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CHARACTERIZATION OF *RHIZOBIUM* ISOLATES FROM *SESBANIA* RHIZOSPHERE AND THEIR ROLE IN BIOREMEDIATION OF GLYPHOSATE AND MONOCROTOPHOS

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ABSTRACT

Monocrotophos and glyphosate are widely used as plant protection agents worldwide. The present study deals with the characterization and efficiency of three *Rhizobium* isolates (SR G, SR I, SR 01) obtained from the root nodules of *Sesbania rostrata* for biological remediation of these pesticides. Identity of these Gram's negative rod shaped isolates was established using biochemical as well as 16S rRNA sequencing. These isolates showed good ability of growth when the tested pesticides were used as a sole source of carbon and or phosphorous. The sensitivity of these three rhizobial isolates in response to different types of antibiotics with various concentrations indicated SR 01 to be resistant against most number of antibiotics. 16S-rRNA genes sequence analysis revealed maximum similarity of SR G with *Rhizobium* sp. SCAUS14 (KF836037.1), SR I with *Sinorhizobium saheli* OP3-1 (JX855185.1), and SR 01 with *Ensifer* sp. AC01b (JF450128.1). These isolates showed sigmoid growth pattern in MS1 medium supplemented with glucose (20mM). However, when grown in glyphosate (30mM), and monocrotophos (20mM) supplemented MS1 medium, they showed a typical and uncommon growth curve. The rhizobial isolates grown in pesticide-supplemented medium showed comparatively longer lag phase; and a clear-cut demarcation between other growth phases of the batch cultures was missing. HPLC analysis of the filter sterilized supernatant revealed SR G to be most efficient in removal of glyphosate (43.99%), followed respectively by SR I (40.82%) and SR 01 (38.70%). However, removal of monocrotophos was maximum by the isolate SR G (34.79%) which was closely followed by SR 01 (33.25%) and SR I (26.99%). The results revealed the potentiality of these bacterial isolates for bioremediation of environmentally unsafe xenobiotic compounds.

KEYWORDS: Glyphosate, Growth Curve, Harmful, Monocrotophos, Pesticides

INTRODUCTION

The extensive and indiscriminate use and disposal of pesticides in agricultural fields and large plantation is posing serious threat to the sustainability of ecosystems due to the problem of environmental contamination and related health hazards. Pesticides after being sprayed can become airborne and may ultimately end up in soil or water. Glyphosate [N-(phosphonomethyl) glycine] is a globally used broad-spectrum herbicide. It represents the phosphonic acid group of compounds, which is characterized by a direct carbon to phosphorus (C–P) bond. The C–P linkage is chemically and thermally very stable and renders the molecule much more resistant to non-biological degradation in the environment than its analogues with O-P linkage [1]. This herbicide is a potent inhibitor of the enzyme 3-enol-pyruvylshikimate-5-phosphate synthase (EPSP synthase, EC 2.5.1.19) and a competitive inhibitor of EPSPS with respect to phosphoenolpyruvate, which

are respectively involved in the biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan and block import of the EPSPS preprotein into chloroplast, the subcellular location of the shikimic acid pathway [2, 3].

Monocrotophos [(3-hydroxy-N-methyl-cis-crotonamide) dimethyl phosphate] is a broad-spectrum organophosphate insecticide widely for agricultural and household purposes and works systemically and on contact [4]. It has been classified as highly toxic pesticide, its half-life in soil has been observed to be 40–60 days, and has an LD50 value of 20 mg/kg for mammals [5]. Monocrotophos is water-soluble and affects the central nervous system by inhibiting cholinesterase, an enzyme essential for normal nerve impulse transmission. Excessive use of monocrotophos, therefore, may pose serious threat to the survivability of the human beings as it acts by both systemic and residual contact properties. It causes severe poisoning effect besides being a reproductive, mutagenic, and carcinogenic agent [6], and thus, may disturb biodiversity of the agro-ecosystems. Microbial degradation is considered one of the most important pesticide transformation processes controlling their persistence in soil [7].

