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Profenofos degradation and plant growth promoting potential bacterial isolate S. *Paucimobilisdn*-5

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Abstract
In the agricultural field, many pesticides are used to control various pests in crops. Pesticides consist of many chemicals which are also harmful to human health and caused environmental pollution. Organophosphates are the most widely used group of pesticides. Profenofos is one of the most largely used organophosphate insecticides on a variety of crops including cotton and vegetables. The World Health Organization classified this compound as moderately hazardous (Toxicity class II). Many microbes are capable of the biodegradation of profenofos from pesticides contaminated soil. In context to the same, in the present study, we used two bacterial strains which are isolated from the pesticides contaminated soil by enrichment culture technique and identified by morphological characteristic and biochemical reactions were found to be *Acinetobacter ursingii*DN-4 and *Sphingomonas paucimobilis* DN-5. In this study, both bacterial strains were used to check plant growth-promoting activities like the production of IAA, ammonia, hydrogen cyanide, siderophore, phosphate solubilization, etc. which is important for the plant growth promotion, soil fertility, and development of eco-friendly sustainable agriculture. Later, both bacterial strains were also used for the biodegradation of the pesticides contaminated soil, and degradation was analyzed by HPLC. Based on plant growth-promoting activities *S. paucimobilis* DN-5 was selected for the growth experiment on *Vigna radiata*.

Keywords: profenofos, organophosphate insecticide, *Acinetobacter ursingii*, *Sphingomonas paucimobilis*, biodegradation, HPLC

Introduction
The plant is predominantly photosynthetic eukaryotes of the kingdom Plantae. The plant is the primary and only producer of food. With the increasing global population, it becomes necessary to increase the production of food to meet the hunger of consumers. Globally, an average of 35% of potential crop yield is lost to pre-harvested pests (Oerke 2005) [14]. The reduction of current yield losses caused by pests, pathogens, and weeds is a major challenge to agriculture production. The intensity of crop protection has increased considerably with the use of pesticides worldwide (Oerke 2005) [14].
Pesticides are the compounds used the control pests invading food crops. The earliest pesticides that were used included either inorganic or plant-derived products. However, in the past few years the use of chemical pesticides has increased tremendously (Ajaz and Rasool, 2005). Chemical pesticides consist of many chemicals which are harmful to pests. The deliberated application and intensive use of pesticides in the environment have been leading to the contamination of air, soil, surface, and groundwater (Bampus and Aust, 1987) [3]. These pesticides are non-biodegradable. Various pesticides like Organophosphates, Carbamates, Organochlorine insecticides, Pyrethroid, etc. are chemical pesticides. Organophosphates are the main compound of herbicides, pesticides, and insecticides. They contain C-P, S-P, O-P, and N-P linkage. C-P linkage is thermally and chemically inert and is resistant to thermal hydrolysis (Graves and Letcher, 2017) [9]. Acute and chronic exposure to OPs can produce varying levels of toxicity in humans, animals, plants, and insects.
Profenofos is an organophosphate insecticide. It is a liquid with a pale yellow to amber color and garlic-like order. It can be used on a variety of crops including cotton and vegetables such as maize, potato, soybean, and sugarbeet (McDaniel and Moser, 2004) [12]. Profenofos was first registered in the United States in 1982 (USEPA, 2000c) [18] to combat pests that showed resistance to chlorpyrifos and other Ops (Gotoh *et al*., 2001). Like another organophosphate, the profenofos mechanism of action is via the inhibition of the

acetylcholinesterase enzyme (Fukuto, 1990; Akerblom, 2004; Costa *et al*., 2008) [8, 2, 6]. A 2007 World Health Organization report found no adverse effects to workers from routine exposure to profenofos and no teratogenicity or carcinogenicity. Based on a study of patients poisoned with profenofos, the compound has been described as "of moderately severe toxicity", causing respiratory failure. A United States Environmental Protection Agency report identified profenofos as toxic to birds, small mammals, bees, fish, and aquatic invertebrates. (USEPA, 2002) [19].

The harmful effects of profenofos have led to a rapid increase in research on methods to remove this xenobiotic from the environment. Bioremediation is the most successful technique that uses microbial metabolism to remove pollutants (Chatterjee and Dutta, 2003; T. K. Dutta, 2007) [5, 11]. A profenofos has been reported to be degraded by *Pseudomonas putida* and *Burkholderia gladioli* (Malghani *et al*., 2009) [10], *Bacillus subtilis*, and *Pseudomonas sp.* (Salunkhe *et al*., 2013) [15]. 4-Bromo-2-chlorophenol was identified as major metabolite during profenofos degradation (Siripattanakul-Ratpukdi *et al*. 2015) [16].

Bacteria having the ability to grow in presence of profenofos (either using it as a sole source of carbon or using it in presence of other cometabolites) can be exploited to bioremediate soil contaminated with a high concentration of profenofos and other related OPs. In the present study, we used screened and identified bacterial isolates from pesticide-contaminated soils having the ability to degrade pesticides that lower the toxic effects of these pesticides and possess the property of plant growth promotion. These traits included the production of phytohormones such as IAA that stimulate cell proliferation and enhance the host's uptake of minerals and nutrients from the soil (Vessey JK, 2003) [20], solubilizing phosphate for root uptake and nitrogen fixation. Collating efficient biodegradation potential along with multiple biological properties, these isolates have the potential to develop into valuable candidates for the development of bioremediation strategies.

Materials and Methods

Pesticide

A technical grade profenofos (50% E.C.) an organophosphate (OP) pesticide used in this study was procured from "KhetiVikas Kendra", Satapar, Ta.- Jam Jodhpur, Dist. - Jamnagar.

Chemicals and Media

Bushnell Haas Broth, Gram's Reagent. King's Medium Base, Luria Broth, Chromoazyrol Sulphonate Agar,
Pikovskaya agar medium, Salkowski's reagent, Antibiotics, Acetone, and Hexanewere used for studies. Chemicals used in this study were of analytical grade. All media, media constituents, and testing kits used for bacterial cultivation, culture maintenance, and study culture characterization were purchased from Hi-Media Laboratory Pvt. Ltd, Mumbai India, and Sisco Research Laboratories Pvt. Ltd. (SRL), India.

Sample Collection

Agricultural fields from Rajkot district of Gujarat state used to cultivate cotton and groundnut and having minimum 8 years history of using profenofos as pest control agent was selected for sampling sites. The samples were collected from 10-15 cm below the soil surface. The samples were air-dried and sieved (<2 mm) and stored at 4 ºC till further analysis.

