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LIST OF PUBLICATIONS

1) Participated in:

a) 2nd International Conference on Recent Advances in Biotechnology and Nanobiotechnology 2022 (Oral Presentation)

b) Participated in the National Conference on “Healthcare advancement by optimizing pharmaceutical and educational sector by PRISAL 2022 (oral presentation)

c) Participated in the International Conference on "Emerging Role of Pharmacist in Innovation, Discovery, and Development of Biological Products and Bio-Pharma," Gurukul Kangri Haridwar Uttarakhand (25th-27th September 2021) (Oral Presentation)

d) Participated in the International e-Conference on "Emerging Innovation and Advancement in Biological Science, Human Welfare, and Agriculture Research in the Current Era" (EIABSHWAR 2020) (Oral Presentation)

e) 3rd international conference on INCD 2018 (Poster presentation)

2) Paper published:

a) Rani A, Mathur A, Gupta S. Isolation and screening of actinobacterial isolates from chemical pesticides usage fields of the uttarakhand region. Journal of Advanced Scientific Research. 2022 Jul 1;13(6).

b) Rani A, Mathur A, Gupta S. Screening of PGPR traits and monocrotophos pesticide degradation properties of actinobacterial isolates. IJPSR, 2023; Vol 14(2): 731-739

c) Mathur A, Rani A, Gupta S. Isolation and Screening of Actinobacterial Isolates: Promising agents for Biodegradation of Monocrotophos pesticides. Zeichen, 2022; Vol. 8;593 -601

CERTIFICATES

Amity Institute of Biotechnology, Amity University Madhya Pradesh, Gwalior

CERTIFICATE

This certificate is awarded to Ms. Ankita Rani from HIHT University, Jolly Grant, Dehradun, Uttarakhand for her presentation of research paper titled Screening of actinobacterial isolates from soil samples of field areas of Uttarakhand region having prevalent usage of monocrotophos pesticides in the 2nd International Conference on Recent Advances in Biotechnology and Nanobiotechnology (Int-BIONANO-2022) during 10-11 February 2022.

Prof.(Dr.) Rajesh Singh Tomar
Convener

Lt. Gen. V. K. Sharma, AVSM (Retd.)
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Gurukula Kangri (Deemed to be University), Haridwar, Uttarakhand

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WORLD PHARMACISTS DAY

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OF APPRECIATION

This is to certify that **Ms. Ankita Rani** has participated as a **Delegate** and awarded for **Oral Presentation** on the topic of **Study of the bioformulation of actinobacterial isolates and their degradation ability of Monocrotophos pesticide concentration in soil samples** in the **Three-days International Conference on "Emerging Role of Pharmacist in Innovation, Discovery & Development of Biological Products & Bio- Pharmaceuticals"** held on **September 26** on the occasion of **World Pharmacist Day-2021** from September 25-27, 2021 organized by Department of Pharmaceutical Sciences FMSH, Gurukul Kangri (Deemed to University), Haridwar. We appreciate his/her remarkable contribution in this field. Gurukula family wish him/her all success in future endeavors.

Prof. S. K. Rajput	**Prof. R. C. Dubey**	**Prof. Roop Kishor Shastri**
Organizing Secretary	Chairman	Patron
Head, Dept. of Pharmaceutical Science	Dean, Faculty of Med. Science	Hon'ble Vice-Chancellor,
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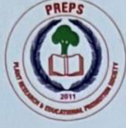

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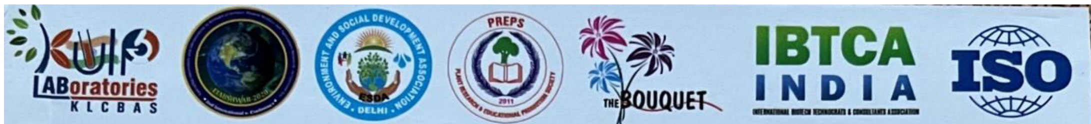

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To

Ms. Ankita Rani

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for her outstanding contribution in the field of

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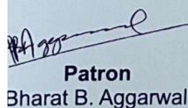
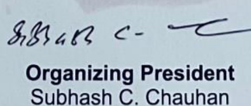
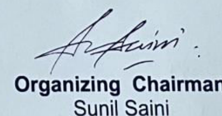

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Research papers

Short Communication

Isolation and Screening of Actinobacterial Isolates: Promising agents for Biodegradation of Monocrotophos pesticides

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Abstract: *Actinobacterial isolates are the effective microbes responsible for production of several metabolites and drugs. There are different remarkable properties of Actinobacteria for which these are explored and thus are the first choice of microbiologists and researchers working on natural and novel compounds. In the present study, actinobacterial isolates were isolated to explore the biodegradation properties for degradation of monocrotophos pesticides. In the present study, total 120 Soil samples were aseptically collected from different field regions of Uttarakhand viz. Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi and Haridwar where prevalent usage of monocrotophos pesticides was done. Amongst all the samples, 280 microbes were isolated; out of which 24 isolates of Actinobacteria (8.57 %) were isolated which belong to the genera viz. Micromonospora (65%), Actinomycetes (25%) and Streptomyces (10%) meant to be responsible for biodegradation of monocrotophos pesticides.*

Keywords: Chemical toxicity, Monocrotophos pesticides, organophosphate, biodegradation, bioremediation, actinobacterial isolates, pesticides degradation.

