day

Bacterial community of *Artemisia herba-alba* rhizosphere:

Total DNA that characterize bacterial community of *Artemisia herba-alba* rhizosphere digest with Hae III restriction Enzyme for analysis fingerprint of bacterial community by TRFLP

Bacterial community in *A. herba-alba* rhizosphere in all dry sand and water treated with distilled sterile water for 10 and 20 days of incubation in room temperature was 188 only 13 bacterial strains were presence in all condition with percentage 6.9 % of total bacterial community

Also, rhizosphere of *A. herba-alba* rhizosphere immersed in water for 20 day was richness community with different 51 bacterial strains as shown in table No 3 and fig. No 5



	1	2	3	4	5	6	7	8	9	10	11	12	13
DNA length	194	224	187	189	191	192	201	203	205	215	218	219	374
A herba-alba ct 10	133	687	97	52.5	149	337.5	79	126	280.5	60	108.5	149.5	56
A herba-alba ct 20	110	398	90.5	67.5	91	169.5	72	80.5	154.5	212.5	66	197	97.5
A herba-alba M 10	94	255	56	49	83	120	43	32	73	348	81	202	105
A herba-alba M 20	80	249.5	94	81	73.5	99.5	56.5	42.5	79.5	323	55.5	276	135.5

Fig No 5: *A.herba-alba* dry sand and microcosms Fragments shared by its DNA length and adjacent area for 10 and 20 day

Table No 3: Microbial community richness of TRFLP for *A. herba-alba* microcosms experiment sand rhizosphere of Saint Catherine Mountain

sample	**Total bacterial community**	**Percentage with all communities**	**Percentage of shared DNA**	**Persentage of diff. DNA**
A. herba-alba cont. 10	40	21.2766	32.5	67.5
A. herba-alba cont 20	49	26.06383	26.53061	73.46
A. herba-alba	48	25.53191	27.08333	72.91

M10				
A. herba-alba M20	51	27.12766	25.4902	74.50
Sum of total community	188	100	6.914894	

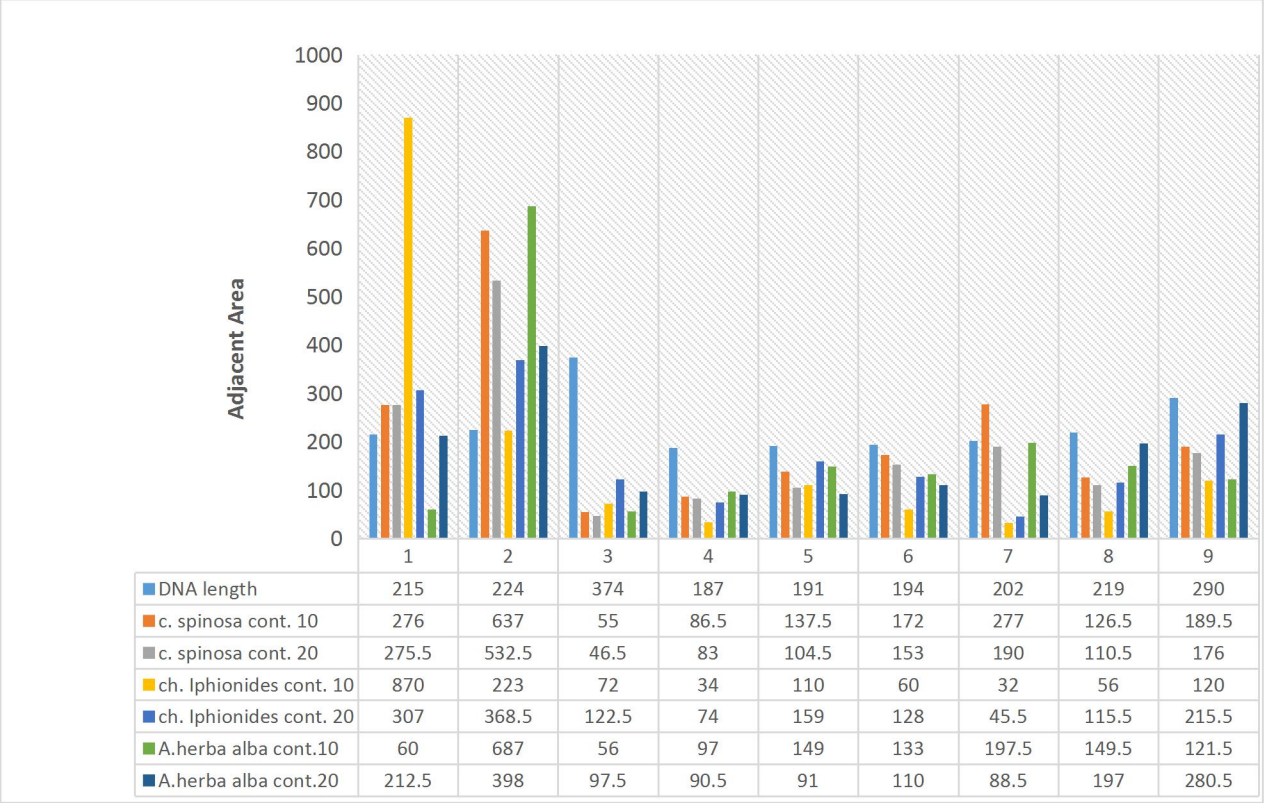
Terminal restriction fragment length polymorphism (TRFLP) results for all dry rhizosphere samples:

Total bacterial community in all control samples rhizosphere in all dry sand and microcosms with distilled sterile water for 10 and 20 days of incubation in room temperature was 253 only 9 bacterial strains were presence in all condition with percentage 3.5 % of total bacterial community as shown in table No 4 and fig No 6

Table No 4: Microbial richness community of Terminal restriction fragment length polymorphism (TRFLP) results for all dry rhizosphere samples

Samples	Total bacterial community	Percentage with all communities	Percentage of shared DNA	Persentage of diff. DNA
C. spinosa 10 day	40	15.81028	22.5	77.5
C. spinosa 20 day	42	16.60079	21.42857	78.57143
Ch. iphionoides 10 day	37	14.62451	24.32432	75.67568
Ch. iphionoides 20 day	45	17.78656	20	80
A. herba-alba 10 day	40	15.81028	22.5	77.5
A. herba-alba 20 day	49	19.36759	18.36735	81.63265
Sum of total	253	100	3.557312	

community				



	1	2	3	4	5	6	7	8	9
DNA length	215	224	374	187	191	194	202	219	290
c. spinosa cont. 10	276	637	55	86.5	137.5	172	277	126.5	189.5
c. spinosa cont. 20	275.5	532.5	46.5	83	104.5	153	190	110.5	176
ch. Iphionides cont. 10	870	223	72	34	110	60	32	56	120
ch. Iphionides cont. 20	307	368.5	122.5	74	159	128	45.5	115.5	215.5
A.herba alba cont.10	60	687	56	97	149	133	197.5	149.5	121.5
A.herba alba cont.20	212.5	398	97.5	90.5	91	110	88.5	197	280.5