Screening of bio fertilizer activities of plant growth-promoting rhizobacteria

The isolated strains were further characterized based on their substrate specificity and gram character.

Indole Acetic Acid (IAA) production Standard curve of Indole acetic acid

Standard solution of IAA consisting various concentrations (5, 10, 20, 50 and 100µg/ml) from stock consisting
1000 µg/ml were prepared. 1 milliliter of each standard was mixed with 2 milliliters of Salkowski's reagent and incubated for 25 minutes at room temperature. The intensity of color was measured at λ535 nm. Values of optical density (OD) obtained were used to prepare a standard curve.

Determination of IAA production by *Acinetobacter ursingii* DN-4and *Sphingomonas paucimobilis* DN-5.

LB-broth (50 ml) supplemented with tryptophan (25, 50, and 75 µg/ml) was inoculated with 50µl of actively growing cells of *Acinetobacter ursingii*DN-4 and *Sphingomonas paucimobilis* DN-5 (having OD of 0.5 value at Λ 600 nm) in triplicate and incubated (35 °C, 120 rpm for 72 hours). After incubation of 48 and 72 hours, the reaction mixture was centrifuged. 2ml of supernatant was mixed with 4ml of Salkowski's reagent. The intensity of color was measured at λ535nm and the amount of IAA produced was quantified using a standard curve. The effect of profenofos on the IAA production capability of the microbes was evaluated by supplementing the LB- broth with 100ppm of profenofos in presence of 100 µg/mL tryptophan.

Ammonia Production

Centrifuge tubes containing 10ml peptone water supplemented with profenofos (100ppm) were inoculated with 5% actively growing *Acinetobacter ursingii*DN-4 and *Sphingomonaspaucimobilis*DN-5and incubated at 35 °C for 96 hours. The experiment was performed in triplicate. The formation of yellow color and its intensity in the bottle upon addition of Nessler's reagent indicated the production of ammonia.

Phosphate Solubilization

The ability of *Acinetobacter ursingii*DN-4 and *Sphingomonas paucimobilis* DN-5 to solubilize phosphate was determined by plating *Acinetobacter ursingii*DN-4 and *Sphingomonas paucimobilis* DN-5 on Pikovskaya agar medium. The presence of a clear zone around bacterial colonies after one week of incubation at 35 °C indicated the phosphate solubilization ability of *Acinetoba ter ursingii* DN-4 and *Sphingomonaspaucimobilis*DN-5. Phosphate solubilization activity was measured by calculating the Solubilization Index (SI) (the ratio of Total diameter: Colony diameter).

Protease Activity

Proteolytic activities of all isolates were assayed using gelatin agar and exhibited as the diameter of the clear zone. Gelatin agar plate assay is a qualitative type of test of protease. A gelatin agar plate supplemented with 3% agar powder was used. A spot of isolates DN-4 and DN-5 was

inoculated on the middle of the plate and a clear zone was observed on incubation for 24 hours at 37 °C.

Siderophore production

For the detection of siderophore production, Chromoazyrol sulfate agar plate assay is used (Mathurot Chaiharn *et al*., 2008) [13]. For the preparation of Chromoazyrol sulfate agar, Take 0.06 g Chromoazyrol sulfate dye (CAS dye) in 50ml D/W and 0.07 g Hexadecyltrimethyl Ammonium Bromide. (HDMT) in 40 ml D/W then mixed it. Add 0.03 ml Hcl in 100ml D/W and take 10ml prepared Hcl solution then mixed with 100ml solution of 0.026 g ferric chloride. After previously prepared CAS dye and HDMT solution mixed with ferric chloride and Hcl solution then make the final solution of CAS reagent 200 ml. Take 35 ml CAS reagent and mixed with 100 ml of King's agar and thus Chromoazyrol sulfate agar plate is prepared. The spot of isolates DN-4 and DN-5 was inoculated on the middle of the plate and it is incubated at 28 °C for 5 days. Assayed through a change of color from blue to yellowish-orange around the spot.

Hydrogen cyanide production: King's base media is used for the detection of Hydrogen Cyanide production. For this isolate, DN-4 and DN-5 were streaked on the king's base agar plate. Filter paper impregnated with picric acid and sodium bicarbonate is put inside of the king's base media plate. The change in color of the filter paper disc from yellow to light brown or reddish-brown was recorded after 3 days or a week. Is an indication of moderate or strong HCN production by each strain respectively.

Amylase production

In the amylase production test, we use starch agar, which is a differential nutritive medium. The test organisms are inoculated onto a starch plate which is supplemented with 0.5% starch and incubated at 30 °C until growth is seen (i.e. up to 48 hours). The Petri plate is then flooded with an iodine solution. If there is no enzyme present, and therefore no hydrolysis, the amylase, and iodine react together to form a blue color. When bacteria are capable of producing amylase, they secrete enzymes into the surrounding areas and hydrolyze the starch. They give a clear zone of hydrolysis surrounding the colonies.

Antibiotic assay

Nutrient Agar Plate was prepared supplemented with 3% agar-agar and active culture of DN-4 and DN-5 were spread on the plate. HiMediaDoDeca Disk of Dodeca Universal-III and G-IIIV Minus was put on the plate and incubated for 24 hr at 37 °C. The zone of inhibition was observed around each disk. Therefore, the sensitivity and the resistance of the antibiotic in the isolates are decided based on the zone of inhibition obtained.

Bacterial degradation of profenofos microorganisms

Sphinogomonas paucimobilis DN-5 isolated and screened from profenofos contaminated soil were used for degradation study.

Biodegradation studies

Inoculum preparation for Profenofos Degradation

Actively growing cultures of *Sphingomonas paucimobilis* DN-5 was prepared in nutrient broth for 20 or 24 hours at 37 ºC temperatures under shaking culture condition and suspended in a complete medium.

Degradation analysis

Sphingomonas paucimobilis DN-5 was cultivated in BHB containing 100 ppm profenofos for 30 days under shaking culture conditions at 37 ºC. Extraction of unutilized profenofos and degradation products generated from profenofos was carried out in hexane and subjected to HPLC.