Sesbania rostrata is a tropical stem- and root-nodulated legume that possesses high rates of N_2 fixation and may adapt to adverse climatic conditions [8, 9], which may be due to their association with the nitrogen fixing rhizobia and other plant growth promoting rhizobacteria. Rhizobia obtained from the plants growing under tropical and alkaline soil show significantly higher salt and temperature tolerance and possess mechanisms to function as excellent plant growth promoting rhizobacteria [10]. These bacteria promote plant growth either by production of metabolites such as antibiotics, HCN, or siderophores or by production of plant growth regulators, improvement in plant nutrients uptake, or promote induced systemic resistance of the plant [11], and hence, are important in long-term sustainability of the agro ecosystems.

Global use of glyphosate (agricultural plus non-agricultural) has rose more than 12-fold from about 67 million kg in 1995 to 826 million kg in 2014. Nearly 6.1 billion kgs of glyphosate have been applied in the last decade, which accounts for 71.6 % of total pesticide used worldwide from 1974-2014 [12]. However, monocrotophos is one of the most widely used insecticides as total sales for monocrotophos worldwide accounts to nearly 3% of all insecticide-product sales [6]. Therefore, a need was felt to isolate rhizobia from the rhizosphere of *Sesbania rostrata*, which bioremediate these pesticides in one hand and on the other may be useful for promoting plant growth. In this background, the present study was undertaken to obtain and characterize *Rhizobium* isolates from the rhizospheric soil of *Sesbania rostrata* for degradation of glyphosate and monocrotophos.

MATERIALS AND METHODS

Isolation of *Rhizobium*

Ten grams of soil samples collected from the rhizosphere of *Sesbania rostrata*, growing in the fields of Nirma University, Ahmedabad, Gujarat (India), was put in flask containing 50 ml sterile distilled water, mixed properly, and the volume was adjusted to 100 ml. Following serial dilution 100 µl of soil suspension was spread-plated over pre-sterilized petriplates containing nutrient agar medium following standard plating method in triplicates, and incubated at 37±2 ^{0}C. After 2-4 days of incubation, colonies that failed to absorb congo red dye and showed circular, smooth white or beige, convex, semi-translucent or opaque colonies were selected and maintained on congo red yeast extract mannitol agar (CRYEMA) slants as pure isolates for further analysis.

Biochemical Characterization of the Isolates

Bacterial isolates were screened for the production of exopolysaccharide (EPS) by observing formation of

gummy/mucoid colonies on CRYEMA medium. Gram's reaction, Methyl Red- Voges Proskauer (MRVP) test, and tests for citrate utilization and lactose fermentation was done following the method of Cappucino and Sherman [13]. Presence of catalase enzyme was tested by taking 24 h old bacterial colonies on glass slides and adding one drop of H_2O_2 (30%). Appearance of gas bubble indicated positive test for catalase production [14]. Oxidase reaction was carried out by touching and spreading a distinctly isolated colony on the oxidase disc (HIMEDIA, India). Peptone utilization test was carried out following method of Kleczkowska and Nutman [15] whereas ketolactose production was tested by replacing mannitol with lactose in CRYEMA medium followed by autoclaving and streaking a loopful of sample on the ketolactose agar media and after incubation (3 to 4 days at 28^0 C) flooding the plates with a shallow layer of Benedict's solution. Triple sugar iron test and urease production test was done as per the method of Cappucino and Sherman [13]. The phenylalanine agar medium, a nutrient medium enriched with 0.2% phenylalanine, was used to test the presence of enzyme phenylalanine deaminase, which was needed to use the amino acid phenylalanine as a carbon and energy source for growth [16]. Starch hydrolysis test was performed to determine the ability of microorganisms to use starch as a carbon source [17] and antibiotic resistance assay was done to test the sensitivity of bacteria to various antibiotics using antibiotic discs (HIMEDIA, India).

Acclimation and Screening for Glyphosate and Monocrotophos Bioremediation

The exponentially growing cultures (OD_{600} = 0.6-0.8) were first acclimatised in MS1 medium [18] containing 5.0 mM of glyphosate and monocrotophos, separately and pH adjusted to 6.8±0.2. After 48 hrs of incubation at 35±2°C, the surviving cultures were inoculated into MS1 medium with higher concentrations of glyphosate and monocrotophos, successively with the following concentrations 5.0, 10.0, 15.0, 20.0, 25.0, and 30.0 mM for the purpose of screening. The cultures could sustain 30 mM of glyphosate whereas they could only tolerate 20 mM of monocrotophos. Therefore, 30 mM of glyphosate and 20 mM of monocrotophos supplemented MS1 medium were finally selected for biodegradation studies of these pesticides by the isolates.

