Journal of Advanced Scientific Research

Available online through https://sciensage.info

ISSN 0976-9595

Research Article

BIODEGRADATION OF PESTICIDES THROUGH METAGENOMIC APPROACHES

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Received: 12-08-2021; Revised: 06-2-2022; Accepted: 14-02-2022; Published: 31-03-2022

 https://doi.org/10.55218/JASR.202213205

ABSTRACT

The idea behind the work is to check for the residence of pesticide degrading genes in plasmids and to transform them on an expression vector allowing them to degrade the pesticide more efficiently. Seven different commercially used toxic pesticides were used for bioremediation study. Around 20 organisms were first isolated and based on their pesticide tolerance, 11 organisms were further used for analysis. Each of the organisms was first subjected to plasmid curing and confirmed for their ability of pesticide degradation ability on plasmids. From the isolated 6 organisms based on their efficiency, 3 isolates containing plasmids were further chosen. Their plasmids were extracted and transformed to *E.coli* DHα. Upon subjecting them to agarose gel electrophoresis the result implies the successful transformation of only two plasmid DNA to the host cell. The pesticide degradation rate of the genetically modified strain was found to be higher than that of the wild type adapted strain. Morphometeric and germinating ability of *Venia radiata* was tested in pesticide contaminated soil and was found to support the growth of the plant.

Keywords: Metagenomic approaches, Morphogenetic analysis, Pesticide degradation, Plasmids and pesticides, Transformation.

1. INTRODUCTION

Pesticides are manmade chemicals that are said to be developed as a part of pests treatment involved in reducing the agricultural revenues. Pesticides are necessary to protect crops and losses that may amount to about 45% of total food production worldwide. It is also normal that pests do develop natural resistance against these pesticides over time. Since, these pesticides are said to harbour aromatic rings, they are carcinogenic in nature. When such carcinogenic chemicals are said to be accumulated in the agricultural environment there are all possible probabilities of them reaching the human system, where it is capable of having very serious catastrophe. Apart from being carcinogenic, the organo-pesticides are found to be inhibiting acetylcholinestrase enzyme thereby interfering with the transmission of nerve impulses. Such is the impact of the pesticides has become life threatening with regard to the current situation. Without any proper awareness, the farmers have started over exploiting the usage of these pesticides without any quanta limit. This has led to the spread and accumulation of pesticides from the agricultural land to the food products developed, percolated water reaching the water table along with the pesticides wash water from the agricultural land reaching the natural water systems etc. The excessive use of pesticides leads to uptake and accumulation of toxic compounds in the food chain and drinking water, which negatively impacts the current and future generations [1]. Dichlorodiphenyltrichloroethane (DDT) is one such chemical pesticide that is banned over many countries due to its after effects. In order to curb the menacing effects of these chemical pesticides, it is highly necessary that it has to be remediated in with immediate effect, also ensuring the process to be a safe entity. Effective bacterial strain can easily degrade largest pollutants [2]. Microbial bioremediation is said to be an effective procedure that could put an end to this pollution that is ubiquitous in nature (by travelling through the food

chain). It should be noted that utilizing pesticide as a part of its food chain has been evolved of late for all the microorganisms. Also these evolving genes are a part of the plasmid that these organisms harbour. It is evident that the evolution and spread of these pesticide-degrading plasmids play a major role in the widespread occurrence of microbial populations capable of the degradation of these potential environmental pollutants. Well-known genetic and biophysical properties of pesticide degrading plasmids would enhance the bioremediation process. For the pesticide part or the entire degradative pathway is carried on conjugative plasmids (designated TFD plasmids) [3]. Soil isolated bacterias are having the capacity to degrade pesticides [4]. Studies have shown that pesticide-degrading bacteria applied as consortia can increase degradation of Pesticide [1]. With most the genes requisite for the mineralization of pesticides residing within the plasmid, it provides a unique opportunity in building up a genetic pattern in a wild type microorganism capable of triggering an effective response with enhanced activity through the process of transformation. This is because of the fact that there are genes, could end up enhancing the pesticides reducing capability of the modified organism. This gives us an opportunity in dealing with different classes of pesticides at the same time. With the background information the broad objectives of the work carried out has been elaborated. The specific objectives that are deployed in the work are the bioremediation of textile effluents to select microbes and enhance its potential through transformation of plasmids that have effective in reducing pesticides found in agricultural land. The work also analyses the probability of the degraded products and its effect on the plant growth.