Extraction protocol

Every after 6 days of incubation, the culture was harvested (centrifuging at 6000g for 10 minutes) from respective reaction flasks, and cell-free supernatant was collected to estimate unutilized profenofos and check the presence of intermediate metabolites produced due to its degradation. The extraction of unutilized profenofos (and other intermediate metabolites generated during this incubation period) was carried out twice using an equal volume of analytical-grade hexane. The extract evaporated to dryness by a rotary evaporator. The dried samples were dissolved in an appropriate volume (1ml) of hexane and then filter sterilized (through sterile disposable syringe filter, Millipore make, 0.22 µm diameter pore size) before GC-MS analysis. Identification of intermediates was performed by HPLC. High-performance liquid chromatography (HPLC) is a form of column chromatography that was used for quantitative analysis of profenofos degradation. Sunfire C18 column with 3.4 µm, 4.6* 100mm was used. Equilibration was carried out with 0.0%glacial acetic 3.7 pH, Acetonitrile (10:90) Equilibration was done by passing 10 column volumes of the same buffer. And then20µl of Sample was loaded into the injector. For the standardization of the technique, 0.55ml/min flow rates were maintained.

Growth experiments with *Vigna radiate* (L.) R. Wilczek

Pot experiments were conducted to study the effects of profenofos on the germination of seeds of *Vigna radiata* in the presence and absence of *Sphingomonas paucimobilis* DN-5. Seeds of *Vigna radiata* were surface sterilized (70% ethanol solution for 5 minutes followed by washing with sterilized distilled water). Sterile and unsterile soil samples (4 kg) were spiked with profenofos (200 ppm/1000 grams of soil) and then inoculated with a 5% actively growing culture of *Sphingomonaspaucimobilis*DN-5. Sterilized seeds of *Vigna radiate* were sown in the respective pots and then the soil was moistened with water. Care was taken to ensure that the pots were kept at ambient light and temperature. The seed germination was monitored daily and was allowed to grow for 6 weeks. The following parameters were recorded during the process:

1. Seed germination (%)
2. Shoot length (cm)
3. Root length (cm)
4. Leaf length (cm)
5. Shoot fresh weight (g)
6. Root fresh weight (g)
7. Leaf fresh weight (g)
8. Shoot dry weight (g)
9. Root dry weight (g)
10. Leaf dry weight (g)

Results

Plant growth-promoting activities of bacterial isolates DN-5 and DN-6. Indole acetic acid production

IAA production is dependent on the presence of L-Tryptophan which is utilized by the organism and produces indole acetic acid. The results of IAA were compared with the standard curve of Tryptophan in the range of 5- 100 µg/mL, the results obtained were an increasing amount of IAA. The absorbance was measured at 535 nm which on plotting the graph of concentration of Indole acetic acid produced versus absorbance.

The two isolates DN 4 and DN 5 showed significant production of IAA. After 48 to 72 hours of incubation, the concentration of IAA was increased. Isolate DN 4 showed the concentration of IAA production was 50, 39, 53 µg/ml in the presence of 25, 50, and 75 µg/ml concentrations of L-tryptophan respectively. Isolate DN 5 showed the concentration of IAA production was 17 µg/ml in the presence of 25, 50, and 75 µg/ml concentrations of L-tryptophan respectively. Based on the above result it can be found that the concentration of IAA production was increased with the increasing concentrations of L-tryptophan.

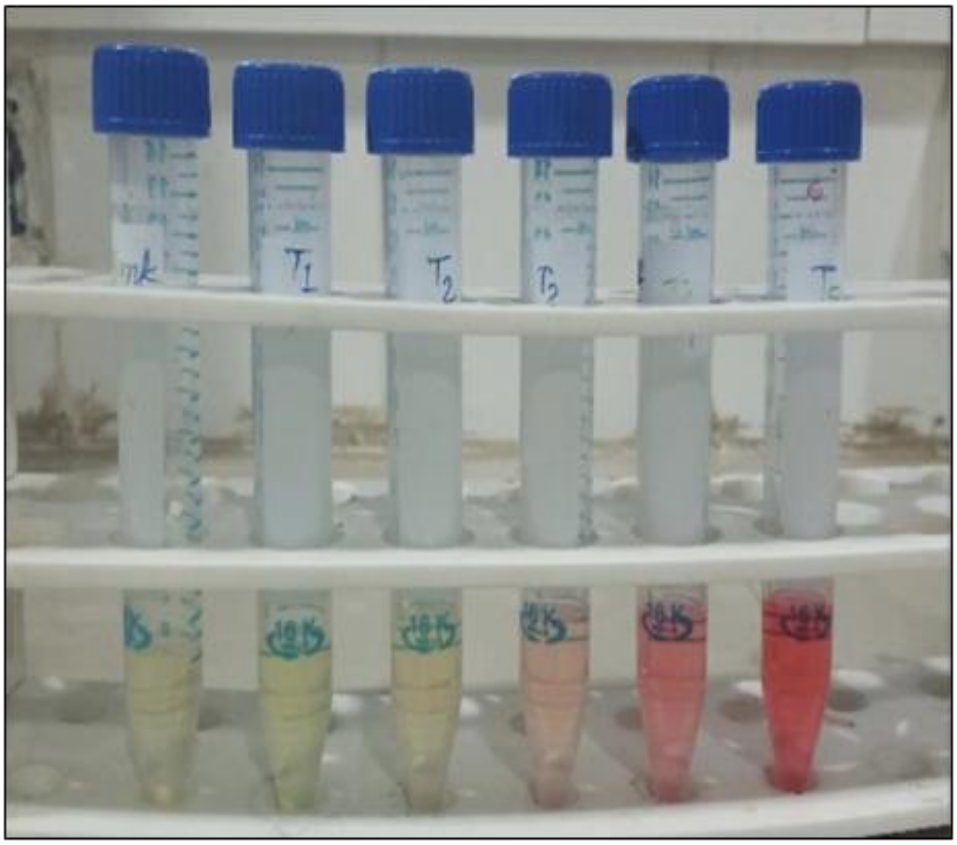
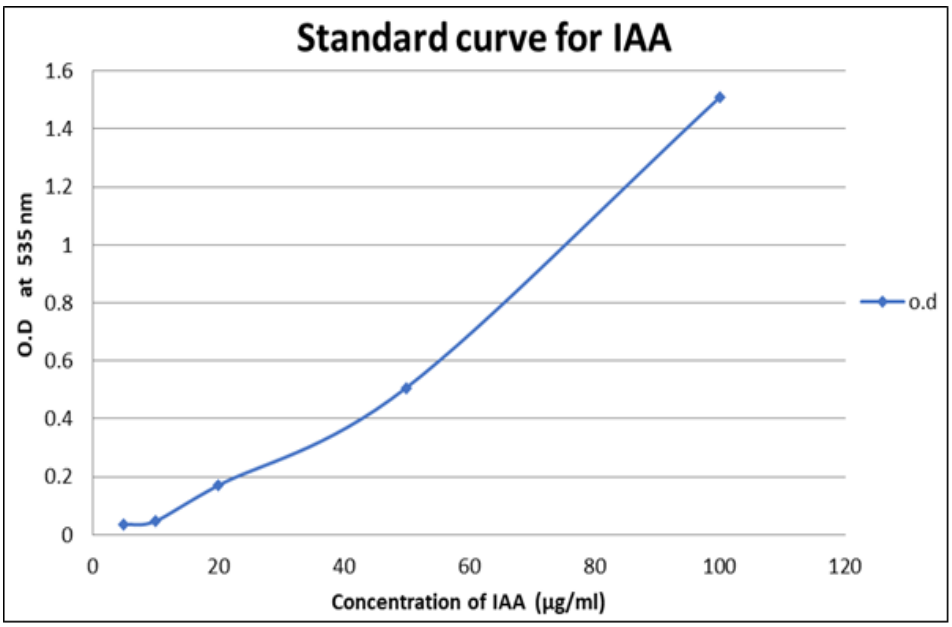