Molecular Characterization

Single colony of the pure culture was inoculated into LB broth and incubated for 24 hours for isolation of genomic DNA using HipurA™ Bacterial genomic DNA purification kit (HIMEDIA, India) following manufacturer's instructions. PCR amplification of the 16S rDNA gene was carried out with the universal bacterial primers; F27: (5-AGAGTTTGATCATGGCTCAG-3') and R1492R: (5'-TACGGTTACCTTACGA CTT-3') in a final volume of 20 µl having 1XTAE buffer (with 15 mM MgCl2), 10 mM of deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP), forward and reverse primers (10 pico moles each), 1 U of Taq DNA polymerase (Bangalore Genei Ltd., India) and 100 ng of genomic DNA. Amplification was achieved in a Master Cycler (Eppendorf, USA) programmed for initial denaturation (94°C for 4 min); followed by 35 cycles composed of denaturation (94°C for 1 min), annealing (51°C for 1 min), extension (72°C for 2 min); and a final extension of 5 min at 72°C and subsequent cooling at 4°C. The amplified PCR products were purified using QIAquick Gel Extraction Kit (Qiagen) and 16S rRNA gene sequencing was performed using Big Dye terminator cycle sequencing and products were resolved on an automated DNA sequencer (ABI3730XL, Applied BioSystems, USA). These sequences were compared for similarity with the reference species present in NCBI database and BLASTn algorithm [19] and dendrograms were constructed by neighbour joining method using MEGA5 [20].

Growth Curve and Quantification of Glyphosate and Monocrotophos Bioremediation

To study the growth pattern, single colony of the log phase cultures (SR G, SR I, SR 01) were placed separately in

MS1 medium (initial pH adjusted to 6.8±0.2) containing respectively, glucose (20mM), glyphosate (30mM), and monocrotophos (20mM), and were incubated at 35±2°C at 120 rpm for 36 hrs in shaker incubator (GeNei, Bengaluru). Culture samples were withdrawn aseptically at regular time intervals for measurement of growth, which was analyzed by taking absorbance at 600 nm using uninoculated media as blank spectrophotometrically and the growth curve was plotted using Microsoft Excel.

The above set of cultures were maintained for a week; after which, the loss of glyphosate from the media was monitored by High Performance Liquid Chromatography (HPLC) (Shimadzu Class-VP) on a reverse phase C-18 with a mobile phase of acetonitrile (80%) and 0.05% H_3PO_4 in water (20%). Chromatography was carried out at an ambient temperature at a flow rate of 1.0 ml/min, wavelength of 210 nm and run time of 10 minutes. The concentration of pesticide was calculated using commercial standards (Monsanto's Roundup) [21]. However, estimation of residual monocrotophos was done by HPLC (Agaram – LC 2010, solvent delivery pump) having 250mm × 4mm SS column packed with Lichrosphere 100 RP-18 endcapped (5 µm) in LichroCART (Merck, India) and UV detector (Agaram – LC 2020) at 210 nm. The data was processed on a Chemito 5000 integrator (Toshniwal Instruments Pvt. Ltd., India). The operating conditions were as follows: ambient column temperature, 28^{0} C; mobile phase, 20% acetonitrile in water; flow rate of 0.5 ml/min; and standard aqueous solution of monocrotophos [22]. For glyphosate analysis we took 15mM as a standard which had a retention time of 1.498 minutes and area under curve was 895059units. However, for monocrotophos we took 10mM as a standard with retention time 6.148 minutes and 28511818 was area under curve. The percentase of glyphosate and monocrotophos removal was calculated by formula: % Bioremediation = 100 – (Area under curve of unknown / Area under curve of standard) x 100.