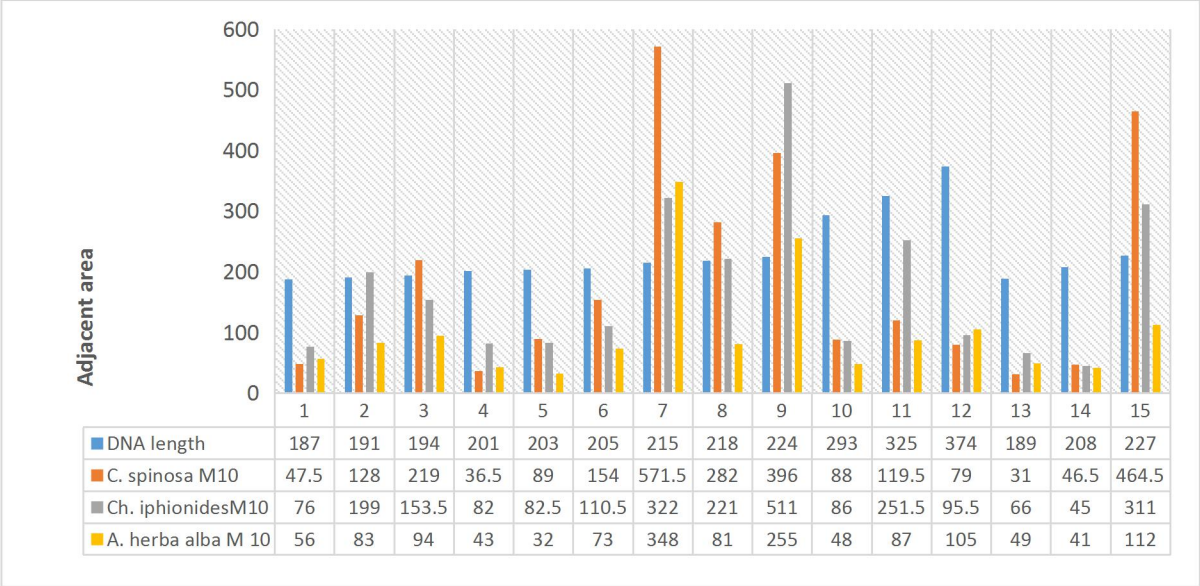
Fig No 6: Terminal restriction fragment length polymorphism (TRFLP) Adjacent Area results for all Dry sand samples

Terminal restriction fragment length polymorphism (TRFLP) results for all water treated samples after 10 day:

Total bacterial community in all dry rhizosphere samples after 10 days of incubation in room temperature was 133 only 15 bacterial strains were presence in all condition with percentage 11.27 % of total bacterial community**.** Also, dry rhizosphere of *A. herba alba* was richness community with different 48 bacterial strains as shown in table No 5 and Fig. No 7

Table No 5: Terminal restriction fragment length polymorphism (TRFLP) results for all water treated samples after 10 day

Samples	Total bacterial community	Percentage with all communities	Percentage of shared DNA	Persentage of diff. DNA
***C. spinosa* M 10 day**	43	32.33	34.88	65.11628
***Ch. iphionoides* M 10 day**	42	31.57	35.71	64.28571
***A. herba-alba* M 10 day**	**48**	36.09	**31.25 %**	68.75
Sum of total community	133	100	11.2782	



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DNA length	187	191	194	201	203	205	215	218	224	293	325	374	189	208	227
C. spinosa M10	47.5	128	219	36.5	89	154	571.5	282	396	88	119.5	79	31	46.5	464.5
Ch. iphionidesM10	76	199	153.5	82	82.5	110.5	322	221	511	86	251.5	95.5	66	45	311
A. herba alba M 10	56	83	94	43	32	73	348	81	255	48	87	105	49	41	112

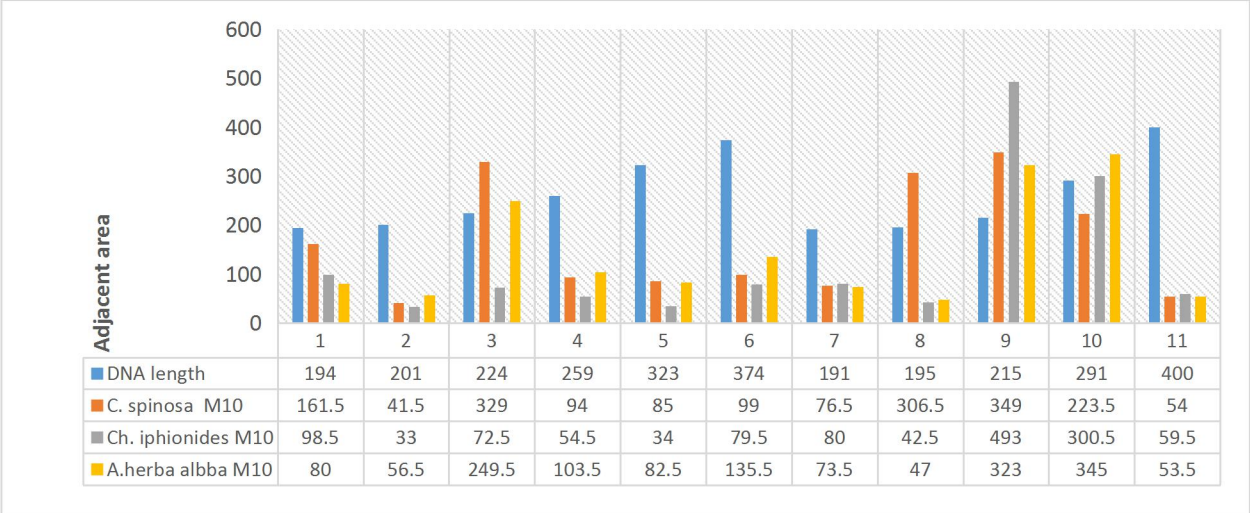
Fig No 7: Terminal restriction fragment length polymorphism (TRFLP) results for all water treated samples after 10 day