Fig 1: Standard Indole Acetic acid Production



Graph 1: Standard curve of IAA

Table 1: Production of Indole Acetic Acid by *Acinetobacter ursingii* DN-4 and *Sphingomonas paucimobilis* DN-5

Sample	*Tryptophan concentrations* (µg/ml)	Absorbance (535 nm)	IAA Concentration (µg/ml)
DN 4 T1	25	0.494	50
DN 4 T2	50	0.387	39
DN 4 T3	75	0.53	53
DN 5 T1	25	0.129	17
DN 5 T2	50	0.122	17
DN 5 T3	75	0.123	17

Ammonia production

Isolates DN-4 and DN-5 showed higher intensity of yellow color after the addition of Nessler's reagents as compared to blank, So, that it was indicated that isolates DN-4 and DN-5 were ammonia producers.

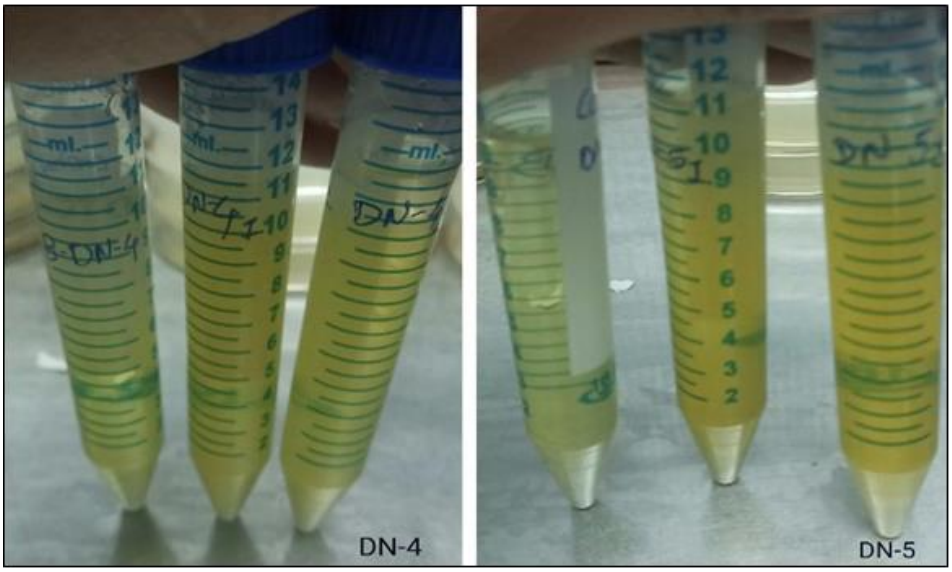


Fig 2: Ammonia production by DN-4 and DN-5

Siderophore production

Siderophore production by the isolates DN-4 and DN-5 was carried on a CAS agar plate, by performing this test, no color change was observed by isolates DN-4 and DN-5. So, this was proved that isolates DN-4 and DN-5 showed negative results towards siderophore production and showed their incapability towards it.

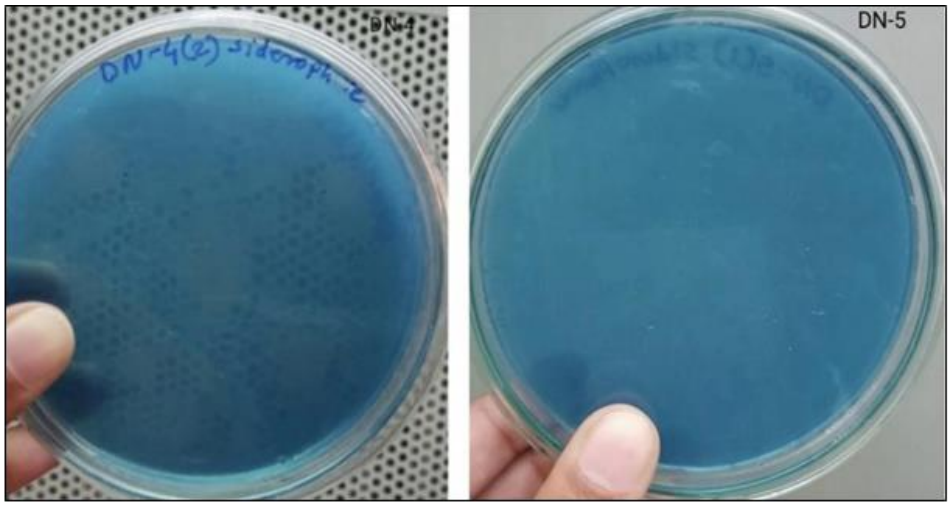


Fig 3: Siderophore production by DN-4 and DN-5

Hydrogen cyanide production

HCN production by fungal and bacterial biocontrol agents was tested quantitatively following the method of Bakker and Schipper (1987). A change in color of the filter paper from yellow to light brown, brown, or reddish- brown was recorded as a weak (+), moderate (++), or strong (+++) reaction respectively.