1. INTRODUCTION

The over usage of chemicals and pesticides toxicity have been a meager issue in agriculture and farming practices. These chemicals not only leave toxic residues in the soil but also enters in the food chain and ecosystem. Monocrotophos is poisonous organophosphates observed all cross the country and are widely used for agriculture. It is a direct acting cholinesterase inhibitor capable of penetration through the skin. Symptoms are similar to those of other organophosphate compounds but effect can be observed within minutes or in a day. Its cholinesterase inhibiting activity causes nervous system effects. Cases of human poisoning are characterized by muscular weakness, blurred vision, profuse perspiration, confusion, vomiting, pain, and small pupils. This may involve vomiting, diarrhea, nausea, headache, abdominal cramps etc. Severe poisoning due to monocrotophos causes cardiac arrest or respiratory failure which leads to death of person in the severe cases [1-5]. The two main Organizations related to health and agriculture (FAO) and WHO encouraged countries to list out pesticides having highly hazardous components. Many countries involved Australia, China, the European Union, Cambodia, Laos, Indonesia, Philippines, Vietnam Sri Lanka, Thailand; the United States of America banned the use of monocrotophos. To take off this from market urgent steps should be taken. Many developing countries of Asia also have banned the use of monocrotophos as it causes high health risks. India is very much familiar with the threats of pesticides. But in the fields of rural India, pesticides like monocrotophos is

continuously produced, used and exported in India. The reason behind this is that it is cheap and necessary for agricultural productivity [6-10].

2. MATERIALS AND METHODS

2.1 Collection and preparation of soil sample

In the present study, total of 120 Soil samples till date were aseptically collected from different field regions of Uttarakhand viz. Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi and Haridwar having dominant usage of monocrotophos pesticides. Soil samples (approx. 500 g) were collected using some clean, dry and sterile polythene bags along with sterile spatula, marking pen rubber band and other accessories. These samples were air-dried for 1 week, crushed and sieved. The sieved soils were then used for actinomycetes isolation as per the series wise methods as follows-

2.2 Isolation of Actinomycetes

About 5 grams of the soil were suspended in 50 ml of Normal saline (NaCl-0·85g/L). The soil suspension was incubated in an orbital shaker incubator at 28 ^{0}C with shaking at 200 rpm for 3 minutes. Actinomycetes were isolated by spread plate techniques following the serial dilution of soil in YIM6 Starch- casein medium.

Different salt mixtures viz. NaCl- 100-150 g; KCl- 20 g; $MgCl_2$- 30 g; $MgSO_4$- 5 g; K_2HPO_4- 1g; Starch- 20 g; Casein/milk powder- 10 g.

The pH of each of the above medium was maintained from 10-12. In each of the medium, nalidixic acid (25-50 g/liter) was added. Isolated plates were incubated at 28°C for 25-35 days for the observation of growth of Actinomycetes [11-15].

These are discussed below:

2.2.1 Aerial Mass Color

The colour of the mature sporulating aerial mycelium is recorded in an exceedingly straightforward method (White, grey, red, green, blue and violet). Once the aerial mass color falls between two colors series, both the colors are recorded. If the aerial mass color of a strain to be studied shows intermediate tints, then also, both the colors series are noted [16].

2.2.2 Melanoid Pigments

The grouping is formed on the assembly of melanoid pigments (*i.e.* light-green brown, brown black or distinct brown, pigment changed by alternative colors) on the medium. The strains are grouped as melanoid pigment created (+) and not created (-) [17].

2.2.3 Reverse Side Pigments

The strains were divided into two groups, consistent with their ability to provide characteristic pigments on the reverse aspect of the colony, namely, distinctive (+) and not distinctive or none (-). In case, a color with low saturation like yellowness, olive or yellowish brown occurs, it is included in the latter group (-) [18-20].

2.2.4 Soluble Pigments

The strains are divided into two groups by their ability to provide soluble pigments apart from melanin: particularly, produced (+) and not produced (-). The color is recorded (orange, red, green, violet, blue and yellow) [21-22].

2.2.5 Spore Chain Morphology

With relevancy to spore chains, the strains are sorted into "sections". The species belonging to the genus Streptomyces are divided into three sections (Shirling and Gottlieb 1966), particularly recti-flexibiles (RF), retina-culiaperti (RA) and spirales (S). Once a strain forms two types of spore chains, both are noted [23-25].