RESULTS

Isolation and Biochemical Characterization of the Bacterial Isolates

Fifty-two bacterial isolates were collected from the petriplates having CRYEMA medium that failed to absorb congo red dye and showed circular, smooth white or beige, convex, semi-translucent or opaque colonies. Out of these 52 isolates, 32 produced exopolysaccharide and of these EPS producing isolates, 22 were gram negative. These 22 isolates were further characterized biochemically starting with Gram's staining; followed by Methyl Red-Voges Proskauer test, tests for production of citrate, catalase, lactose fermentation, oxidase, peptone utilization, ketolactose, triple sugar iron test, urease, phenylalanine deaminase, and urease. Only nine isolates were lactose non-fermentors and of which only three isolates were negative for citrate utilization. Each of these three isolates (SR G, SR I, SR 01) showed production of exopolysaccharides and were negative for Gram's reaction, keto lactose test, Methyl Red-Voges Proskauer test, triple sugar iron test and citrate test (Table 1) which are required for qualitative characterization and to justify the closeness of these isolates to rhizobia.

The sensitivity or resistance of selected rhizobial isolates in response to different types of antibiotics with various concentration indicates that all the isolates were sensitive to Streptomycin (10 mcg), Rifampicin (5 mcg), and Amikacin (30 mcg). However, the isolate SR 01 showed resistance against most number of antibiotics, SR I showed resistance against Cefpodoxime (10 mcg), Levofloxacin (5 mcg), Clindamycin (2 mcg), and Tetracycline (30 mcg), and the isolate SR G was resistant only to Augmentin (30 mcg) (Table 2).

Molecular Characterization

The Bacterial Genomic DNA showed presence of single distinct genomic DNA without shearing and the A260/280 was between 1.7-1.9. The PCR amplification of the 16S rRNA gene when resolved on 1.2% agarose gel along with O'gene ruler produced a band of about 1500bp. The resulting 16S-rRNA gene sequences were submitted in the NCBI GenBank with accession numbers KY118087, KY118088, KY118089. BLAST search analysis of 16S-rRNA genes sequences revealed maximum similarity of SR G with *Rhizobium* sp. SCAUS14 (KF836037.1), SR I with *Sinorhizobium saheli* OP3-1 (JX8551851), and SR 01 with *Ensifer* sp. AC01b (JF450128.1) as per GenBank databases (Figure 1).

Growth Curve of Isolates and Glyphosate and Monocrotophos Removal Curve

The growth curve of the three isolates obtained with MS1 medium supplemented with glucose (20mM) shows sigmoid pattern. The lag phase for the isolate SR G was up to 10h, log phase from 10-16h, stationary phase from 16-28h followed by death phase. Whereas the other two isolates (SR I and SR 01) respectively showed lag phase up to 12 and 8h, log phase from 18-20 and 8-16h, stationary phase from 20-30 and 16-34 hr, and death phase after 30 and 34h of incubation. However, isolates SR G, SR I, and SR 01 grown in glyphosate (30mM), and monocrotophos (20mM) supplemented MS1 medium showed a very typical and uncommon growth curve. As compared to the glucose-supplemented medium, the pesticide-supplemented medium (respectively, glyphosate and monocrotophos) represented longer lag phase, which was respectively 12 and 14, 12 and 14, and 14 and 16h for the three isolates (Figure 2). Moreover, the isolates under pesticides treatment could not show a clear-cut rise in growth rate or exponential growth so as to justify a log phase. After lag phase, the cultures fail to show a steep rise in growth rate, which is required for the onset of log phase in the bacterial growth curve. Additionally, a clear-cut demarcation between other growth phases of the bacterial batch cultures was missing.

Bioremediation of Glyphosate and Monocrotophos

After an incubation period of a week, the cell free supernatant was filter sterilized and were subjected to HPLC analysis. Of the three isolates, SR G proved maximum efficient in degradation of glyphosate (43.99%), which was followed respectively by SR I (40.82%) and SR 01 (38.70%) (Table 3). However, degradation of monocrotophos was also maximum by the isolate SR G (34.79%) which was closely followed by SR 01 (33.25%) and SR I (26.99%). Overall, the isolate SR G proved most efficient in bioremediation of both the pesticides, glyphosate and monocrotophos (Figure 3).