2. MATERIAL AND METHODS

2.1. Pesticides used

The commercial grade pesticides namely Choloro-pyrifos, Cypermethrin Glyphosphate, Diammonium phosphate, Dimethoate, Hycrofos, and Imidacloprid were procured and used for the study. The soil used for the enrichment and isolation of pesticide degrading bacteria was obtained from the six different agricultural lands located in and around Erode.

2.2. Isolation of pesticide degrading organism

About 1 gm of each soil sample collected was added into a 250 ml flask containing 30 ml of sterile liquid Minimal Salt Medium (MSM) along with 10ppm of each pesticide used for the study (Choloropyrifos, Cypermethrin, Glyphosphate, Diammonium phosphate, Hycrofos, Dimethoate, Imidacloprid).

Minimal Salt Medium: The media had the following composition in (g/L): KH_2PO_4- 4.8g; K_2HPO_4 - 1.2g; NH_4NO_3- 1.0g ; $MgSO_4.7H_2O$ -0.2g; Ca $(NO_3)_2.4H_2O$- 0.04g; and Fe $(SO_4)_3$- 0.001g with pH 7.0 and incubated at 37°C for 7 days in orbital shaker [2].

2.3. Enrichment technique

From every flask, 1 ml of the above sample was inoculated into 30ml of sterile liquid Mineral Salt Medium (MSM) with 10ppm of corresponding pesticide and incubated at 28±2°C for 7days (1st enrichment). From every flask, 5ml was re-inoculated to the flask with same medium composition aseptically and further incubated at 28°C for 7 days on shaking conditions (2nd enrichment). Two stages of enrichment were done mainly to provide every possible opportunity for all the minority groups of microbes or in general the metagenomic microbes to flourish in the ambience and to select the most efficient strain for the study. From every flask, a loop full of culture was streaked on Minimal agar plate and the plates were incubated 28°C for 48 hours to get isolated colonies of bacteria capable for tolerating the pesticide. The isolated colonies were grown on sterile nutrient agar slants as pure cultures and maintained as stock cultures. Based on morphology and staining, the colony characteristics were identified [2].

2.4. Secondary Screening for bacterial strains capable of tolerating a wide range of pesticides

The isolated organisms were inoculated in nutrient agar plates containing 1000 ppm of all the seven different pesticides and incubated at 37°C for 24 hours. The microorganism capable of tolerating all the pesticides was further identified and used for the study. The organisms thus selected were identified further through basic microscopic and biochemical tests.

2.5. Analysis of pesticide degradation and estimation of released phosphate by Fiske Subbarow method

Principle of Fiske Subbarow method is that phosphate is converted into phosphomolybdic acid in 0.5 M sulfuric acid and reduced with l-amino-2-naphthol-4-sulfonic acid. The total organically bound phosphates sample must be brought into inorganic form. This is usually accomplished by wet incineration in the presence of a strong acid and an oxidizing agent. The presence of

inorganic phosphorous is directly proportional to the degradation of chemical pesticides. The following table gives you the standard chart for the phosphate released, which could be used for further analysis. In a series of test tubes working standard was pipetted out ranging from 1 to 10ml. The volume of each tube was made upto 10ml using distilled water. To each tube, 1ml of ammonium molybdate and 0.5 ml of 1, 2, 4-amino naphtholsulphonic acid was added. The mixture was thoroughly mixed and allowed to stand for 10 minutes and the absorbance for each solution was determined using spectrophotometer at 690 nm (Table 1).

