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ISOLATION & CHARACTERIZATION OF MICROORGANISM FROM AGRICULTURAL SOIL FOR IDENTIFYING POTENTIAL BIOFERTILIZER FOR DRY HARSH CLIMATE OF KACHCHH, GUJARAT, INDIA

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ABSTRACT

The use of Chemical fertilizers has helped farmers in increasing crop yields since the 1930's. Though chemical fertilizers have an important role in increasing plant nutrients in adverse conditions or during times when crops need more nutrients, there are several adverse effects of chemical fertilizers. Some of the harmful chemical fertilizers cause soil and water pollution, chemical burns to plants, increasing air pollution, disturbing the pH of the soil and mineral leaching from the soil. The above-mentioned problems can be avoided by increasing our reliance on biofertilizers. This paper aims to find out the suitable micro-organism that can be used as potential biofertilizers which are indigenous to the harsh and arid climate of the Kachchh area of Gujarat. The Fifty-six bacterial strain and four actinomycetes strain were isolated from the soil of different fields from Kukuma village of Bhuj- Kachchh (Gujarat). From the isolated organisms some strains are plant growth-promoting and some are used in to maintain nutrient stability in soil. Some strains with the greatest effects if they are used as a biofertilizer inoculum. These strains were identified to be of the genera *Pseudomonas, Chryseobacterium, Escherichia, Rhizobium, Bacillus, Stenotrophomonas and Streptococcus*. 16s rRNA gene sequence was used for species-level identification. Among

these, some strains produced a different type of hormone, enzyme and can be used to increase crop production significantly if developed and used as biofertilizer.

KEYWORDS: Biofertilizers, Rhizosperic soil, Phosphate solubilizing bacteria, Nitrogen-fixing bacteria.

INTRODUCTION

The chemical fertilizers since 1930's have supported farmers to increase the output of their crops through difficult environment but excess use of the fertilizers has led to the downfall of soil and groundwater quality and lead to various problems associated with the synthetic fertilizers like leaching, pollution of ground water sources nearby air pollution, soil pollution.[1]

To overcome these problems associated with the chemical fertilizers, microscopic organisms that allow the more efficient nutrient use or increase nutrient availability can provide sustainable solutions for present and future agricultural practices.

This paper aims to provide the names and characteristics of some microbes that have the capacity to be used as potential biofertilizers isolated locally use those as examples of possible choices for practical large-scale applications which are indigenous to soil and needs no new adaptations to survive in the dry arid region. In India, the farmers mainly used nitrogenous fertilizers and some phosphorous and very few to none use potassium in crop production. Biofertilizers are one of the best tools for sustainable agriculture. Biofertilizers are applied in the agricultural field as a substitute or in tandem with chemical fertilizers.[2] It helps to enhance the sustainability of the system. One possible mechanism for the effectiveness of biofertilizers, such as mobilization of sparingly available plant mineral nutrients nitrogen fixer, phosphorus, potassium and zinc Solubilizers, production of growth promoting substances, enhanced and induced resistance to environmental stress factors and direct or indirect suppression of plant pathogens.[3]

The eco-friendly agricultural system has emerged as an important priority area globally in view of its quality like growing demand for safe and healthy food and long-term soil-environmental sustainability and concerns on environmental pollution associated with indiscriminate use of agrochemicals.[4]

The main objective of the study was to isolate important microorganism by collecting different types of agricultural soil samples from various sources to find out their characteristics and application for nitrogen fixation and phosphate solubalizing and then test them to find out their species by using 16s rRNA gene sequencing.

MATERIALS AND METHODS

Soil Sample collection

The collection of soil sample from different farm of Bhuj (Gujarat) was done during the months October and December 2015. The samples were collected from Kukuma (N 23°12' 50" E 69°46' 54"), Madhapar (N 23 °26' 13" E 69 °72 '50") and Purasar (N 23 °26' 13" E 69 °72 '50") village of Bhuj-Kachchh, Gujarat. Almost 50 gram of soil was needed and was collected from the surface of soil after the top layer was scraped off and then collected. Total twenty samples were collected randomly from a different fields (Table 1) at a depth of no more than 10 to 20 cm and placed in sterile zip-lock polythene bags and labeled properly. The bags were placed in a cool box, transported to the laboratory and stored at 4 °C till further analysis was carried out. Sampling was done carefully in a manner which ensures that no cross-contamination can occur between samples.[5] These samples were then analyzed to determine the temperature, pH and Electrical Conductivity (EC) using Hach analyzer. Water holding capacity was done using the gravimetric method.

Table (1):- Sample source and site.