2.2.6 Reproductive Structure Surface

Spore morphology and its surface options ought to be determined under the scanning electron microscope. The cross hatched cultures arranged for observation under the light microscope can be used for this purpose. The electron grid ought to be cleaned and adhesive tape should be placed on the surface of the grid. The mature spores of the strain ought to be rigorously placed on the surface of the adhesive tape and gold coating should be applied for half an hour and also the specimen is examined under the electron microscope at completely different magnifications. The reproductive structure silhouettes are characterized as spiny, smooth, warty and hairy [26].

3. RESULTS AND DISCUSSION

In the study, field areas of Uttarakhand region (Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi and Haridwar) having dominant usage of monocrotophos pesticides. Amongst these samples, **total of 280 microbes were isolated;** out of which **24 isolates of Actinobacteria (8.57 %)** were isolated. The results are shown in **Table 1** and **Figure 1**. The actinobacteria isolates were screened on specific agar media and characterized by morphological colonies appearance and staining procedures. The actinobacteria isolates were categorized on the basis of a) type of pigment production **(Table 2)** and colony and color **(Table 3) and Figure 2.** These actinobacterial isolates were further screened for their identification by molecular. The results revealed the strains of the genera viz. Micromonospora (65%), Actinomycetes (25%) and Streptomyces (10%). The actinobacterial isolates were found in high density in the soil enriched with monocrotophos pesticides [27-28].

4. CONCLUSION

The study revealed that, actinobacterial isolates density gets increased as per the availability and accumulation of pesticides in the soil. The studies revealed that, actinobacterial isolates can be utilized for the biodegradation of pesticides. The studies are however required in order to isolate and explore such microbial strains for biodegradation of monocrotophos pesticides.

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Table 1: Actinomycetes isolates (percent diversity) on YIM6 starch- casein agar medium

Soil sample	Total no. of microbes isolated	Actinobacteria isolates	Percent diversity of microbes isolated	Percent diversity of actinobacteria isolated
120	256	24	91.42	8.57

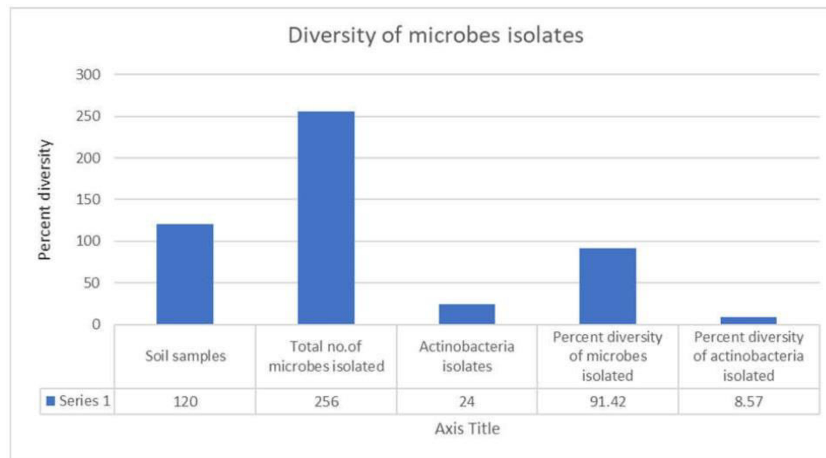

Figure 1: Actinomycetes isolates (percent diversity) on YIM6 starch- casein agar medium

Table 2: Characterization of isolatedActinobacterial strains on the basis of pigment production

s.no.	Strain code	Pigment production		
		Melanoid pigment	Reverse side pigment	Soluble pigment
1	ASUK03	+	+	+
2	ASUK07	-	+	+
3	ASUK254	-	+	+
4	ASUK145	+	+	+
5	ASUK67	+	+	+
6	ASUK86	+	+	+
7	ASUK46	+	+	+
8	ASUK34	+	+	+
9	ASUK23	+	+	+
10	ASUK60	+	+	+
11	ASUK79	+	+	+
12	ASUK224	-	+	+
13	ASUK185	-	+	+
14	ASUK145	-	+	+
15	ASUK76	-	+	+
16	ASUK216	-	+	+
17	ASUK237	-	+	+
18	ASUK259	-	+	+
19	ASUK263	-	+	+
20	ASUK283	+	+	+
21	ASUK292	+	+	+
22	ASUK308	-	+	+
23	ASUK315	+	+	+
24	ASUK423	-	+	+

***+, Presence -, Absence**

Table 3: Screening of isolated actinobacterial strains on the basis of color of pigment, mycelium and appearance of colony and identified genera