Table 1: Preparation of Standards for Fiske Subbarow Method

S. No	Volume of working standard(ml)	Concentration of working standard (µg)	Volume of distilled water (ml)	Reagents	OD at 690nm
Blank	-	-	10	1ml of ammonium molybdate + 0.4 ml ANSA	0.0
1	1.0	8	9	1ml of ammonium molybdate + 0.4 ml ANSA	0.140
2	2.0	16	8	1ml of ammonium molybdate + 0.4 ml ANSA	0.230
3	3.0	24	7	1ml of ammonium molybdate + 0.4 ml ANSA	0.270
4	4.0	32	6	1ml of ammonium molybdate + 0.4 ml ANSA	0.420
5	5.0	40	5	1ml of ammonium molybdate + 0.4 ml ANSA	0.580
6	6.0	48	4	1ml of ammonium molybdate + 0.4 ml ANSA	0.640
7	7.0	56	3	1ml of ammonium molybdate + 0.4 ml ANSA	0.720
8	8.0	64	2	1ml of ammonium molybdate + 0.4 ml ANSA	0.824
9	9.0	72	1	1ml of ammonium molybdate + 0.4 ml ANSA	0.890
10	1.0	90	0	1ml of ammonium molybdate + 0.4 ml ANSA	0.980

2.6. Preparation of sample

The analysis of phosphorus in form of inorganic phosphate (PO_4^{3-}) was carried out on UV-Visible spectrophotometer (ELICO SL 244) at 690nm, using Fiske-Subbarow method. For analysis of pesticide degradation, the isolated microorganism was incubated with suitable pesticide at 37°C for 4 days on a shaker at 200rpm. Control was maintained without inoculums. Trichloro acetic acid [5%(wt/vol), 9.50ml] was placed in a centrifuge tube with addition of 1.0 cm^3 of filtrate of broth culture medium. The mixture was stirred using stirring rod and was allowed to stand for 5minutes. The mixture was centrifuged for 5 minutes at 1500 rpm. The clear supernatant (5.0 ml) was transferred into a test tube, followed by addition of 1.0 ml of ammonium molybdate and 0.5 ml of 1,2,4-aminonaphthol sulphonic acid. The mixture was thoroughly mixed and allowed to stand for 10 minutes. The procedure was repeated for filtrates. The absorbance for each solution was measured at 690nm against distilled water. Analyses were carried out in duplicates. Spectrophotometric analyses of filtrate of broth culture medium and control were also carried out. The O.D values were measured and compared with the standard graph [5].

2.7. Isolation of plasmid DNA by alkaline lysis method

About 5 ml Leuria Bertani medium containing proper antibiotics were inoculated with a single bacterial colony. The tube was incubated at 37°C overnight with vigorous shaking at 360 rpm. Bacterial pellet was taken from the culture which was centrifuged at 10,000 rpm for 5 minutes at room temperature. Supernatant was discarded. Bacterial pellet was resuspended in a 1 ml ice-cooled solution I (50 mm) and vortexed. To this, added about 2 ml of 0.2 N NaOH/1.0% SDS to the suspension at room temperature, mixed thoroughly by repeated gentle inversion. 1.5 ml ice-cold Solution III was added to the lysate and mixed thoroughly by repeated gentle inversion. The mixture was centrifuged at 15,500 rpm for 30 minutes at 4°C and the supernatant were recovered. 2.5 volume isopropanol was added to the precipitate and mixed thoroughly by repeated gentle inversion, centrifuged at 15,500 rpm for 30 minutes at 4°C, and the resulting supernatant was removed. The pellet obtained was plasmid DNA. The pellet was rinsed in ice-cold 70% ethanol and allowed to air-dry for 10 minutes to evaporate ethanol. 10-20 µl of TE was added to dissolve the pellet. After addition of 2µl RNase (10mg/ml), the mixture was incubated for 20 minutes at room temperature to remove RNA.

2.7.1. Reagents

Solution I (Lysis buffer I) was prepared by adding 50 mM glucose, 10 mM EDTA pH 8.8 and 25 mM Tris HCl to 85.5 ml H_2O. It was autoclaved at 121°C for 15 minutes and store at 4°C.