Sr. No	Sample type	Source	Location
1	Panjabi grass	Rhizospheric	Kukuma
2	Maize	Rhizospheric	Kukuma
3	Castor	Rhizospheric	Kukuma
4	Wheat	Rhizospheric	Kukuma
5	Potato	Rhizospheric	Kukuma
6	Gram	Rhizospheric	Kukuma
7	Alfa alfa	Rhizospheric	Kukuma
8	Potato	Rhizospheric	Madhapar
9	Coriander	Rhizospheric	Madhapar
10	Cow domb	Rhizospheric	Kukuma
11	Thorn based	Rhizospheric	Kukuma
12	Gobar liquid sludge	Rhizospheric	Kukuma
13	Gobar compost solid	Compost	Kukuma
14	Vermi compost	Compost	Kukuma
15	Narip compost	Compost	Kukuma
16	Carrot field	Rhizospheric	Purasar
17	Onion root	Rhizospheric	Purasar
18	Pashu amrit	Solution	Purasar
19	Hariyali	Solution	Purasar
20	Termin	Solution	Purasar

Table (2):- Physical parameters of soil samples

Sr. No.	Parameters	Values
1	Temp °C	25
2	pH	7.8 to 8.3
3	EC	1.82 to 0.81
4	Water holding capacity	Low to medium

The sample was then processed before dilution and was enriched in different broth such as Nutrient broth, Pikovskaya broth, Abshys mannitol broth for one week in a shaker incubator. Then the calculation for the total numbers of bacteria was done by plating soil dilutions on nutrient agar and total numbers of nitrogen-fixing bacteria and phosphate solubilizer were counted by plating of soil dilutions on nitrogen-free medium Abshys mannitol Agar (AMA), Nutrient Agar (NA) and Pikovskaya (PVK). 100 µl of the suspension from each dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}) was taken and poured into the nutrient agar media, phosphate solubilizer media, and the nitrogen-free media on Petri dish separately Then incubated the plates at 30 ˚C for 48 hours.

Isolating pure culture from the plates

The total count of the microorganisms was obtained by multiplying the number of cells per plate by the dilution factor, which was the reciprocal of the dilution. The pure culture of bacteria was isolated by taking discrete well-developed and separated colonies from the surface of a nutrient medium plate and transferred separately into a specific media containing plates such as AMA, PVK, and NA medium. Each of these new cultures represents the growth of a single bacterial species.

The colonies, which are different in appearances and characters were picked and purified. From these, the isolation of nitrogen-fixing and phosphate solubilizing microorganism was done, the strains were studied using the methods of Cerney (1993)[6] including their morphological and physiological features. The microbes were grown on the nutrient agar plate for overnight incubation at 37 ˚C. The bacterial culture was taken out by a sterile loop and was spotted on specific media containing Plates such as PVK plate, AMA plate, and NBRIP plate.

After spotting incubate the plate on 37 ℃ for 15 days for visualization of the clear zone on the plate. The plates were checked every day and their growth and clear zone on the plate was noted and gram staining of the same was carried out.

Qualitative assay of phosphate solubilizing activity

The now-grown pure culture of phosphate solubilizing bacteria was spot inoculated in plates containing Pikovskaya agar medium. The plates were incubated at 30 ℃ for 7 -10 days. The colonies forming more than 2.0 mm zone of solubilizing were stocked, the zone of phosphate solubilization (mm) formed around colonies was recorded after every 24 hours for 10 days.

The efficiency of solubilizing of the microorganism was readily calculated by the following formula.[7]

$$Solubilising\ efficiency\ (\%\ s.e) = \frac{Z - C}{C} \times 100$$

Where C=size of the colony diameter in mm, Z= solubilizing zone in mm.

Quantitative assay of phosphate solubilizing activity

The quantitative estimation of P Solubilizers bacterial isolates was carried out by the vanadamolybdophosphoric yellow color method in NBRIP broth containing 1000 microgram/ml tricalcium phosphate (TCP).[8-10]

The National Botanical Research Institute Phosphate (NBRIP) broth in 100 ml aliquots containing 1000 microgram /ml in the form of TCP was inoculated aseptically with 1ml of culture broth having OD at 600 nm.

The aliquots were incubated with shaking at 28 ℃ up to 10 days 5 ml of the growth medium from each flask was taken out on 3rd, 5th 7th and 10th day, filtered through Whatman no.1 filter paper, and centrifuged at 10000 r.p.m for 20 minutes.

Phosphorus in the cell-free culture supernatant was determined by the above-mentioned method, for this, 0.5 ml of the supernatant was taken, 2.5 ml of Barton's reagent was added and volume was made up to 50ml with double distilled water. After 10 minutes, the intensity of yellow color was read on a spectrophotometer at 430 nm and the amount of Phosphate solubilized from the standard curve.

Physiological studies of the selected strains and Quantitative assay of Nitrogenase activity and ammonia production

Nitrogen fixation capacity of nitrogen fixers was quantified indirectly by Acetylene reduction assay measuring the reduction of acetylene to ethylene. Next qualitative detection of ammonia production was done by the method given by Bakker and Schippers (1987)[11] using

Nessler's reagent. And the physiological activities of the selected strains were tested through oxidase, catalase, and nitrate reduction by Collee and Miles (1989).[12]

Purification and preservation

On obtaining the pure isolates, the cultures were submitted to Bank-A-Bug, microbial repository as per repository guidelines and unique BAB Ids were allotted. Their colony characteristics were noted down and then preserved in 20% glycerol PBS and stored properly.