S.no.	Strain code	Pigment color/mycelium/appearance of colony		
		Color of pigment	**Mycelium**	**Appearance of colony**
1	ASUK03	Yellow	Rough	Dirty based
2	ASUK07	Whitish yellow	Smooth	Round
3	ASUK254	Whitish green	Rough	Thick
4	ASUK145	White	Hairy	Thread like
5	ASUK67	Whitish pink	Branched	Wrinkled
6	ASUK86	Yellowish pink	Branched	Wrinkled
7	ASUK46	Whitish creamy	Branched	Wrinkled
8	ASUK34	Yellowish creamy	Branched	Smooth
9	ASUK23	Whitish concave	Spherical	Smooth
10	ASUK60	White cotton	Spherical	Smooth
11	ASUK79	Whitish	Spreader	Flattened
12	ASUK224	Whitish thread	Branched	Flattened
13	ASUK185	Whitish point	Aerial	Smooth
14	ASUK145	Whitish cotton like	Branched	Smooth
15	ASUK76	Purple spreader	Granular	Wrinkled
16	ASUK216	Whitish yellow cotton like growth	Rough	Flattened
17	ASUK237	Whitish cotton	Spherical	Smooth
18	ASUK259	Whitish scanty	Smooth	Smooth
19	ASUK263	Pinkish white	Flattened and spherical	Wrinkled
20	ASUK283	Whitish spreader	flattened	Wrinkled
21	ASUK292	Whitish yellow spreader	flattened	Wrinkled
22	ASUK308	Yellowish white spreader	flattened	Wrinkled
23	ASUK315	Whitish spreader	flattened	Wrinkled
24	ASUK423	Whitish brown spreader	flattened	Wrinkled

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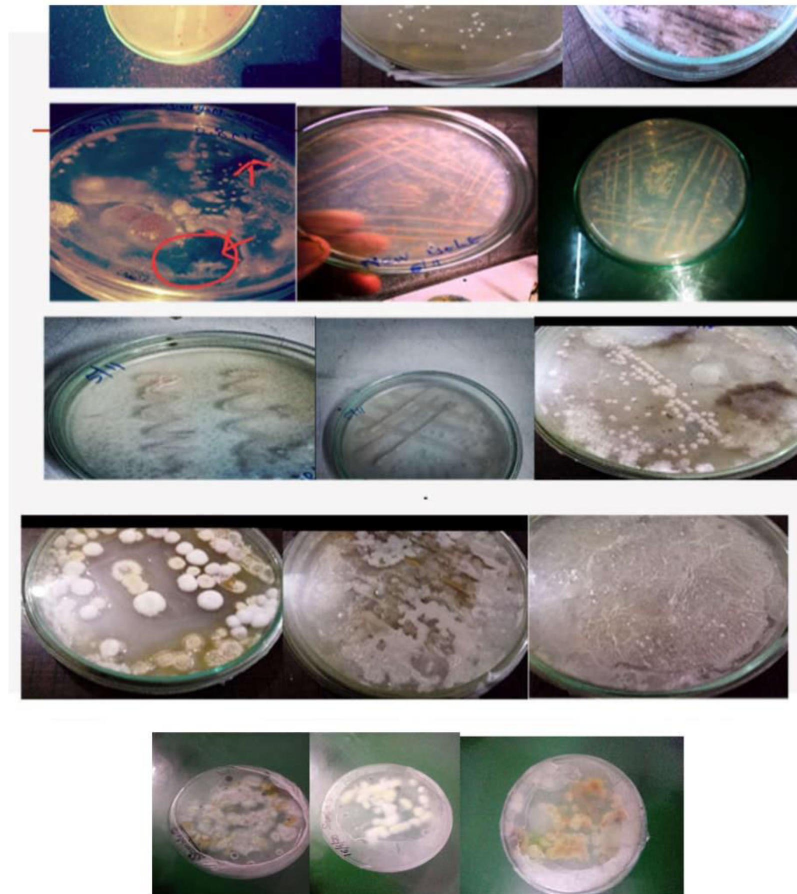

Figure 2: Actinobacterial isolates as isolated on Y1M6 starch- casein agar medium (1-24) *
each plate showing single isolate; Numbering from stain 1 to strain 24*.**

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SCREENING OF PGPR TRAITS AND MONOCROTOPHOS PESTICIDE DEGRADATION PROPERTIES OF ACTINOBACTERIAL ISOLATES

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Keywords:

Actinobacterial isolates, PGPR traits, Monocrotophos pesticides, Bioremediation, Rhizoremediation, Reduction in soil toxicity, Recombinant cells, Least toxic pesticide derivatives

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ABSTRACT: Bioremediation of organic compounds and heavy metals has been recognized to be a successful and effective technique in rhizoremediation of soil toxicity using the beneficial microbes which produce different metabolites and enzymes to degrade the toxic compounds/ pesticides available in the soil. In the present study, actinobacterial isolates were explored and identified for bioremediation of soil toxicity available in the soil. The actinobacterial isolates were utilized for the promising PGPR traits and ability to degrade the monocrotophos pesticide residues available in the soil. The "*opd*" gene of interest responsible for monocrotophos pesticide degradation trait was isolated and cloned in vector DNA to produce recombinant DNA. The recombinant DNA was transformed in *E. coli* cells to produce multiple copies in E. coli cells resulting in recombinant (transformed) and non-recombinant (non-transformed) colonies. The transformed *E. coli* cells were inoculated in nutrient broth having pesticide concentration. The transformed cells degraded the pesticide, and the HPLC method determined the derivatives produced. The formulations based on actinobacterial isolates were tested, and field applications were done to determine the reduced soil toxicity, if any, observed.