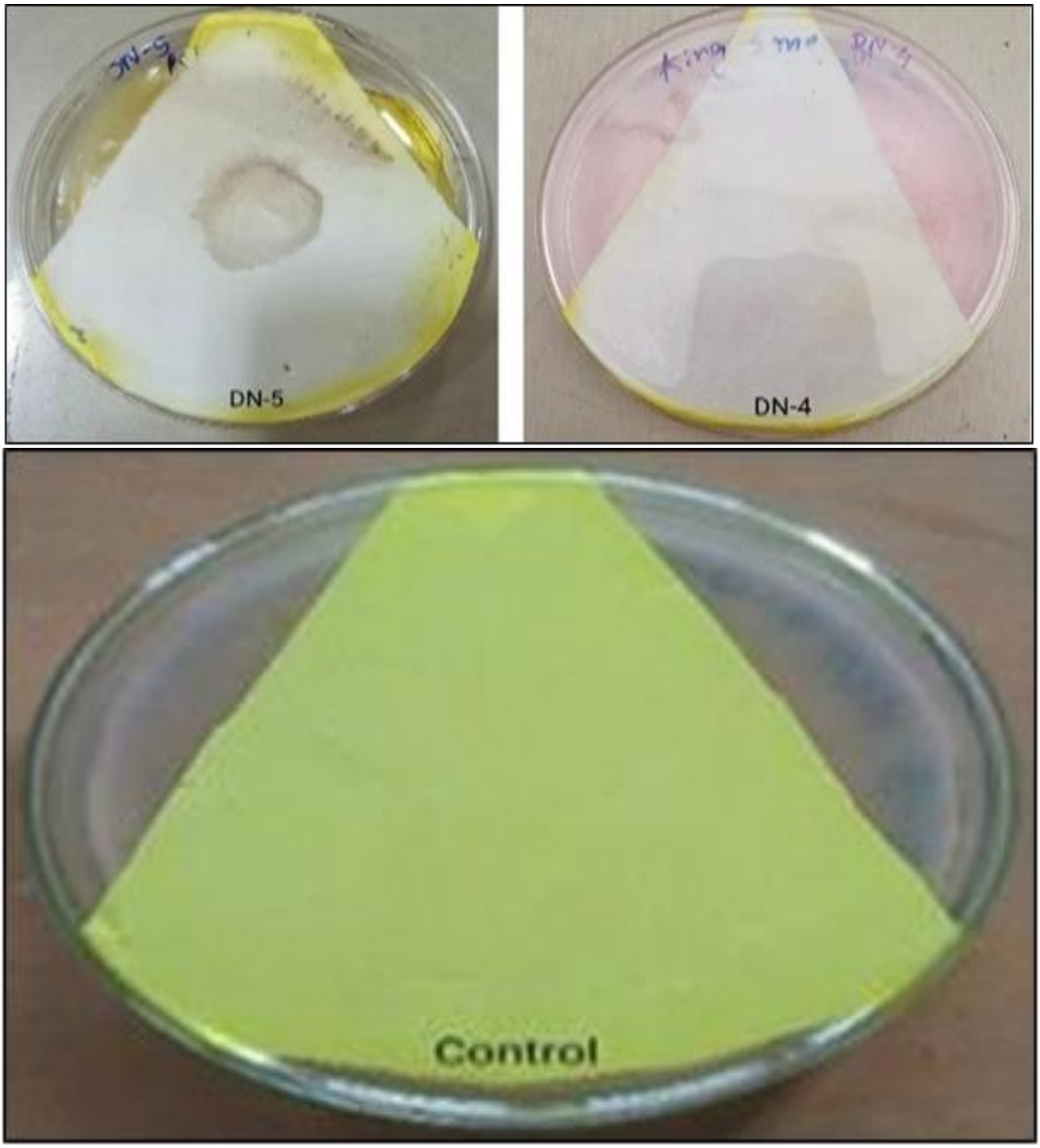


Fig 4: Hydrogen cyanide production by DN-4 and DN-5

By performing this test we observed that isolates DN-4 and DN-5 were able to produce HCN in moderate amounts. The color of the filter paper was changed from yellow to an off-white and brown color.

Protease Production

Protease activity was observed from the zone of hydrolysis observed on a gelatin agar plate. Isolates DN-4 and DN-5 give negative results in protease production, they do not produce a zone of clearance at surround them.

Phosphate Solubilization

By performing this test as shown in fig. 10 *Sphingomonas paucimobilis* DN-5 was able to solubilize phosphate. The phosphate solubilization activity of *Sphingomonas paucimobilis* DN-5 was indicated by producing zone surrounding the colony on Pikovskaya's agar plate. Following are the calculations of the Solubilization Index (SI).