Genomic DNA Isolation of Microorganism and Amplification of 16S rRNA

DNA isolation of bacteria and actinomycetes was carried out according to Sam-brook Protocol and its Genomic DNA was run on a gel. The Bacterial and Actinomycetes 16S rRNA gene primers and their sequences are detailed as follows: for the bacterial samples the Bacterial primers.

Table (3):- Bacterial and actinomycetes primers.

Sr.No	Sources	Primer	Sequence of Primer	References
1	Bacteria	8F	5'-AGAGTTTGATCCTGGCTCAG-3'	[13]
2	Bacteria	1492R	5'-GGTTACCTTGTTACGACTT-3'	[13]
3	Bacteria	704F	5'-GTAGCGGTGAAATGCGTAGA-3'	[14]
4	Bacteria	907R	5'-CCGTCAATTCCTTTRAGTTT-3'	[14]
5	Actinomycetes	243F	5'-GGATGAGCCCGCGGCCTA-3'	[15]
6	Actinomycetes	1360R	5'-CTGATCTGCGATTACTAGCGACTCC-3'	[16]

Amplification of 16S rRNA gene was done after multiplying the sequence in a PCR. PCR was carried out using 1X final concentration of Ready-mix™ Top Taq PCR Reaction Mix and template DNA (50 ng/ µL). The reaction was carried out in Thermal cycler (Applied Bio systems Veriti®) with PCR condition for 16s rRNA amplification using bacterial 8F/ 1492R primers were done as follows: Initial Denaturation at 95˚C for 3 min was done then subsequent 15 cycles of denaturation at 95˚C for 40 seconds followed by annealing at 55 ˚C for 1 min and extension of the template was done at 72˚C for 2 minutes, and after completion of the 15 cycles the next 10 cycles where carried out with initial Denaturation at 95˚C for 40 seconds followed by annealing at 50 ˚C for 1 min and extension of the template was done at 72˚C for 2 minutes after the end of the last cycle the final extension was carried out at 72˚C for 10 minutes ending the pcr process and sample was held at 4 degree Celsius till removed from the thermocycler. 16s rRNA gene in Bacteria was amplified in a volume of 20 µL containing 10 µL Taq PCR reaction mix,10 p/mole forward primer (243F), 10pmole reverse primer (1360R), 50 ng template DNA and sterile ion-free water. Amplification using 243F/

1360 R primer was done with conditions as follows: Initial denaturation at 95˚C for 3 min was done then subsequent 15 cycles of denaturation at 95˚C for 40 seconds followed by annealing at 64 ˚C for 1 min and extension of the template was done at 72˚C for 2 minutes, and after completion of the 15 cycles the next 10 cycles where carried out with initial denaturation at 95˚C for 40 seconds followed by annealing at 60 ˚C for 1 min and extension of the template was done at 72˚C for 2 minutes after the end of the last cycle the final extension was carried out at 72˚C for 10 minutes ending the pcr process and sample was held at 4 degree Celsius till removed from the cycler.

Preparation of samples and scanning of gels and purification of amplified products

Electrophoresis was carried out on the PCR product. The gel images were recorded by using gel documentation system (Biorad, USA). The gels were analyzed by using the software Image lab version 3.0 (Biorad, USA).After visualizing the gel image noted the positive band and processed it.the Purification of, 16s rRNA gene (bacteria, actinomycetes) amplified products were done using the EXO-SAP method, using following procedure.In which we take 4 µL EXO- SAP and 10 µL of PCR product.PCR product.

Cycle sequencing

Purified PCR products were subjected to cycle sequencing reaction by adding 4 µl of Big Dye 3.1 cycle sequencing mixture, 0.5 µl of Forward primer (10 pmol)/ Reverse primer (10 pmol), 1 µl 5x Sequencing buffer, 4 µl of nuclease-free water and 5 µl of purified DNA Template in two different PCR tubes. Cycle sequencing was performed using bacterial primers 704F and 907R, whereas action sequencing was performed using a different primer that is 243F and 1360R primers taken of 1 µl of Forward primer(10pmol)/Reverse primer(10 pmol).

Cycle sequencing purification

After completion of cycle sequencing, the product was purified using Big Dye X Terminator Purification Kit. 10 µl of Big Dye X Terminator was added to 45 µl of SAM solution for each well and 10 µl of cycle sequencing product was added into the mixture. Two reaction tubes were prepared for forward sequencing primers and reverse sequencing primers. In this experiment16s rRNA gene amplified product (704F, 907R, 243F, 1360R) served as sequencing primers Plate was sealed and subjected to vortex for 30 minutes at 2000 rpm. Sequencing was done on 3500xL Genetic Analyzer using POP 7.

Amplification of 16S rRNA gene in bacteria and actinomycetes were carried out in Thermal cycler (Applied Biosystems Veriti®). Reactions were amplified through 35 cycles with varying annealing Temperatures (55°C/50°C)**,** bacteria, actinomycetes respectively conditions were.

PCR conditions for cycle sequencing of 16S rRNA gene in bacteria

Initial denaturation at 96˚C for 1 min followed by the 25 cycles of denaturation at 96˚C for 10 seconds. Then annealing at 50˚C for 5 sec, followed by the end of a cycle with Extension At 60˚C for 4 min. After the end of all the 25 cycles, the sample was held at 4 ˚C till removed from the thermocycler.