DISCUSSIONS

Indiscriminate use of xenobiotic compounds generates undesirable environmental concern due to the harmful side effects. The degradation of such harmful complex chemical compounds is a desirable indicator for functional, healthy and sustainable ecosystems. The herbicide glyphosate is mostly used as a total killer and is applied often to control weeds in grasslands or in fallow land whereas monocrotophos is an organophosphorus compound used worldwide to control insect-pests. The intensive use of pesticides has become a matter of environmental concern in modern agriculture, partially because of the adverse effects of these chemicals on crops and soil microorganisms [23].

Although, natural degradation of persistent pesticides normally takes longer time, the process may be expedited through bioremediation as it is a effective tool to stimulate the biodegradation in contaminated soils [24]. A wide variety of microorganisms present in soil may play an important role in pesticide degradation and may use these substances as a

source of carbon, energy and other nutrients, which in turn promote microbial growth [25]. Moreover, the glyphosate/monocrotophos-degrading strains used in the bioremediation studies should be non-pathogenic for mammals and should not exhibit integral toxicity and phytotoxicity. In the present study, the isolates on the one hand bioremediate the persistent and harmful pesticides whereas on the hand they belong to a group of bacteria; which functions as plant growth promoting rhizobacteria [9]. The decrease in the glyphosate content during bioremediation by bacteria lead to restoration of soil biological activity, as is evident from a more than twofold increase in the dehydrogenase activity of indigenous soil microorganisms and their biomass (1.2-foldand 1.6-fold for saprotrophic bacteria and fungi, respectively) [26].

In the present study, the isolates were tested for exopolysaccharides production. A number of bacteria including rhizobia produce variety of exopolysaccharides, which due to remarkably high moisture holding capacity help to maintain sufficient moisture in their immediate environment. It protects these microbes from desiccation and serves as a potential energy reserve [27]. Ketolactose test was performed to rule out the presence of *Agrobacterium* because *Rhizobium sp.* are often confused with *Agrobacterium sp.* However, unlike agrobacteria, rhizobia are ketolactose negative i.e. they lack 3-ketolactase enzyme which converts lactose to 3-ketolactose, which in turn gives yellow colouration when reacted with Benedict's reaction [28]. Also, rhizobia are lactose non-fermenters, peptone slow utilizers and citrate non utilizers unlike enterobacters [29] and may produce important metabolic enzymes like urease and phenylalanine deaminase.

Out of several isolates selected for biochemical characterization, only three isolates were presumed to be similar to the *Rhizobium* sp. due to their positive tests for exopolysaccharides production, catalase, oxidase, urease, and phenylalanine deaminase and negative results for Gram's staining, ketolactose and MR-VP tests, citrate and lactose fermentation, peptone utilization, triple sugar iron test, and that for starch hydrolysis. Therefore, these three isolates were subjected to the 16SrRNA gene sequence analysis.

The antibiotic profile of these three isolates indicates their competitiveness in the soil environment or agro ecosystems due to their inherent ability to resist against various types of antibiotics produced by other bacteria in the environment. The isolate SR 01 may prove comparatively more robust in competing in the pesticide affected soil or agro ecosystems due to its inherent resistance against maximum number of antibiotics treatment (Cefpodoxime, 10 mcg); Levofloxacin, 5 mcg; Clindamycin, 2 mcg; and Tetracycline, 30 mcg). The 16S rRNA gene sequence analysis confirms the similarity of these isolates to *Rhizobium*. The 16S rRNA gene sequences are assumed to be molecular chronometer due to the conserved nature of these sequences and which are most widely used tool in confirming species identity [30].

The three isolates selected after being acclimatised could survive in the minimal salt medium supplemented with glyphosate and monocrotophos as a sole carbon/phosphorous source either due to tolerance to the pesticide or due to their ability to degrade it. Glyphosate is moderately persistent [5] and its mineralization is related to both the activity and biomass of soil microorganisms [31]. Microbial degradation of glyphosate produces the major metabolite amino methyl phosphonic acid and ultimately leads to the production of CO_2, phosphate and water [7, 32]. Degradation or detoxification of organophosphorus pesticides by the action of microorganisms is hydrolysis of P–O alkyl and P–O aryl bonds. This reaction is considered as the most significant step in the detoxification of organophosphorus compounds. The hydrolase enzyme responsible for catalysing this reaction is referred to as an esterase or phosphotriesterase [33] or phosphatase [34], and is considered the most important enzyme in the bacterial metabolism of organophosphates. Isolate

SR G degraded highest amount of glyphosate and monocrotophos (43.99 and 34.79%, respectively) as compared to SR I (40.82 and 26.99%, respectively) and SR 01 (38.70 and 33.25%, respectively).