INTRODUCTION: Bioremediation is also used to convert hazardous substances into less toxic components. Microorganisms have a vital role in the breakdown and mineralization of these contaminants. Bioremediation as sustainable technology is significant in examining and reducing manmade chemicals released into the environment. Bioremediation is the microorganisms used to attain the function of bioremediation. Bioremediation technology has been commonly categorized as *ex-situ* and *in-situ* bioremediation.

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In situ bioremediation comprises the treatment and elimination of the contaminated material at the site, whereas, in *ex-situ*, the contaminated are treated somewhere else. bioventing, bioleaching, biostimulation, land farming, composting, bioaugmentation, rhizofilteration, and phytoremediation are a few examples of bioremediation technologies [1-5]. Biodegradation and bioremediation are similar methods up to an extent since both of these approaches employ microorganisms for the alteration or breakdown of pesticides.

The only difference between these two is that biodegradation is a natural process whereas bioremediation is considered technology. Various aspects are limiting factors for pesticide degrading microorganisms, such as pH, temperature, nutrients, water potential and a number of

metabolites or pesticide content in soil [6-8]. Actinobacterial isolates produce non-antibiotic molecules which exhibit bioactivities, such as immuno-suppressors, phytotoxins, bio-pesticides, Nano-particles, probiotics, enzyme inhibitors and different enzymes and proteins that are involved in the degradation of complex polymers and biomolecules [9-12], that single isolates can mineralize a limited number of these xenobiotic pesticides. Still, consortia of bacteria are mostly required for complete degradation of the process. Members of this group of gram-positive bacteria have been found to degrade pesticides with widely different chemical structures, including organochlorines, *s*-triazines, triazinones, carbamates, organophosphates, organ phosphonates, acetanilides and sulfonylureas. Single isolates can mineralize a limited number of these xenobiotic pesticides, but consortia of bacteria are often required for complete degradation [13-15].

MATERIALS AND METHODS:

Screening of Isolates for PGPR Traits: The actinobacterial isolates were screened for PGPR traits [16-20].

A. IAA Production: Indole acetic acid (IAA) production was quantitatively estimated by Salkowski method. The actinobacterial cultures were grown on Luria broth liquid medium at 36±2 °C. Fifty milliliter of Luria Bertani (LB) broth containing 0.1% DL tryptophan were inoculated with 500 µl of 48 h old actinobacterial cultures and incubated in refrigerated incubator shaker at 30±0.1°C at 180 rpm for 48 h in dark. Fully grown bacterial cultures were centrifuged at 10,000 rpm for 10 minutes at 4°C. Estimation of IAA production in the supernatants was done using a colorimetric assay. One milliliter (1 ml) of supernatant was mixed with 100 ml of 10 mM orthophosphoric acid and 2 ml of the Salkowski reagent (1 ml of 0.5 M FeCl3 in 50 ml of 35% HClO4) at 28±2 °C for 30 minutes. The development of pink colour in test tubes at the end of the incubation indicated IAA production. The pink colour absorbance measured the quantification of IAA at 530 nm after 30 minutes in UV/VIS spectrophotometer. The results were tabulated as Higher IAA producers (+++); Medium IAA producers (++), and Lowest IAA producers (+).

B. Phosphate Solubilization: A loop full of isolated pure fresh actinobacterial cultures was streaked on the Centre of agar plates modified with Pikovskaya agar with insoluble tricalcium phosphate (TCP) and incubated for 120 h at 28±2°C 18. The halo zone around the bacterial colonies indicated positive phosphate solubilization ability (Pikovskaya, 1948). The results were tabulated as Higher phosphate solubilizers (+++); Medium phosphate solubilizers (++), and Lowest phosphate solubilizers (+).

C. Siderophores and HCN Production: Qualitative estimation of siderophore production by the actinobacteria isolates was determined by adopting the modified chrome azurol sulphonate (CAS) assay method. Production of siderophore was determined by developing an orange halo zone around the actinobacterial colonies. In addition, all the bacterial isolates were screened for HCN production. The Colour change of the filter paper from deep yellow to reddish-brown colour indicated the production of HCN. The results were tabulated as Higher Siderophores and HCN producers (+++); Medium Siderophores and HCN producers (++) and Lower Siderophores and HCN producers (+).