The PCR condition for cycle sequencing

16 s RNA gene in Actinobacteria

Initial denaturation at 98˚C for 2 min followed by the 25 cycles beginning with denaturation at 98˚C for 20 seconds, then Annealing at 55˚C for 15 sec followed by the end of a cycle with Extension At 60˚C for 4 min. After the end of all the 25 cycles, the sample was held at 4 ˚C till removed from the thermocycler.

Capillary electrophoresis

Capillary electrophoresis of cycle sequenced products was Performed on 3500 XL platform (Applied Biosystems) Instrument software 3500 was used to give commands to the instrument. Dye Set Z and the Sequencing Install Standard, BigDye® Terminator v3.1 Kit was used for creating the BigDye® Direct spectral calibration information to apply to the data. Big Dye® mobility and calibration files were used for optimal base calling with the BigDye® Cycle Sequencing Kit v 3.1. Capillaries were filled with POP-7™ polymer.Selecting following parameters capillary electrophoresis was performed.

Sequence analysis and NCBI submissions

Sequence analysis was done using sequencing analysis version 5.4 (Applied Biosystems) and Bio Edit 7.2.5. Bio Edit was used to align and merge the DNA fragments in order to reconstruct the original sequence since the sequencing of 16S rDNA of isolates was done using both the forward and reverse primer. Assembly of forward and reverse sequences were performed using Cap Contig Assembly Program of Bio Edit software. These sequences were subjected to Sequence match analysis in form of FASTA using Basic Local Alignment Search Tool (BLAST) on NCBI.

FASTA file of the sequences was uploaded. Consensus sequences which showed significant match with the maximum identified data on NCBI were submitted to NCBI according to the guidelines provided in NCBI submission guideline.

BLAST analysis

The assembled sequence of the 16S rRNA gene of each isolate was analyzed using non-redundant BLAST search program at the NCBI. The BLAST analysis of 16S rRNA gene sequences of the selected strains showed alignments of these sequences with reported 16S rRNA genes in the Gen Bank.

Phylogenetic analysis:

Phylogenetic analysis and tree reconstruction were performed using MEGA 5.2. The 16S ribosomal DNA sequences of isolates were used for the phylogenetic analysis as they are evolutionary conserved and results were used for tree reconstruction. Analysis preferences can be selected from the dialogue box, tree for the isolates were constructed by neighbor-joining method a bootstrap confidence analysis was performed on 1000 replicates to determine the reliability of the distance tree topologies obtained. Jukes-Cantor model was used. The tree was constructed using 16S rDNA sequences of all the isolates of bacteria and actinomycetes sequences were taken as outgroup. The tree obtained by the data is given in results and discussion section figure.1.

RESULT AND DISCUSSION

Soil samples were found to be dark brown to black in color. As shown in table (Table 2.) Ph of soil samples ranging from 7.4 to 8.3 which is highly alkaline in nature with low to medium water holding capacity, which indicates the extreme nature of the soil.

The various bacterial and actinomycetes species were found from various sources (Table 1.) and their identification was carried out using 16s rRNA sequencing with the list of primers given in table (Table 3.), and their colony characteristics, Accession number, and gram nature were found out and given in the table (Table 4.) as well as their nitrogen fixing and phosphate solubilizing efficiency was found out (Table 5.).

In which several are plant growth promoting in the form of fixing of nitrogen and phosphorus in soil and different type of antibiotic-producing, and also shows an inhibitory effect against plant pathogen that make them a useful source of organic biopesticide. Even though

biopesticides cover only about 1% of the entire products for plant protection worldwide, their growth rate as and their number is increasing, this increasing trend in the past two decades.[17] Generally insect pathogenic bacteria occur in the families of Bacillaceae, Pseudomonadaceae, *Enterobacteriaceae*, *Streptococcaceae*, and *Micrococcaceae*. Member of *Bacillaceae*, particularly Bacillus spp., have received maximum attention as microbial control agents[18], some of the species were isolated belonged to some of these families. Some of these can be used in bioremediation as well. The name of plant growth promoting culture is under *Chryseobacterium arthrosphaerae, Chryseobacterium hispalense, Bacillus amyloliquefaciens,Bacillus niacin, Fictibacillus phosphorivorans, Lysinibacillussphaericus, Bacillus pumilus, Pseudomonas plecoglossicida, Stenotrophomonas maltophilia, Streptomyces pactum, Streptomyces diastaticus, Bacillus velezensis, Pseudomonas plecoglossicida, Luteibacter yeojuensis, Bacillus amyloliquefacien., Microbacterium takaoensis* And various others listed in the table above (Table 5).

Some have shown the potency to fix macronutrients in the soil but are pathogenic to cattle, some precautions can be taken to avoid infection from them and can be readily used as a liquid inoculum for agricultural practices.[19]

We also find some bacteria that produce antibiotic, but the main aim of the study is to find out such type of bacteria from Kachchh soil who have the ability to fix macronutrient in soil because they have no adverse effect on plant and soil and need no special conditions as they are indigenous to the soil and well adapted to survive and perform in the soil. The phylogenetic three made from the 16s rRNA sequencing shows interrelatedness of various microbes isolated.