Solution II (Lysis buffer II) was prepared by adding 0.2

N NaOH, 1% SDS, to 100ml distilled water and stored at room temperature.

Isopropanol: Stored at -20.0°C

Solution III (Lysis buffer III) was prepared by dissolving 3M potassium acetate pH 6.0, 11.5 ml glacial acetate to 28.5 ml H_2O.

TE Buffer recipe comprised of 10mM Tris pH 8.0, 1mM EDTA

2.8. Analysis of Presence of Plasmid from Isolated Bacterial Strains through Agarose Gel Electrophoresis

The sample isolated through the extraction procedures was loaded along with loading dye, and marker on the first lane. A constant electric current of 50V/cm was applied and mobility of the dye was tracked. The gel was stained 2 to 5 minutes with ethidium bromide and then de-stained with sterile water. The gel was placed on an UV transilluminator and the bands were observed to check for the presence of plasmid in the isolated bacterial strain.

2.9. Transformation of Plasmid to a Versatile Bacterial Strain

An overnight *E. coli* DHα cells culture was taken. The culture was transformed to centrifuge tubes and kept in ice for about 20 minutes; it was then centrifuged at 4°C for 10 minutes (3000 rpm). The supernatant was discarded and 20ml cold 0.1M $CaCl_2$ was added to the pellet, kept in ice for about 30 minutes. The solution was centrifuged at 4°C for about 10 minutes (3000 rpm), the supernatant was discarded. The prepared competent cells were transferred to an eppendroff tube and were stored at 4°C.

The competent DH5a cells were thawed on ice. The cells were mixed gently with the pipette tip and aliquot of 50µl of cells for each transformation were added to 1.5ml tubes that have been pre-chilled on ice. About 1-5 µl DNA was added to the cells and was mixed gently and the tubes were incubated on ice for 30 minutes. Heat shock was applied at 42°C for exactly 30 seconds without shaking and then tubes were placed on ice for 2 minutes. About 250 µl of pre-warmed LB was added and kept on shaker at 37°C for 1 hour. After incubation, about 20 µl of each transformed strain was spread on LB plates with appropriate antibiotic and incubated at 37°C. An agarose electrophoresis was run to check for transformation.

2.10. Analysis of Pesticide Degradation through Pot study experiments

Biodegradation of pesticide was also studied by pot culture experiment. The effect of pesticide was analyzed in *Vigna radiata*. The pesticide contaminated soil was taken and autoclaved at 121°C for 15 minutes. The following three treatment procedures were carried out.

2.10.1. Trial I

Sterile soil in which seeds were sown and plant growth was determined.

2.10.2. Trial II

To the sterile pesticide contaminated soil, 10ppm of each of the individual pesticide (chlorpyrifos, cypermethrin, glyphosphate, di ammonium phosphate, dimethoate, Hycrofos, Iminocloprid) was incorporated and seeds were sown, the germination percentage and plant growth was observed.

2.10.3. Trial II

To the sterile pesticide, contaminated soil 10ppm of each of the individual pesticide (chlorpyrifos, cypermethrin, glyphosphate, di ammonium phosphate, dimethoate, Hycrofos, Iminocloprid) was incorporated and genetically modified strain was inoculated and seeds were sown and plant growth was observed.

2.11. Effect of degraded pesticide on germinating ability of the treated and untreated soil samples

The plant growth was estimated by using percentage of germination and root & shoot length was determined.

The percentage of germination ability was calculated by the following formula:

Percentage of germinating ability = (Total number of seeds germinate)/(Total number of seeds shown) X 100

2.12. Morphometric parameters (Root and shoot length)

Plants were collected from each pot at 14thday. Root and shoot length of plant was measured and expressed in cm.