SI = Total diameter of solubilization (mm) /colony diameter (mm): 3.7/2.2 = 1.6

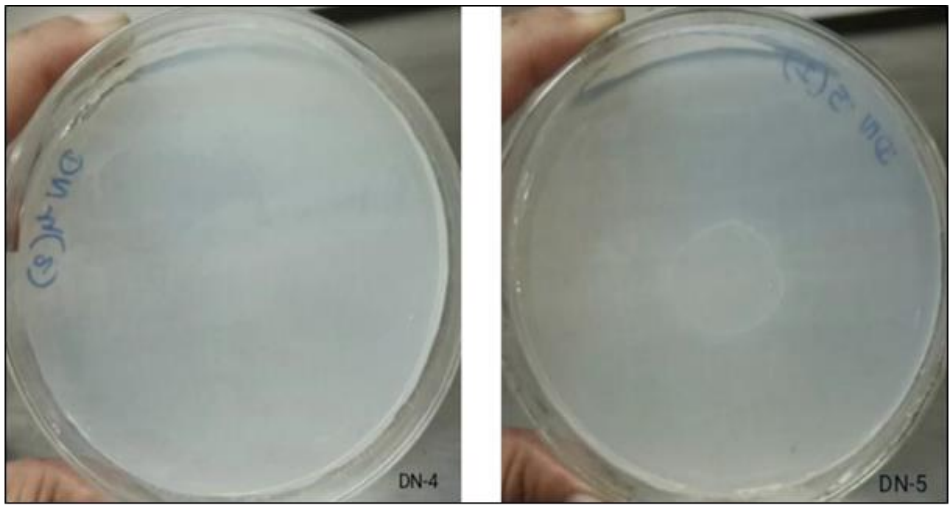


Fig 5: Protease production by DN-4 and DN-5

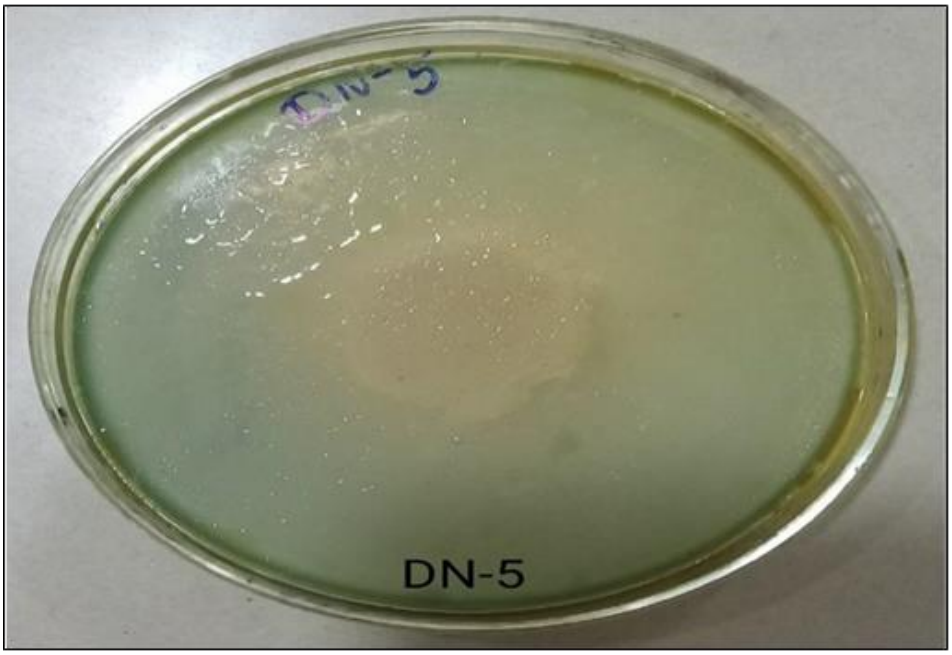


Fig 6: Zone of phosphate solubilization surrounding the colony of 5

Amylase production

Bacteria can produce extracellular Isolate *Sphingomonas paucimobilis*DN-5 give a clear zone of hydrolysis so that they can produce extracellular amylase. *Acinetobacter ursingii*DN-4 were not given a zone of hydrolysis so they are not able to produce extracellular amylase.