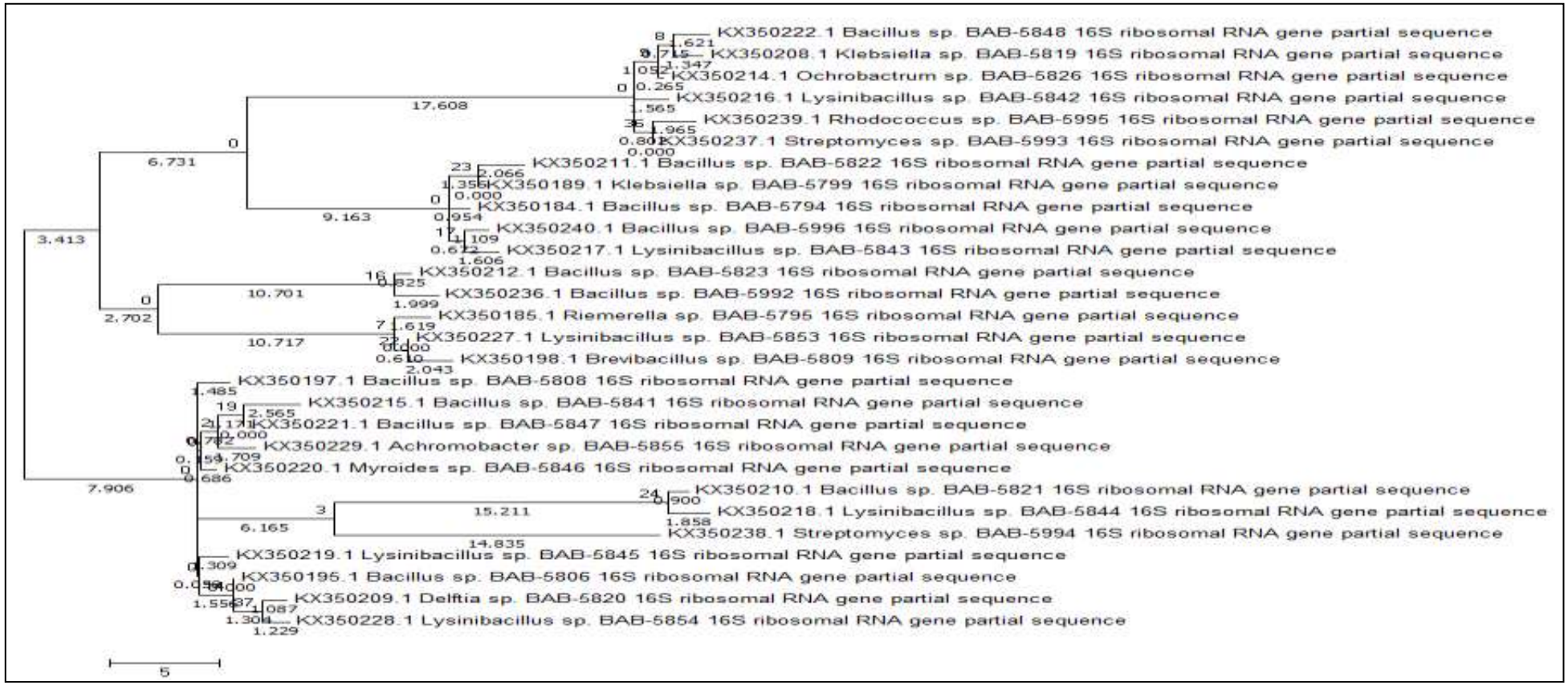

Figure (1):- Phylogenetic analysis of the isolates using 16s rRNA sequence.

Table (4):- Nature of the collected microbes and their details.

Serial No.	Species Name	BAB ID	Accession number	Colony Morphology	Aerobic / Anaerobic	GRAM +VE/ -VE
1	*Bacillus licheniformis*	5793	-	round,circular,pinpoint, creamish white, moist,flat,entire	Aerobic	+ve
2	*Bacillus licheniformis*	5794	KX350184	Round,circular,pinpoint, Dirty white, rough, raised,undulate	Aerobic	+ve
3	*Riemerella anatipestifer*	5795	-	circular, rounded, 2mm,orange colour, moist, raised, entire	Aerobic	-ve
4	*Acinetobacter pitti*	5796	-	irregular, pinpoint, creamy, white, mucoid, raised, curled	Aerobic	-ve
5	*Chryseobacterium arthrosphaerae*	5797	KX350187	circular,rounded,2mm,yellowish, mucoid, smooth, entre	Aerobic	-ve
6	*Chryseobacterium hispalense*	5798	KX350188	rounded,circular,2mm,yelllowish, moist, entire, curled	Aerobic	-ve
7	*Klebsiella pneumoniae*	5799	KX350189	rounded,circular,3mm,white, translucent, mucuiod, raised, entire	Facultative Anaerobic	-ve
8	*Luteibacter yeojuensis*	5800	KX350190	rounded, circular, dotlike, whiteish, moist, convex, entire	Aerobic	-ve
9	*Bacillus amyloliquefaciens*	5801	KX350191	irregular, filamentous ,pinpoint, white, rough, raised,undulate	Aerobic	+ve
10	*Microbacterium takaoensis*	5802	KX350192	rounded, circular, pinpoint, pinkish, moist, flat, entire	Aerobic	+ve
11	*Bacillus niacin*	5803	KX350193	circular, rounded, dot like, white translucent, mucousy, flat, entire	Facultative Anaerobic	+ve
12	*Fictibacillus phosphorivorans*	5804	KX350194	irregular, rhizoid, pinpoint, dirty white, rough, raised, undulate	Aerobic	+ve
13	*Pseudomonas indica*	5805	-	circular, rounded, 2mm,translucent white, moist,flat,raised	Aerobic	-ve
14	*Bacillus licheniformis*	5806	KX350195	irregular, rhizoid, pinpoint, white,	Aerobic	+ve