3. RESULTS AND DISCUSSION

3.1. Isolation of pesticide degrading organism-Enrichment technique

The bacterial densities were monitored spectrophotometrically at every 24 hours. There was considerable increase in OD at 600 nm up to 3rd day suggesting the

growth of microorganisms due to carbon source available in the minimal media initially. However, from 4th day onwards, there was a decrease in OD value, as only those microorganisms which were capable of utilizing the pesticide as the sole source of carbon were present in the medium. When the organisms present in the media were further enriched with the corresponding pesticide, only those capable of tolerating the pesticide toxicity and capable of utilizing it by degrading the pesticide were present. The bacterial colonies were then serially diluted and spread plated on to plates containing a higher concentration of the corresponding pesticide (1000ppm). The purpose of spread plating is to isolate individual bacterial cells that can degrade pesticide on minimal media and a total of 20 isolates were obtained in this study. The isolated colonies are sub cultured in nutrient agar and slants in order to obtain pure culture. Biological removals of chemo-pollutants has become the method of choice, since microorganism's uses a variety of xenobiotic compounds including pesticides for their growth and mineralizes and detoxify them [6]. Each colony was streaked on to nutrient agar plate containing 1000 ppm of different pesticides in order to check pesticide tolerating capacity. Among the 20 strains obtained from the preliminary screening, a total of 11 different isolates were able to grow on at least 4 different pesticides used for the study. This secondary screening is used to ensure the microbes that are capable of utilizing a wide variety of pesticides. According to Rashmi *et al*., (2015) [7], the bacterial isolates are able to grow in medium in the presence of added pesticides and may therefore be used for bioremediation of pesticide contaminated soil. The present study suggests that the use of potential microorganisms in the treatment system can successfully overcome many of the disadvantages associated with the conventional method used for the degradation of pesticides.

3.2. Identification of isolated bacterial strains

Identification of bacterial isolates was carried out by the routine bacteriological methods as gram staining and biochemical identification. Investigations were focused on to the isolation and characterization of the bacterial stains with pesticide tolerance to identify potential candidates for pesticide bioremediation [8]. The bacterial isolates were morphologically and biochemically typified and properties were as listed in Table 2. The isolate 2, 4, and 10 showed similar biochemical results as that of isolate 9, isolate 8 and isolate 3 respectively. The difference in colony morphology may be attributed to the addition of xenobiotics compounds to the media which could have brought about the change in colony morphology.

Table 2: Biochemical and Microscopic analysis of selected bacterial strain

Test method \ Bacterial isolates	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Gram Staining	-	+	-	-	-	+	-	+	+	-	+
Shape	rods	rods	rods	rods	rods	cocci	rods	cocci	rods	rods	rods
Indole test	-	-	-	-	-	-	-	-	-		-
MR test	+	-	+	+	+	-	+	-	-	+	-
VP test	-	+	-	-	-	-	-	-	+	-	+
Glucose fermentation	-	-	-	-	-	-	-	-	-	-	-
Sucrose fermentation	-	-	-	-	-	-	-	-	-	-	-
Lactose fermentation	-	-	-	-	+/-	-	-	-	-	-	-
Mannitol fermentation	-	+	-	-	-	+	-	+	+	-	+
Citrate utilization	+	-	+	+	+/-	-	+	-	-	+	-
Urease test	-	-	-	-	-	-	-	-	-	-	-
Catalyse test	+	-	+	+	+	+	+	+	-	+	-
Oxidase test	+	-	+	-	+	+	+	-	-	+	-
Nitrate test	+	-	+	+	+	-	+	-	-	+	-
Starch test	-	+	-	-	-	-	-	-	+	-	+

Bacterial isolate No.1,3,4,7,10 are tentatively identified as Pseudomonas sp., Isolate No.2,9,11 are tentatively identified as Bacillus sp., Isolate No.5 are tentatively identified as Paracoccus sp., Isolate No.6 are tentatively identified as Planococcussp., Isolate No.8 are tentatively identified as Staphylococcus sp.,

Though so far no literature was found in accordance to the above statement, but during the study we observed the difference in colony morphology of certain bacterial strains were found to be different in the presence of xenobiotics. Therefore, the isolates which showed close proximity in biochemical results were ruled out. Though, there is no strong reason for eliminating those strains as the species level identification was not performed, it was done for our convenience. So from the 11 bacterial strains, only 8 different strains were used for further study.