D. Catalase Activity: Actinobacterial cultures were grown in a nutrient agar medium for 48 h at 28°C. The 48-hour-old bacterial colonies were added with 2-3 drops of hydrogen peroxide (3%) on a clean glass slide and mixed using a sterile toothpick. Oxygen evolution as effervescence indicated catalase activity (Rorth and Jensen, 1967). The results were tabulated as Higher Catalase producers (+++); Medium Catalase producers (++), and Lower Catalase producers (+).

Screening of Actinobacterial Isolates for Monocrotophos Pesticides Degradation:

Primary Screening:

Determination of Zone of Clearance of Pesticide Degradation: The screening of actinobacterial isolates for monocrotophos pesticide degradation was performed as per the method described. The growth of isolated actinobacterial cultures were inoculated separately in 100 ml Mineral Salt Media (MSM) enriched with an addition of 25 ppm MCP pesticide. These samples were incubated on a rotary shaker (150 rpm) at 30°C for 7 days. The

growth curve for the actinobacterial isolates was determined via optical density/absorbance using UV- spectrophotometer at various time intervals. After which one, the cultures were frequently transferred every 3-4 days or until increased turbidity was evidenced.

After 3-4 times of repeated sub-culturing, 0.1 ml culture broth was pipette and introduced in wells punctured within pesticide agar plates. Cultures were incubated at 30°C for 5-8 days. Pesticide degrading isolates were then screened, which developed a clear zone around the wells determining clearance [21].

Secondary Screening:

Determination of Pesticide Degradation by Culture Streak Technique: The pesticide degradation was observed on pesticide-enriched agar by streaking the actinobacterial cultures; the pesticide degradation ability of the actinobacterial isolates was determined as a clearance zone.

Expression of Genes of Promising Isolates of Actinobacteria in *E. coli* Cells: *Escherichia coli* was utilized as one of the organisms of choice to produce recombinant proteins. Its use as a cell factory is well-established and it has become the most popular expression platform. For this reason, there are many molecular tools and protocols for the high-level production of multiple functional proteins. In the present investigation, the genes for monocrotophos pesticide (MCP) degradation were isolated from promising actinobacterial isolates, fused with vector DNA and transformed into E. coli cells. The recombinant *E. coli* cells were further utilized for the biodegradation of monocrotophos pesticides (MCP). The enzymes for degradation of monocrotophos pesticides were identified as phosphodiesterase (PTE) or organophosphorus hydrolase (OPH) specifically encoded by the opd gene. This gene has been Expression in genes of promising isolates of Actinobacteria in *E. coli* cells.

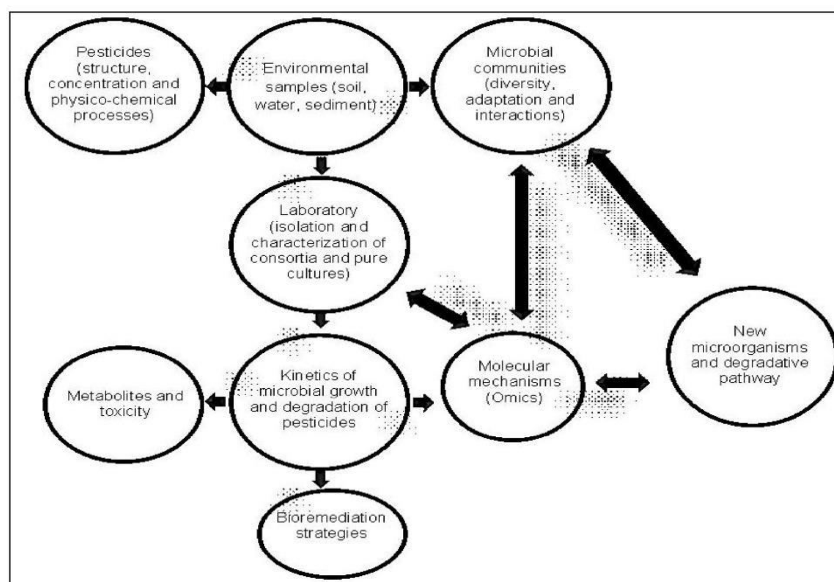


IMAGE 1: REPRESENTATION OF THE RELATIONSHIPS BETWEEN PESTICIDES, MICROBIAL COMMUNITIES AND THE DISCOVERY OF NEW BIODEGRADATION PROCESSES OMICS = HIGH THROUGHPUT-BASED CHARACTERIZATION OF BIOMOLECULES CHARACTERISTIC OF BIOPROCESSES; DNA, GENOMICS; MRNA, TRANSCRIPTOMICS; PROTEIN, PROTEOMICS; METABOLITES, METABOLOMICS

Biodegradation of Monocrotophos Pesticides: The concentrations of monocrotophos pesticide (MCP) at 10 µg/ml were utilized in nutrient broth in different sets, followed by sterilization and inoculation of the E. coli cells and incubation of the broth for 48 hours duration. The degradation of MCP pesticide in the broth culture was determined using TLC and HPLC, as described by Chao *et al.*, 2009. In brief, 4 mL of filtrate was extracted with ethyl acetate from a 5-mL aliquot of culture supernatant. All experimental MCP concentrations were determined at 276 nm by HPLC (PerkinElmer 200 series, CT, USA). The solution filtrate (10 µL) was separated and added with methanol (1:1). Ten, it went across 0.45- µm PVDF filter and injected into the HPLC column (C18, 250×4.6 mm, 5µm, Phenomenex, CA, USA). The 10 µL filtered sample was injected into the HPLC column, and the 0.5% acetic acid and methanol (1:4 v/v) were used as eluent at 1 mL/min flow rate.