				smoooth, raised,curled		
15	*Lysinibacillus sphaericus*	5807	KX350196	rounded,circular,2mm , blackish,rough,raied,entire	Aerobic	+ve
16	*Bacillus pumilus*	5808	KX350197	irregular, rhizod,pinpoint creamish, mucoid,raised,entire	Aerobic	+ve
17	*Brevibacillus brevis*	5809	KX350198	circular,rounded,dotlike,whitish, moist,flat,raised	Aerobic	+ve
18	*Escherichia coli*	5810	KX350199	circular,rounded,pinpoint,creamish, moist,flat,entire	Facultative Anaerobic	-ve
19	*Escherichia coli*	5811	KX350200	rounded,circular,pinpoint, creamish,mucoid,flat,entire	Facultative Anaerobic	-ve
20	*Pseudomonas plecoglossicida*	5812	KX350201	circular,rounded,2mm,creamishwhite , mucoid raised, curled	Aerobic	-ve
21	*Stenotrophomonas maltophilia*	5813	KX350202	circular, rounded, pinpoint, whitish, mucoid, flat, entire	Aerobic	-ve
22	*Chryseobacteriu gleum*	5814	KX350203	circular, rounded, pinpoint, whitish, mucoid, flat, entire	Aerobic	-ve
23	*Brevundimonas terrae*	5815	KX350204	circular, rounded, dotlike, moist, translucent,flat,undulate	Aerobic	-ve
24	*Bacillus cereus*	5816	KX350205	irregular, filamentous, pinpoint, white, rough, raised, undulate	Aerobic	+ve
25	*Brevundimonas diminuta*	5817	KX350206	rounded,circular,2mm,transparent, moist,flat,undulate	Aerobic	-ve
26	*Rhizobium pusense*	5818	KX350207	circular, rounded, dotlike, white, moist, curved, undulate	Aerobic	-ve
27	*Klebsiella pneumonia*	5819	KX350208	irregular, pinpoint, brownish white, moist, curled, undulate	Facultative Anaerobic	-ve
28	*Delftia tsuruhatensis*	5820	KX350209	circular, rounded, dot-like, whitish, moist, flat, entire	Aerobic	-ve
29	*Bacillus subtilis*	5821	KX350210	filamentous, rhizoid, pinpoint, whitish, moist, flat, curled	Aerobic	+ve
30	*Bacillus tequilensis*	5822	KX350211	irregular, irregular, pinpoint, white,	Aerobic	+ve

				moist,flat,entire		
31	*Bacillus licheniformis*	5823	KX350212	irregular, irregular, pinpoint, white, rough, convex, curled	Aerobic	+ve
32	*Bacillus subtilis*	5824	KX350213	irregular,irregular,pinpoint,white, rough,convex,curled	Aerobic	+ve
33	*Bacillus subtilis*	5825	-	irregular, pinpoint, brownish white, moist, flat, curled	Aerobic	+ve
34	*Ochrobactrum intermedium*	5826	KX350214	irregular,pinpoint,creamishwhite, moist,convex,curled	Aerobic	-ve
35	*Bacillus eiseniae*	5841	KX350215	rounded,circularpinpoint,white, moist,curled,undulate	Aerobic	+ve
36	*Lysinibacillus xylanilyticus*	5842	KX350216	rounded,circular,pinpoint,white, moist,curled,undulate	Aerobic	+ve
37	*Lysinibacillus sphaericus*	5843	KX350217	rounded, circular, dotlike, creamyconvex, raised	Aerobic	+ve
38	*Lysinibacillus meyeri*	5844	KX350218	rounded,circular,dotlike,creamy, moist,convex,raised	Aerobic	+ve
39	*Lysinibacillus fusiformis*	5845	KX350219	irregular,rhizoid,dotlike,moist, flat,raised	Aerobic	+ve
40	*Myroides odoratus*	5846	KX350220	rounded,circular,pinpoint, yellowish,moist,flat,entire	Aerobic	+ve
41	*Bacillus subtilis*	5847	KX350221	irregular,rhyzoid,pinpoint, white,moist,flat,entire	Aerobic	+ve
42	*Bacillus enclensis*	5848	KX350222	rounded,circular,2mm,orange, moist,flat,entire	Aerobic	+ve
43	*Shigella flexneri*	5849	KX350223	rounded,circular,3mm,whitish,mucoid, convex,curled	Facultative Anaerobic	-ve
44	*Chryseobacterium gleum*	5850	KX350224	rounded, circular pinpoint, yellow, mucoid, flat, entire	Aerobic	-ve
45	*Empedobacter brevis*	5851	KX350225	Circular, rounded, pinpoint, creamy white, Mucoid, convex, curled	Aerobic	-ve
46	*Leucobacter salsicius*	5852	KX350226	round, circular, 2mm, yellowish,	Aerobic	+ve