3.3. Analysis of pesticide degradation and estimation of released phosphate by Fiske Subbarow method

The determination of rate of utilization of pesticide by isolates was carried out by spectrophotometric analysis using the method of Fiske Subbarow [5]. From the standards, the phosphorus content degraded in the sample supplemented with suitable pesticide was measured. The results depicted that the isolate 3, isolate 8 and isolate 9 showed higher phosphate release in all the pesticide used for the study.

Table 3: Determination of phosphorus content using Fiske Subbarow method

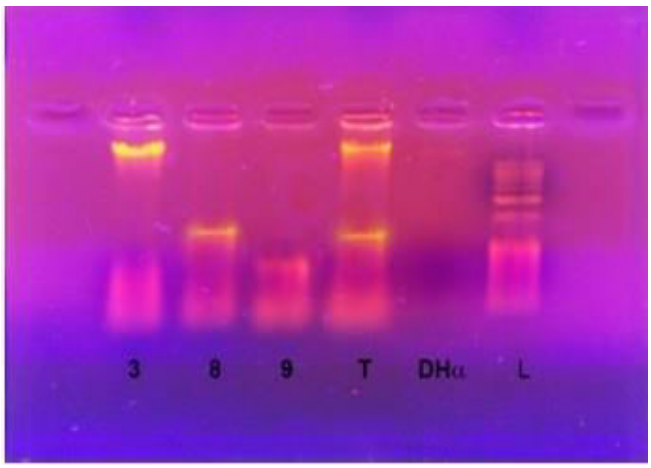
		Chl	Cyb	Gly	DAP	Dim	Hyc	Imi
Isolate 1	Initial	24	22	38	22	35	32	30
	24h	34	35	39	33	43	38	38
	36h	46	43	45	45	56	45	42
	72h	72	67	67	65	76	59	56
Isolate 3	Initial	24	22	38	22	35	32	30
	24h	33	30	42	32	39	42	42
	36h	48	45	55	47	57	67	43
	72h	83	73	78	78	75	75	63
Isolate 5	Initial	24	22	38	22	35	32	30
	24h	36	37	39	36	34	39	34
	36h	47	45	67	45	45	44	62
	72h	65	76	73	72	68	67	78
Isolate 6	Initial	24	22	38	22	35	32	30
	24h	31	32	38	22	41	35	35
	36h	44	40	41	22	47	41	44
	72h	67	61	61	22	54	57	44
Isolate 7	Initial	24	22	38	22	35	32	30
	24h	32	38	34	24	34	34	35
	36h	64	47	57	38	45	56	53
	72h	76	74	69	78	69	80	79
Isolate 8	Initial	24	22	38	22	35	32	30
	24h	33	29	48	26	43	47	56
	36h	52	43	56	34	68	69	47
	72h	87	78	84	76	75	79	65
Isolate 9	Initial	24	22	38	22	35	32	30
	24h	59	39	72	30	47	44	55
	36h	65	47	78	45	69	73	53
	72h	84	80	87	85	89	87	89
Isolate 10	Initial	24	22	38	22	35	32	30
	24h	32	22	39	30	35	32	35
	36h	42	27	44	39	38	37	35
	72h	69	35	47	48	45	37	35
Isolate 11	Initial	24	22	38	22	35	32	30
	24h	29	31	40	34	45	38	30
	36h	41	45	40	41	50	38	30
	72h	71	61	49	54	61	47	30

Chl-Chloropyriphos, Cyb-Cybermethrin,Gly- Glyphosphate, Dim-Dimethoate, Hyc- Hycrophos, Imi- Imidacloprid