Formulations of Actinobacterial Strains: The formulations were prepared as per the following modified methods [22].

1. Alginate-Kaolin Based Granular Formulation (AL-KAO Granular Formulation): Sodium alginate solution was prepared by dissolving 20 g of dry sodium alginate in a minimum volume (10 ml) of sterile distilled water. This mixture was poured into 1 L of swirling, warm, distilled water and allowed to mix on the stirrer for 30 min until a homogeneous suspension was obtained. The alginate solution was sterilized for 15 min at 121 °C and 101 kPa. Twenty grams of the potent dried Actinobacterial isolate propagules were mixed thoroughly with 20 g of previously sterilized kaolin (aluminum silicate), and the mixture was added in small portions (2 g) into 1 L of swirling, sterile distilled water supplemented with 4 drops of Tween 20. The alginate-kaolin-actinobacterial mixture was allowed to swirl in the stirrer until ready for mixing with the sodium alginate solution. A droplet-forming device was constructed by attaching a 1-L reagent bottle with a spout at the bottom to a T-valve outlet system. The entire device was sterilized for 15 min at 121 °C and 101 kPa before use. The sodium alginate solution and kaolin mixture were added to the reagent bottle in 1:1 ratio and stirred continuously. At the same time, the suspension was allowed to drip through an Eppendorf pipette tip, attached to the T- 10 valve, into a sterile solution of 0.1 M $CaCl_2$. The resulting alginate-kaolin beads were then allowed to stand in a fresh 0.1 M $CaCl_2$ solution for 30 min, filtered through a sterile cheese cloth, and washed at least three times with sterile distilled water. The beads were lyophilized at -70 °C, and their dry weight was recorded. This lyophilized granular formulation of mycobacterium (hereafter referred to as beads) was stored in the dark at 4 °C.

2. Flour-Kaolin Based Granular Formulation (FLO-KAO Granular Formulation): The flour and kaolin material were sterilized separately for 15 min at 121 °C and 101 kPa. Upon cooling, 200g of flour, 4 g of kaolin, and 20 g of the inoculum were mixed thoroughly and sieved through a sterile 250 µm pore screen to obtain a homogeneous mixture of the three components. To this mixture, 180 ml sterile distilled water supplemented with 2 drops of Tween 20 was added slowly and mixed thoroughly until a dough was formed. The flakes were lyophilized at -70°C. The lyophilized formulations in liquid hereafter referred to as granules) were stored in the dark at 4°C.

3. Calcium carbonate-CMC Based Powder Formulation (CC-CMC Powder Formulation): The calcium carbonate and CMC material was sterilized separately for 15 min at 121 °C and 101 kPa. Upon cooling, CMC was mixed with 20 g of the inoculum thoroughly and sieved through a sterile 250 µm pore screen to obtain a homogeneous mixture. For this mixture (10 Kg) produced with CMC, the quantity of calcium carbonate was mixed in 90 Kg concentration. The lyophilized formulations in liquid, referred to as powder, were stored in the dark at 4 °C. The material was further utilized to check the shelf life of isolates and the application of powder in the bioremediation of soil toxicity.

RESULTS AND DISCUSSION: As per the studies performed, 120 soil samples were collected from different field areas of the Uttarakhand region (Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi, and Haridwar) having dominant usage of monocrotophos pesticides. Amongst these samples, a total of 280 microbes was isolated; out of which 24 isolates of Actinobacteria (8.57%) were isolated. The actinobacteria isolates were screened on