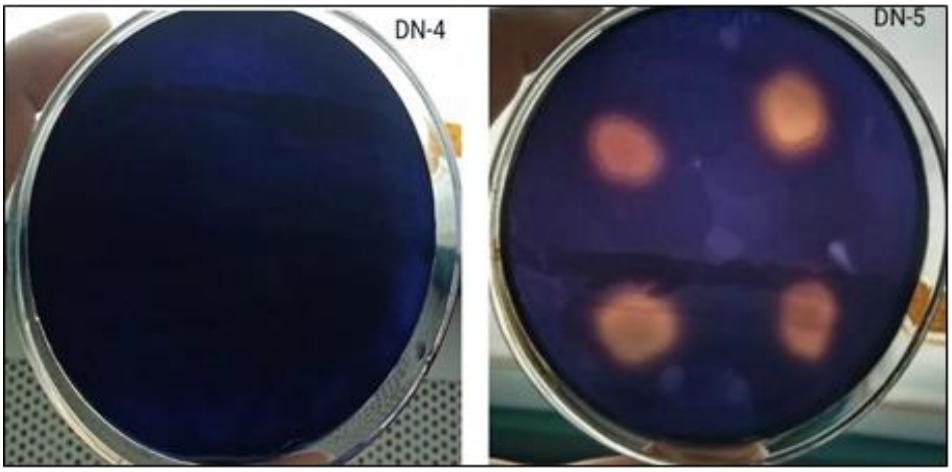


Fig 7: Amylase production by DN-4 and DN-5

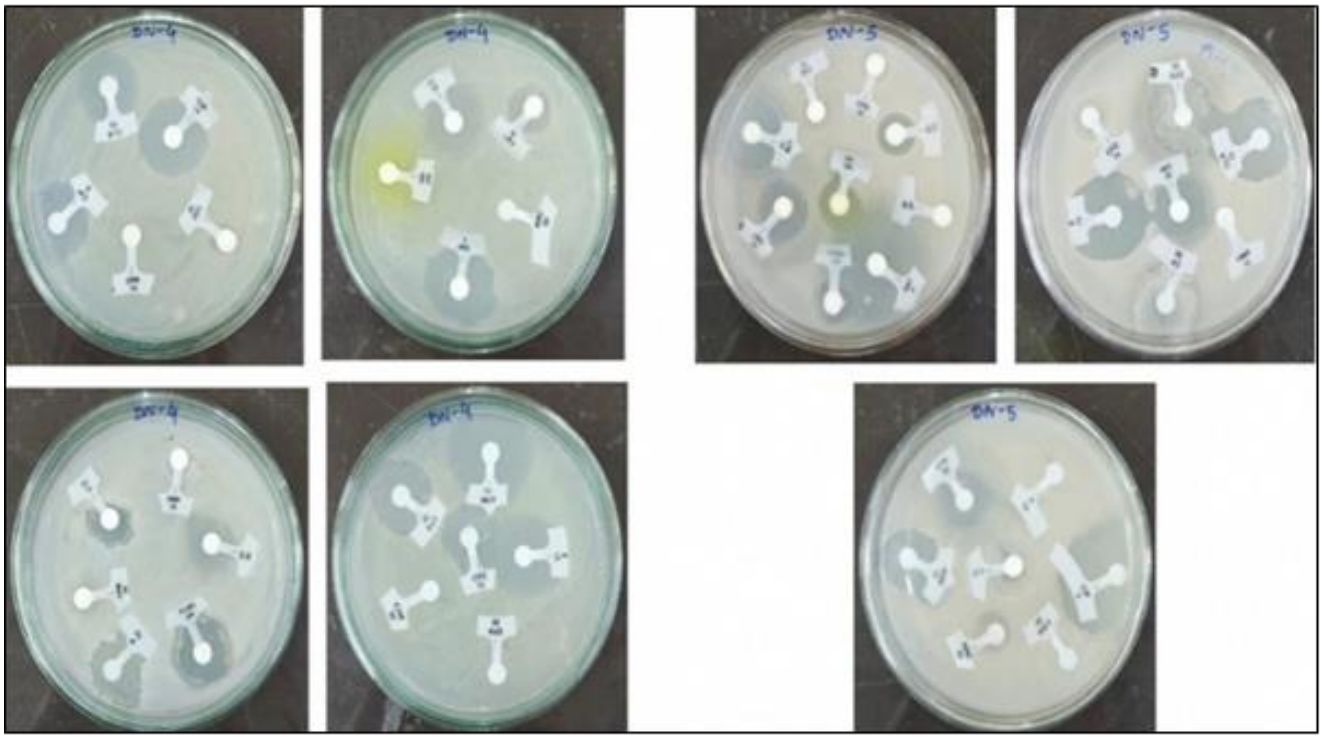


Fig 8: Antibiotioc production by DN-4 and DN-5

Table 2: Dodeca Universal-III antibiotics assay in DN-4 and DN-5

Dodeca Universal-III antibiotics	Symbol	Diameter of zone of inhibition in DN-4 (cm)	Diameter of zone of inhibition in DN-5 (cm)
Ampicillin	AMP	0.0	0.0
Cefuroxime	CXM	0.0	0.0
Cefadroxil	CFR	0.0	2.2
Augmenting	AMC	0.0	1.4
Penicillin	P	1.8	0.0
Cephotaxime	CTX	2.1	0.8
Cefaclor	CF	0.0	0.0
Azithromycin	AZM	3.0	2.0
Erythromycin	E	2.6	2.6
Cefoperazone	CPZ	0.9	0.0
Clarithromycin	CLR	2.1	0.0
Ciprofloxacin	CIP	2.3	3.0

Table 3: Dodeca G-III – Minus antibiotics assy in DN-4 and DN-5

G-VIII - Minus antibiotics	Symbol	Diameter of zone of inhibition in DN-4 (cm)	Diameter of zone of inhibition in DN-5 (cm)
Ampicillin	AMP	0.0	0.0
Ticarcillin	TI	1.3	0.8
Piperacillin/Tazobactim	PIT	1.8	1.4
Ceftazidime	CAZ	0.9	0.0
Cefepime	CPM	0.0	0.0
Cefpodoxime	CPD	0.0	1.9
Gatilfloxacin	GAT	2.5	2.7
Aztreonam	AT	0.0	0.0
Netillin	NET	1.5	1.8
Tobramycin	TOB	2.5	2.6
Colistin	CL	1.8	1.6
Nitrofurantoin	NIT	0.0	1.5

Antibiotics production assay

For the antibiotic assay, HiMediaDoDeca Disk of DodecaUniversal-III and GIIIV-Minus Disk was used and placed on an agar plate which was previously spread with the isolates DN-4 and DN-5 separately. After 24 hours of incubation zone of inhibition was observed which indicates that no growth of organisms was seen around the antibiotic disc so that, the isolate was not resistant to the antibiotics.

Degradation analysis

Bacterial Isolate *Sphingomonas paucimobilis*DN-5 was inoculated with 100 ml Bushnell Haas broth supplemented with 100 ppm profenofos and kept in shaking condition for 30 days and samples were harvested every 6, 12, 18, 24, and 30, days. After centrifugation, each sample was extracted using hexane solution, and filter all the samples were by nylon filter and analyzed through HPLC.

We found that the samples were able to degrade profenofos till 30 days and some other intermediates were also found in the degradation pathways.

Growth experiments with *Vigna radiate* (L.) R. Wilczek: Table 4. summarized the influence of the presence of *S.paucimobilis*DN-5 on seed germination and other plant parts like root length, shoot length, leaf length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight during its development processes in soil contaminated with profenofos

Table 4: The influence of the presence of *S. paucimobilis* DN-5 on seed germination and other plant parts during its development processes.

Particulars	A	B	C	D	E	F	G	H	I	J
Control (sterile)	80	9.03	7.35	4.18	1.04	0.53	4.35	0.32	0.36	1.10
Control (non-sterile)	85	9.85	7.78	3.66	0.97	0.43	2.97	0.31	0.31	0.89
Profenofos (sterile)	95	8.5	7.5	3.8	1.14	0.67	3.5	0.34	0.31	1.12
Profenofos (non-sterile)	100	10.05	7.76	4.32	1.55	0.94	3.64	0.37	0.55	0.88
DN-5 (sterile)	95	8.95	8.18	4.82	1.2	0.65	4.45	0.46	0.44	1.44
DN-5(non-sterile)	90	10.14	7.92	3.8	1.55	0.57	4.78	0.36	0.34	1.09
Profenofos +DN-5 (sterile)	75	8.37	8.1	3.85	0.96	0.51	3.31	0.31	0.34	0.98
Profenofos + DN-5 (non-sterile)	95	8.35	8.56	4.18	1.52	0.72	4.9	0.31	0.30	1.35

Here, A = Percentage (%) germination, B = Shoot length (cm), C= Root length (cm), D= Leaf length (cm), E = Shoot fresh weight (g), F = Root fresh weight (g), G= Leaf fresh weight (g), H= Shoot dry weight (g), I = Root dry weight (g), J= Leaf dry weight (g).

By performing growth experiments with *Vigna radiata*, we could found that *Sphingomonas paucimobilis* DN-5 can degrade profenofos in the soil and simultaneously exhibit its positive effects on plant growth. A pot containing non-sterile soil was given more results as compared to sterilized soil. This may be due to the ability of indigenous soil microbes to degrade profenofos and promote growth.

Profenofos degradation study using HPLC: We found that the isolate DN 5 was able to degrade more than 92% profenofos after 30 days of incubation (Fig 8). The retention time for Profenofos was observed at ~3.69, after 30 days the peak for pesticides was reduced to 0.99% with an unknown peak of 99.01% area. Pesticides-contaminated agricultural soil can be remediated successfully by augmenting it with potential bacterial isolates.