The isolate 9 showed a maximum phosphorus release of 89µg/ml in the pesticide Imidacloprid. Similarly, isolate 3 showed 83µg/ml and isolate 8 showed 87 µg/ml in Chloropyrifos after 72 hour of incubation. From the Table 3, isolate 9, isolate 3 and isolate 8 released highest amount of phosphorous which indicates these two isolates degrades the all seven pesticides effectively. According to Ratna *et al.,* (2013) [9], during the analysis of pesticide degradation, the isolated microorganism showed an increase in phosphorous content. The amount of available phosphorus (in the form of inorganic phosphate) increased with time [5]. Some compounds like organophosphate releases phosphate content as one of the end product in the bioremediation process [9]. Bacterial degradation was mainly through the cleavage of Carbon-Phosphorus (C-P) bond, resulting in the release of sarcosine and a phosphate group [5]. The degradation of each pesticide was represented in the table 2 with each value of organism is compared with control. The amount of phosphorous released determines the degradation of pesticide hence standard phosphorous is compared with the degradation of each organism with 7 different pesticide. Table 2 results suggested that the isolated organism were able to grow more in the presence of 7 different pesticides. The possible mechanism of this would be degrading the compound through breaking of Carbon-Phosphorus (C-P) bond or Carbon-Nitrogen (C-N) bond by the organism [5]. Hence, highest pesticide degrading isolates was chosen and the plasmid DNA was isolated for further metagenomic approaches. The pathway of pesticide degradation suggests the pesticide Profenofos cleaved finally into phosphorus. From the literature, it is evident that the degradative genes are present in the plasmid DNA, and so further step was taken in detection of presence of plasmid DNA in these strains. In accordance to the degradative pattern of the pesticides isolates 3, 8 and 9 were further subjected for plasmid isolation and transformation studies.

3.4. Transformation of Plasmid to a Versatile Bacterial Strain

The plasmid of the three isolates 8, isolate 3, and isolate 9 was used for further study. These isolates were chosen as these strains showed an optimal concentration of phosphorus release in Fiske subbarow method. So in order to enhance the rate of biodegradation of pesticide, transformation of these plasmids were attempted. The transformation of these plasmids was done commercially. Spread plate technique was done and was plated in media containing 1000 ppm of the pesticides. Colonies obtained were confirmed using agarose gel. It was found that competent cells of *E.coli.* DH5α with the isolated plasmid DNAs from pesticide tolerating bacteria were successfully transferred and thus acquired a new extrachromosomal property of tolerating higher concentrations of toxic chemicals. Thus, the genetically modified strain was tested for pesticide degradation (Fig. 1). From the fig. it is evident that only two plasmids have successfully transformed into *E.coli* DHα (from isolate 3 and 8 respectively). This may be due to the fact that the host cell was not able to uptake more than two plasmid DNA available in the media. It may also be due to the fact that on transforming more than two plasmids into the host may bring about damages to the host.



T-Transformed cell of DHα- E.Coli DHα, L-Ladder, 3-Isolate 3, 8 - Isolate 8, and 9 - Isolate 9

Fig. 1: Transformed strain in comparison with other bacterial isolates having the plasmid on an Agarose gel

3.5. Determination of rate of Degradation of transformed strain

The degradation rate of transformed strain was detected by using Fiske subbarow method. The amount of available phosphorus (in the form of inorganic phosphate) increased with time [5] hence, each pesticide degradation was taken in different interval of time and graphical representation was shown in Fig. 2, it was confirmed that degradation of pesticide was increased in transformed strain than wild strain. The results of the fig. 2 indicate that there is increase in rate of degradation of all the pesticides degraded by the transformed strain. The transformed strain showed higher concentration of 98 µg/ml phosphorous content in chloropyrifos and Hycrophos. The developed

consortium can remediate pesticide-contaminated soil effectively with quick action, hence, it is concluded that the developed consortia are efficient pest degrader. Pesticide degrading bacteria applied as consortia can increase the degradation process in soil [10]. As far as metagenomic organisms are considered, they will find it really difficult if left as such in their natural environment and produce the same result. It is only because they are now expressing their capability of pesticide degradation in another expressive organism that they have actually induced so much potential. Also now the gene could be made use of and could be transferred to other organisms through thereby increasing the overall efficiency of the organisms involved.