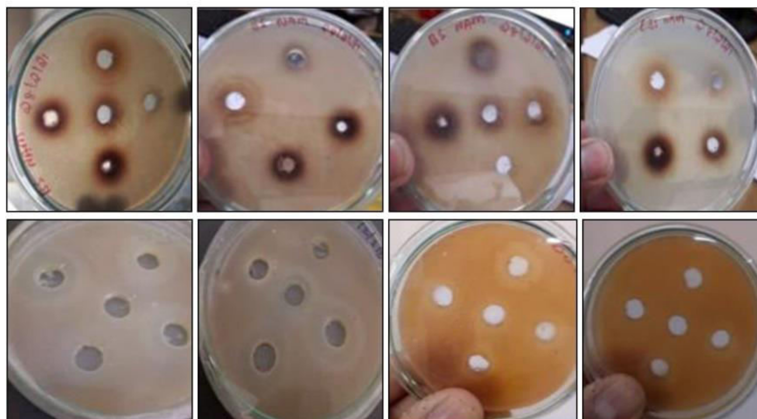


FIG. 2: PRIMARY SCREENING: DETERMINATION OF ZONE OF CLEARANCE OF PESTICIDE DEGRADATION

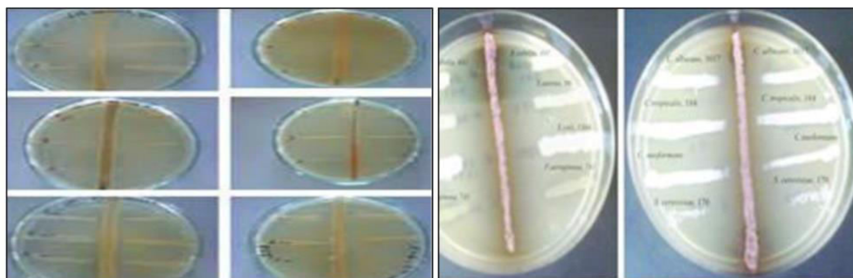


FIG. 3: SECONDARY SCREENING- DETERMINATION OF PESTICIDE DEGRADATION BY CULTURE STREAK TECHNIQUE

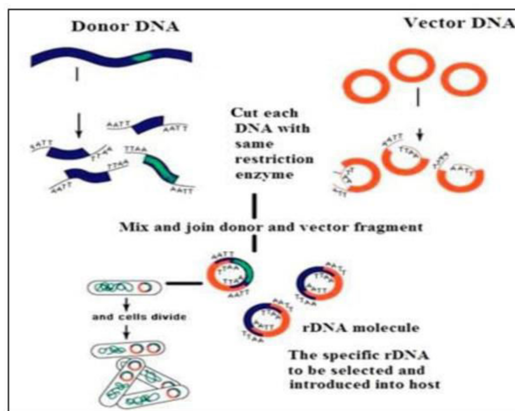
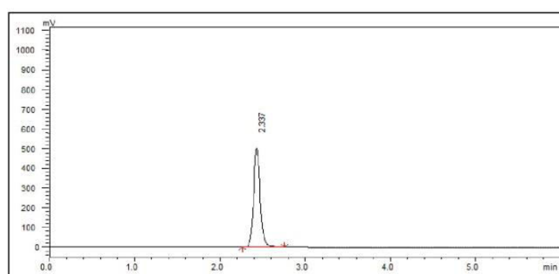


FIG. 4(A): PREPARATION OF RECOMBINANT DNA AND TRANSFORMATION IN E. COLI CELLS

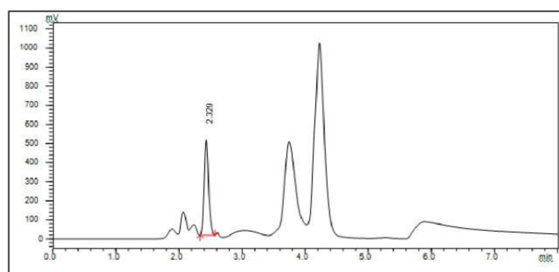
FIG. 4(B): SCREENING OF RECOMBINANT AND NON- RECOMBINANT COLONIES (BLUE COLONIES WERE REGARDED AS NON-RECOMBINANT COLONIES WHILE WHITE COLONIES WERE REPRESENTED AS RECOMBINANT COLONIES)



DETECTOR A CH1 276NM

Peak #	Name	Ret. Time	Area	Height	Area %	Height %
1	RT2.337	2.337	2732519	505147	100.0000	100.0000
Total			2732519	505147	100.0000	100.0000

FIG. 5(A): HPLC CHROMATOGRAM OF ORIGINAL MCP AVAILABLE IN NUTRIENT BROTH



DETECTOR A CH1 276NM

Peak #	Name	Ret. Time	Area	Height	Area %	Height %
1	RT2.329	2.329	2452373	500142	100.0000	100.0000
Total			2452373	500142	100.0000	100.0000

FIG. 5(B): HPLC CHROMATOGRAM OF DEGRADATIVE MCP RESIDUES AVAILABLE IN NUTRIENT BROTH AFTER BIODEGRADATION

The transformed cells degraded the pesticide and derivatives produced were determined by HPLC method **Fig. 5A, Fig. 5B.** Three formulations of actinobacterial strain *viz.,* Alginate-Kaolin Based Granular Formulation (AL-KAO granular formulation), Flour-Kaolin Based Granular Formulation (FLO-KAO granular formulation), Calcium carbonate-CMC Based Powder Formulation (CC-CMC powder formulation) were prepared and utilized in field trials to study the