Terminal restriction fragment length polymorphism (TRFLP) results for all water treated rhizosphere samples after 20 day:

Total bacterial community in all dry rhizosphere samples after 20 days of incubation in room temperature was 140 only 11 bacterial strains were presence in all condition with percentage 7.8 % of total bacterial community. as shown in table No 6 and 7, Fig No 8

Table No 6: Microbial richness community of Terminal restriction fragment length polymorphism (TRFLP) results for all water treated samples after 20 day

Samples	Total bacterial community	Percentage with all communities	Percentage of shared DNA	Persentage of diff. DNA
***C. spinosa* M 20 day**	44	31.42	25	75
***Ch. iphionoides* M 20 day**	46	32.85	23.91	76.08
***A. herba-alba* M 20 day**	**51**	35.71	**22 %**	78
Sum of total community	140	100	7.85	



	1	2	3	4	5	6	7	8	9	10	11
DNA length	194	201	224	259	323	374	191	195	215	291	400
C. spinosa M10	161.5	41.5	329	94	85	99	76.5	306.5	349	223.5	54
Ch. iphionides M10	98.5	33	72.5	54.5	34	79.5	80	42.5	493	300.5	59.5
A.herba albba M10	80	56.5	249.5	103.5	82.5	135.5	73.5	47	323	345	53.5

Fig No 8: Microbial richness community of Terminal restriction fragment length polymorphism (TRFLP) results for all water treated samples after 20 day

Table No 7: Similarity index of microbial community of Terminal restriction fragment length polymorphism (TRFLP) results for all control and microcosms' samples after 10 and 20 day Beginging with C. spinosa

	C10	C20	CM 10	CM20	CH10	CH20	CHM10	CHM20	HE10	HE20	HEM10	HEM20
C10	1	0.77	0.68	0.65	0.40	0.66	0.61	0.43	0.69	0.64	0.56	0.63
C20		1	0.55	0.53	0.41	0.62	0.56	0.35	0.64	0.59	0.44	0.55
CM10			1	0.69	0.37	0.55	0.55	0.39	0.51	0.52	0.46	0.55
CM20				1	0.32	0.40	0.46	0.36	0.48	0.43	0.45	0.46
CH10					1	0.47	0.34	0.56	0.30	0.36	0.20	0.34
CH20						1	0.75	0.37	0.59	0.59	0.22	0.50
CHM10							1	0.38	0.64	0.55	0.47	0.52
CHM20								1	0.31	0.41	0.54	0.47
HER10									1	0.6	0.52	0.51
HER20										1	0.55	0.73
HERM10											1	0.55
HERM20												1

Phylogenetic annotation of metagenomic data sets of the 16S rDNA sequences of *C. spinosa* microbial community rhizosphere

Phylogenetic tree derived from analysis of the 16S rDNA sequences of *C. spinosa* microbial community rhizosphere indicated that the most frequent and dominant microbial species was *Actinomycetales* species followed by *Alphaproteobacteria*.