				moist, convex, curled		
47	*Lysinibacillus macroides*	5853	KX350227	irregular, filament, pinpoint, transparent, moist, flat, entire	Aerobic	+ve
48	*Lysinibacillus mangiferihumi*	5854	KX350228	circular, rounde, 2mm,creamish, moist, raised, entire	Aerobic	+ve
49	*Achromobacter xylosoxidans*	5855	KX350229	circular, rounded, pinpoint, creamy white, mucoid, raised, entire	Aerobic	-ve
50	*Paracoccus pantotrophus*	5856	KX350230	rounded, circular, pinpoint, white, mucoid, curled, convex, undulate	Aerobic	+ve
51	*Acinetobacter pittii*	5857	KX350231	Irregular, rhizoid, pinpoint, transparent ,moist, flat, entire	Aerobic	-ve
52	*Pseudomonas aeruginosa*	5858	KX350232	Irregular, rhizoid, pinpoint, Creamy brown, moist, convex, entire	Aerobic	-ve
53	*Enterobacter cloacae*	5859	KX350233	Circular, rounded, pinpoint, Creamy white, mucoid, raised, entire	Facultative anaerobes	-ve
54	*Streptomyces diastaticus*	5860	KX350234	rounded,filamentous,2mm,white, rough ,entire	Aerobic	+ve
55	*Streptomyces pactum*	5991	KX350235	rhizoid,filamentous,2mm, white , rough ,entire	Aerobic	+ve
56	*Bacillus velezensis*	5992	KX350236	circular, rounded, pinpoint, creamish, moist, flat, undulate	Aerobic	+ve
57	*Streptomyces levis*	5993	KX350237	rounded, circular, filamentous, 2mm,white ,rough ,entire	Aerobic	+ve
58	*Streptomyces coeruleofuscus*	5994	KX350238	irregular,filamentous,2mm, white ,rough ,entire	Aerobic	+ve
59	*Rhodococcusequi*	5995	KX350239	irregular, filamentous, 2mm,white ,rough ,entire	Aerobic	+ve
60	*Bacillus subtilis*	5996	KX350240	Rounded,circular,dotlike, yellow,moist,raised,entire	Aerobic	+ve

Table (5):- Optical density and enzyme activity of microbes and their application.

Serial No	Culture name	OD OF P	OD OF N	CATALASE	NITRATE REDUCTASE	OXIDASE	APPLICATION
1	*Bacillus licheniformis*	0.807	0.689	+	-	+	Stimulus immune system, laundry detergent and antibiotic bacitracin production
2	*Bacillus licheniformis*	0.931	0.812	+	-	+	Stimulus immune system, laundry detergent and antibiotic bacitracin production
3	*Riemerella Anatipestifer*	0.747	0.63	+	-	+	pathogenic to poultry birds
4	*Acinetobacter pitti*	0.592	0.484	+	+	-	human pathogen
5	*Chryseobacterium arthrosphaerae*	0.956	0.859	+	+	+	Nitrogen fixer and used in to enhance crop yielding
6	*Chryseobacterium hispalense*	0.931	0.192	+	+	+	plant growth promoting
7	*Klebsiella pneumonia*	0.701	0.534	+	+	-	pathogen as well as Nitrogen fixer
8	*Luteibacter yeojuensis*	0.694	0.535	-	+	+	Nitrogen fixer, pathogenic
9	*Bacillus amyloliquefaciens*	0.343	0.213	+	+	+	plant growth promoter, protect from plant pathogen and source of bamh1
10	*Microbacterium takaoensis*	0.343	0.225	+	+	+	Cryoprotactant , bioemulsifier , used to stop bioleaching of heavy metals
11	*Bacillus niacin*	0.816	0.003	+	+	+	Nicotin degrading and antibodies production
12	*Fictibacillus phosphorivorans*	0.725	0.612	-	-	+	Nitrogen fixer, phosphorus solubliser
13	*Pseudomonas indica*	0.38	0.266	+	+	+	liquidified petrolium hydrocarbons
14	*Bacillus licheniformis*	0.412	0.297	+	-	+	fungisidal and laundry detergent
15	*Lysinibacillus sphaericus*	0.923	0.803	+	-	-	insecticidal property
16	*Bacillus pumilus*	0.678	0.569	+	+	+	fungicidal and Nitrogen fixer
17	*Bacillus pumilus*	0.963	0.874	+	+	+	antibiotic production
18	*Escherichia coli*	1.044	0.948	+	+	-	used in vaccine production
19	*Escherichia coli*	0.459	0.32	+	+	-	used in vaccine production and antibiotic