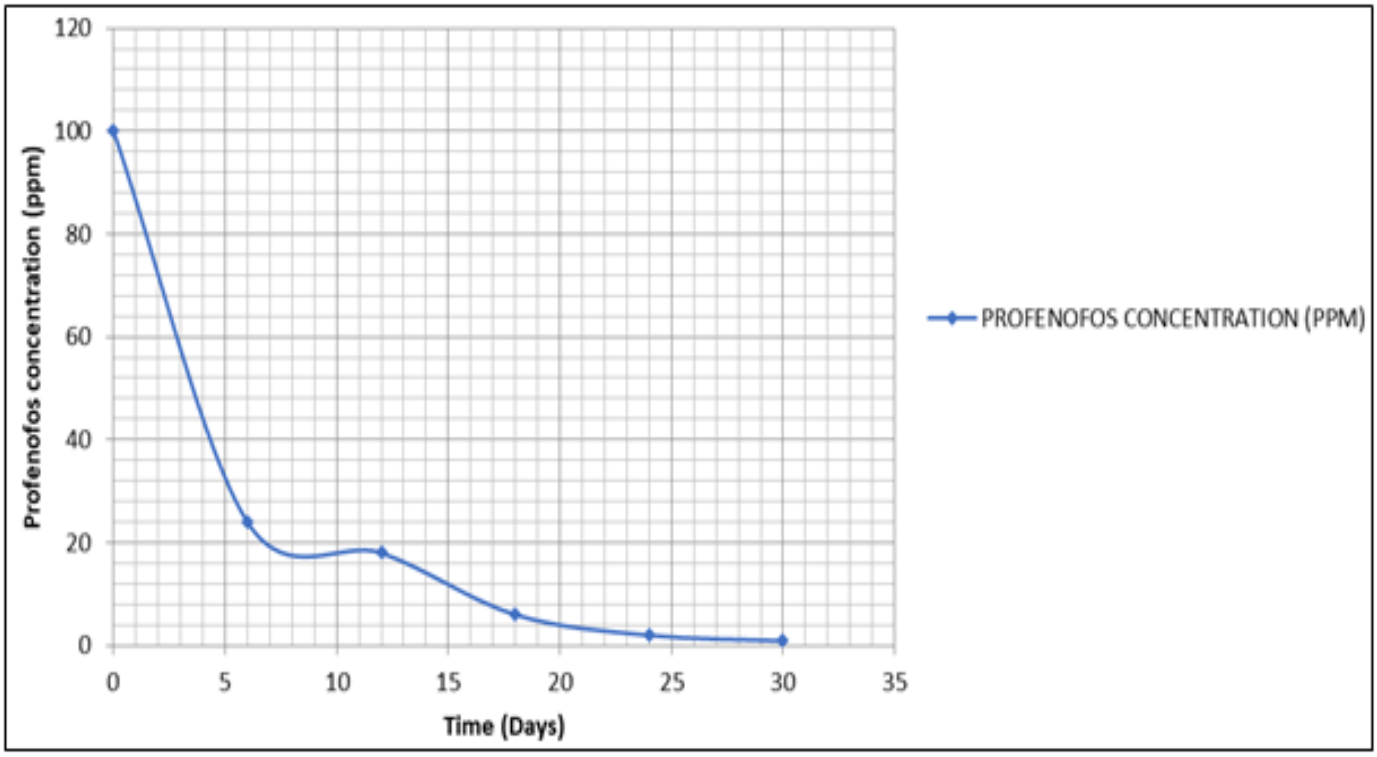


Fig 9: Degradation dynamics of profenofos inoculated with *Sphingomonas paucimobilis*DN-5 at an initial profenofos concentration of 100 ppm

Discussion

The agricultural land biome and its associated niches are among the most primitive man-created landscape designs to fulfill hunger and other essential need (Through the cultivation of crops, vegetables, fruits, etc.), for the human race. The system continues even today apart from acting as an important source of earning revenues and exchanges. The agricultural soil ecosystems are very complex and harbours unique community structures (consisting of microscopic and macroscopic life forms) selected based on thousands of years of intricate processes of coevolution among them.

Rajkot, a district place of Gujarat state situated in the western part of India in no way different from other agriculturally important centers for the cultivation of crops.

Due to its fertile soil, plentiful water resources, and other features, cotton is cultivated here from time immemorial. Today, agricultural fields in Rajkot districts are facing tremendous stress with contamination of several agrochemicals of which OP compounds are most common along with heavy metals and others. This aspect has never been highlighted.

Several but minimal groups of researchers are working on OP compound degradation. This aspect is a very important and challenging scientific endeavor towards the development of suitable bioremediation approaches for the removal of profenofos compounds from their contaminated sites. Bioremediation aims to enhance the natural degradation potential of organisms indigenous to the environment (Chatterjee and Dutta, 2003; Mallick *et al*., 2007) [5, 11]. Profenofosfalls under the xenobiotic category and their degradation under natural conditions are not easy. Moreover, the aerobic or anaerobic degradation of profenofos results in metabolites, which are even more cytotoxic, carcinogenic, and mutagenic than the parental compound.

Currently, no data exist as to what extent these OP insecticides have accumulated in different agricultural niches of Rajkot and around and how they are destabilizing the ecological niches. Worldwide, considerable progress has been achieved in the area of PGPR biofertilizer technology. It has been demonstrated and proved that PGPR can be very effective and are potential microbes for enriching soil fertility and enhancing agriculture yields (Turner *et al*., 2013) [17]. PGPR are excellent model systems that can provide the biotechnologist with novel genetic constituents and bioactive chemicals having diverse uses in agriculture and environmental sustainability (Chaparro *et al*., 2014) [4]. Current and future progress in our understanding of plant growth-promoting bacterial diversity, colonization ability, mechanisms of action, formulation, and application could facilitate their development as reliable components in the management of sustainable agricultural systems.

Conclusion

We attempted to isolate, characterize and identify profenofos degrading bacterial isolates from three different agricultural fields where the cultivation of cotton crop was carried out. Initially, more than 15 isolates bacterial isolates were screened by enrichment culture technique. Later, 10 bacterial isolates were characterized based on growth characteristics, and Gram's reaction and 2 bacterial isolates were tentatively identified using VITEK 2 system. They were further screened based on their ability to grow in presence of profenofos as a sole source of carbon. Further analysis lead to the screening of three bacterial isolates (DN 4 & DN 5) which were able to grow in presence of as high as 1000 ppm profenofos.

We found *S. paucimobilis*DN-5 can degrade up to 99% of profenofos with the optimized nutritional and environmental conditions in a liquid medium within 30 days. By performing HPLC analysis new peaks were observed which indicate the generation of new metabolites.

We found that *S. paucimobilis*DN-5 shows plant growth-promoting activity. It solubilizes phosphate, produces IAA, and ammonia during profenofos degradation. This property of *S. paucimobilis*DN-5 can be exploited to raise the productivity of crops in pesticide-contaminated soil.

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