Phylogenetic annotation of sequencing reads highlighted *Actinomycetales* as the dominating phylum in *C. spinosa* rhizosphere, accounting for 44.9% of all assigned reads (Fig. 10). Further dominating phyla were *Actinomycetales* (33.3%), *Alphaproteobacteria* (20.8%), *Actinobacteria* (12.5.8%) and *Acidobacteria*, Bacteria (Gemmatimonadates) and Bacteria (Chloroflexi) with (8.3%) and finally Bacteria and Bacteria (Deinococci) with (4.1%).

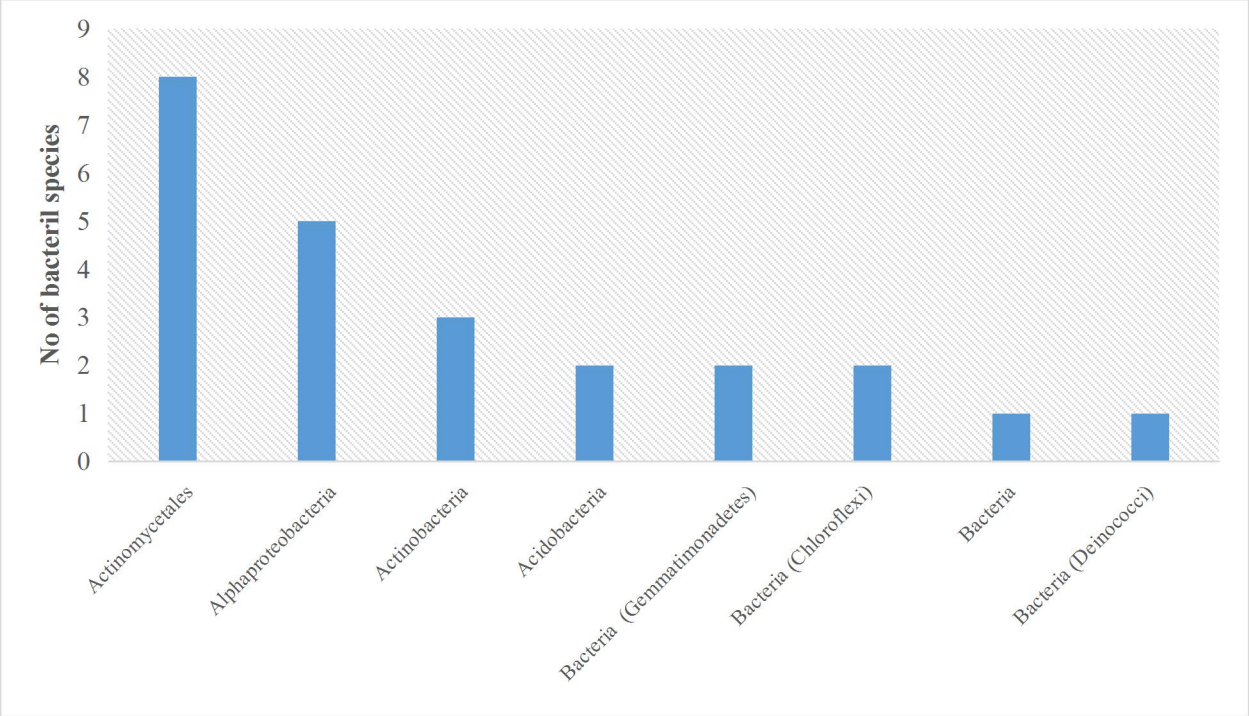


Fig No 10: Phylogenetic annotation of metagenomic data sets of the 16S rDNA sequences of *C. spinosa* microbial community rhizosphere