20	*Pseudomonas plecoglossicida*	0.444	0.321	+	+	+	used in Nitrogen fixation
21	*Stenotrophomonas maltophilia*	1.146	1.001	+	+	-	control from plant pathogen and used in bioremediaton
22	*Chryseobacterium gleum*	0.781	0.636	+	-	+	pathogenic
23	*Brevundimonas terrae*	0.661	0.581	+	+	+	biological control agent,anti fungal,herbicides
24	*Bacillus cereus*	0.776	0.67	+	+	+	cold tollerent plant growth promoter and bio control agent against fungal disease
25	*Brevundimonas diminuta*	1.081	0.97	+	+	+	use in efficiancy of water filter
26	*Rhizobium pusense*	1.062	0.975	+	+	+	pathogenic
27	*Klebsiella pneumonia*	0.89	0.761	+	+	-	Nitrogen fixer and used in toinhance crop yielding
28	*Delftia tsuruhatensis*	0.801	0.653	+	+	+	plant growth promoter and biocontrol agent against plant pathogen
29	*Bacillus subtilis*	1.169	1.093	+	+	-	Nitrogen fixing and plant pathogen inhibitor
30	*Bacillus tequilensis*	1.076	1.002	+	-	+	Nitrogen fixing and plant pathogen ihibitor
31	*Bacillus licheniformis*	0.597	0.46	+	-	+	feather degrading ,antibiotic production
32	*Bacillus subtilis*	0.681	0.564	+	+	-	inhibit planpathogen,antibiotic production, Nitrogen fixer
33	*Bacillus subtilis*	0.662	0.529	+	+	-	Inhibit plantpathogen,antibiotic production, Nitrogen fixer
34	*Ochrobactrum intermedium*	0.778	0.72	+	+	+	oil sludge degrading,use in biodegradation
35	*Bacillus eiseniae*	0.617	0.59	+	+	-	enhance earth worm ablity in degradation of organic material in available form
36	*Lysinibacillus xylanilyticus*	0.961	0.848	+	-	-	used in biodegradation of low level of polythene
37	*Lysinibacillus sphaericus*	1.278	1.134	+	+	-	insecticidal property and used in fermentation
38	*Lysinibacillus meyeri*	1.335	1.08	+	+	+	insecticidal property and used in fermentation
39	*Lysinibacillus fusiformis*	0.974	0.88	+	-	+	pathogenic and detoxified the industrial waste
40	*Myroides odoratus*	0.873	0.743	+	-	+	pathogenic and detoxified the industrial waste
41	*Bacillus subtilis*	0.754	0.67	+	+	-	Nitrogen fixing and plant pathogen inhibitor
42	*Bacillus enclensis*	0.788	0.711	+	-	-	plant growth promoter

43	*Shigella flexneri*	0.618	0.464	+	+	-	Pathogenic
44	*Chryseobacterium gleum*	0.646	0.488	+	-	+	human pathogenic
45	*Empedobacter brevis*	0.81	0.688	+	-	+	pathogenic
46	*Leucobacter salsicius*	0.833	0.695	+	+	-	chromate resistant bacteria
47	*Lysinibacillus macrolides*	1.055	0.948	+	-	+	xylan degrading
48	*Lysinibacillus mangiferihumi*	0.979	0.871	+	+	+	use in bioremediation
49	*Achromobacter xylosoxidans*	0.429	0.328	+	+	+	Pathogenic
50	*Paracoccus pantotrophus*	0.392	0.278	+	+	+	decolourising agent
51	*Acinetobacter pittii*	1.178	1.066	+	-	-	pathogenic to human
52	*Pseudomonas aeruginosa*	1.184	1.066	+	-	+	pathogenic to cattle
53	*Enterobacter cloacae*	0.754	0.62	+	-	-	plant disease controller and biodegration, Nitrogen fixer
54	*Streptomyces diastaticus*	0.668	0.546	+	+	+	arabinofuranase producer
55	*Streptomyces pactum*	0.575	0.455	+	+	-	anti tumor, antibiotic, Nitrogen fixer
56	*Bacillus velezensis*	0.508	0.392	+	+	+	anti microbial activity against plant pathogen, Nitrogen fixer
57	*Streptomyces levis*	1.176	1.051	+	+	+	biological controlling agent, anti fungal, herbicides
58	*Streptomyces coeruleofuscus*	1.086	0.955	+	+	+	antagonostic and antibiotic
59	*Rhodococcus equi*	0.903	0.779	+	+	-	Pathogenic to domestic animal
60	*Bacillus subtilis*	1.072	0.782	+	+	-	Nitrogen fixing and plant pathogen inhibitor

CONCLUSION

The microbes from the different source were isolated and identified using 16s rRNA sequencing, to find out the indigenous microbes that can be used as a potential biofertilizer in the dry arid region of Kachchh Gujarat.

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