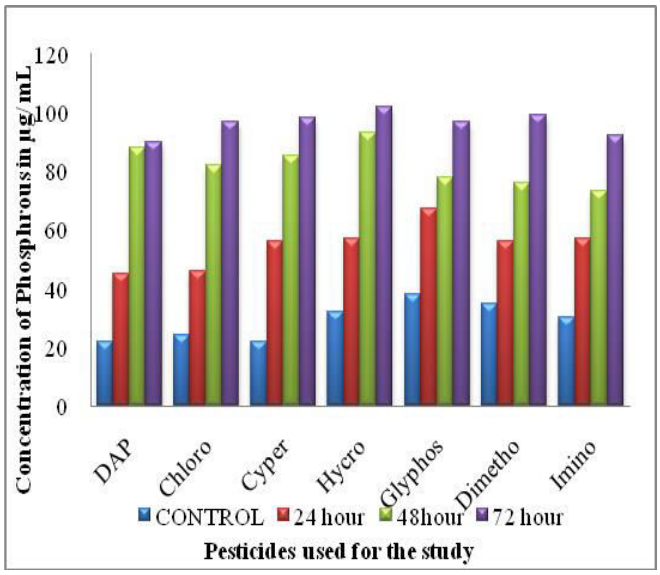


Fig. 2: Determination of phosphorus content of the transformed strain

3.6. Effect of degraded pesticide on germinating ability of the treated and untreated soil samples

The effect of pesticide was analyzed in green gram for its effect on growth of the plant. From the experimental plants, germinating ability and morphometric parameters namely shoot and root length were calculated at 14th day. From the table 4 the germinating ability of Chloropyrifos and Glyphosphate were high comparing with other plants. Table 5 shows the determination of Morphometric parameters of the plant. Glyphosphate and Dimethoate shows increase in root and shoot length. The genetically modified organism can degrade pesticide in contaminate soil and helps for plant growth. These findings were similar to results obtained by Olawae et al., (2011) [1].

Table 4: Evaluation of Germinating ability of the treated and untreated soil samples

Pesticide	Treatment I	Treatment II	Treatment III
Chl	100 %	50%	80%
Cyp	100 %	50%	80%
Gly	100 %	50%	70%
DAP	100 %	40%	60%
Dim	100 %	40%	60%
Hyc	100 %	30%	50%
Imi	100 %	50%	90%

Treatment I-Sterile soil+Seeds, Treatment II-Sterile soil+ pesticide+seeds, Treatment III-Sterile soil+pesticide+ organism+seed

Table 5: Determination of Morphometric parameters of the plants grown with treated and untreated soil samples

Pesticide Treatment	Control		Treated	
	Root (cm)	*Shoot (cm)*	*Root (cm)*	*Shoot (cm)*
Sterile soil + seeds	2.1	12	3.4	6
Chl	1.5	2	1.5	4.5
Cyp	1	3	1.5	6
Gly	2	3.5	3	6.5
DAP	2.5	3.5	3	5.5
Dim	1	1.5	2	5.5
Hyc	1	4	2.5	7
Imi	2	3.5	3	5.5

4. CONCLUSION

Improvised after isolation of potent pesticide degraders from contaminated site they can be used to remediate soil contaminated with pesticide. Pesticide degrading bacteria applied as consortia would increase the degradation process and efficiently minimize the pollutant level in soil [10]. Hence, genetically modified organism helps in pesticide degradation in soil and enhances the growth of the plant. This is due to the fact that the organophosphorus pesticides have converted into inorganic phosphorus, which is the utilizable form for plant growth. Therefore, these kinds of treatments help in the breakdown of the toxic compounds, thereby increasing the soil fertility. As a result of increasing population, there is need for supply of better quality and quantity food to the population. Therefore, the need for synthetic pesticides has also increased, which has lead to the entry of these xenobiotics into the food chain and leading to the cause of dreadful diseases. From the above study it is clear that the degradation of pesticides using bacteria has proven to be effect than the

conventional methods. The step taken to isolate the plasmid DNA which harbours the gene responsible for degradation of the pesticide and transformation of it proves to be an eco-friendly and a cost effective method for the cleanup approach.

Conflict of interest

None declared

5. REFERENCES

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