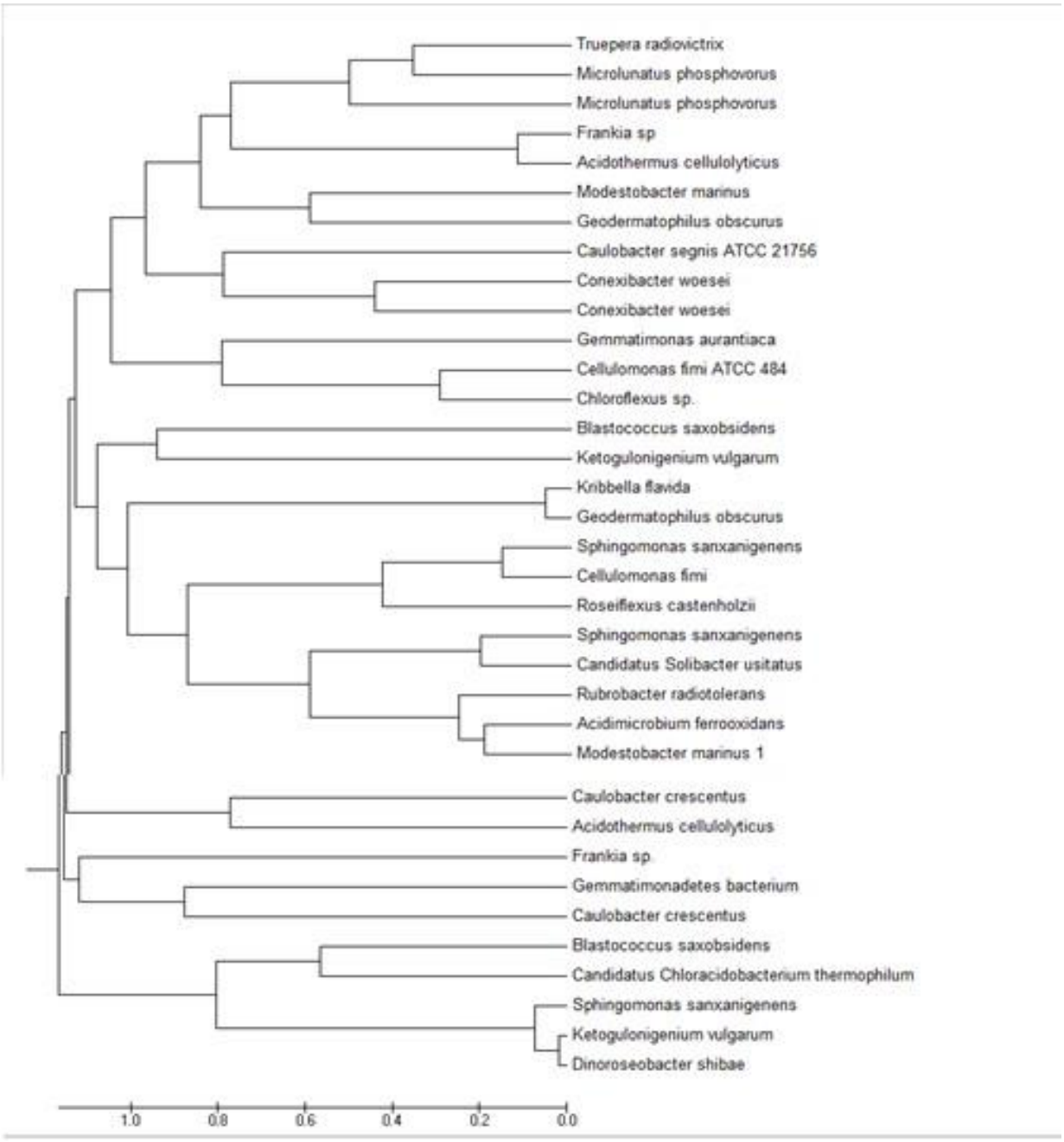


Fig No 11: **Phylogenetic tree derived from analysis of the 16S rDNA sequences of *C. spinosa* microbial community rhizosphere and related sequences obtained from NCBI. Scale bar, 0.02 substitutions per nucleotide position**

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I would like to thank Pro: Max M. Haggblom, Ph.D. Distinguished Professor and Chair, Department of Biochemistry and Microbiology, School of Environmental and Biological Sciences, Rutgers, the State University of New Jersey, United states for supplying and his continuous support

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