The bacterial strains *Achromobacter* sp. Kg 16 (VKM B-2534D) and *Ochrobactrum anthropi* GPK 3 (VKMB-2554D) have been demonstrated earlier to provide a two- to threefold higher rate of glyphosate degradation as compared to indigenous soil microbial community [26]. Several species of bacteria (*Pseudomonas, Flavobacterium, Alcaligenes, Bacillus, Agro bacterium* and *Arthrobacter*) have been isolated earlier, which can degrade glyphosate either co-metabolically or as a source of phosphorus. Similarly, *Aulosira fertilissima, Nostoc muscorum*, *Pseudomonas aeruginosa, Clavibacter michiganense, Pseudomonas*, *Bacillus,* and *Arthrobacter* can utilize monocrotophos as a sole source of carbon. However, this is the first report to investigate the role of rhizobia from sesbania rhizosphere in bioremediation of glyphosate and monocrotophos. *Rhizobium meliloti*, obtained from semi arid regions, is also known to promote plant growth attributes like root and shoot length, dry weight and total nitrogen and seed yield, 100 grain weight, number of root nodules, and nodule fresh and dry weight of the fenugreek crop in such climatic conditions [35]. Moreover, it is suggested by Singh et al. [23] that a judicious use of pesticide should be planned to sustain the recycling and transformation of various elements and to prevent any change in their number or ratio that could potentially prohibit/enhance one or the other reaction chains important for soil fertility. Additionally, use of bio fertilizer in combination with chemical fertilizer has been advocated for balancing soil fertility and crop productivity [36].

CONCLUSIONS

This study holds importance as it indicates that the rhizobia residing in the rhizosphere of *Sesbania rostrata* are instrumental in bioremediation of glyphosate and monocrotophos. This plant can grow under diverse agroclimatic zone and their distribution is widespread across various types of soil and weather situation. Pesticides applied directly to the soil may be washed-off into water bodies or may penetrate through the soil layers and ultimately reach groundwater and may affect the normal functioning of an ecosystem, resulting in economic, social, and aesthetic losses. Therefore, such bacterial isolates if present in the agroecosystems may play an important role in efficient removal of the pesticides residues from the contaminated environment or of degrading the pesticides before it reaches underground water. The results of this study holds potentiality for use of these rhizobia as bioinoculants in vast agricultural fields after evaluating them for various plant growth promoting attributes like, nitrogen fixation, phosphate solubilization, production of plant growth promoting hormones (auxins, gibberellins and cytokinins), and production of siderophore, antibacterial/ antifungal antibiotics and various other enzymes responsible either for combating various plant pathogens or due to their diverse role in biogeochemical cycling of nutrients.

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APPENDICES

Table 1: Biochemical Properties of the Isolates of Rhizobia

Biochemical tests	Isolates		
	SR G	SR I	SR 01
Colony (EPS)	+	+	+
Gram's reaction	-	-	-
Methyl Red	-	-	-
Voges proskauer	-	-	-
Citrate Test	-	-	-
Catalase	+	+	+
Lactose fermentation	-	-	-
Oxidase	+	+	+
Peptone utilization	-	-	-
Keto lactose Test	-	-	-
Triple Sugar iron test	-	-	-
Urease	+	+	+
Phenylalanine deaminase Test	+	+	+
Starch hydrolysis	-	-	-

Table 2: Antibiotic Profile of Isolates

Antibiotic	Concentration	SR G	SR I	SR01
Cefpodoxime	10 mcg	S	R	R
Chloramphenicol	30 mcg	S	S	R
Streptomycin	10 mcg	S	S	S
Vancomycin	30 mcg	S	S	R
Rifampicin	5 mcg	S	S	S
Levofloxacin	5 mcg	S	R	S
Ceftriaxone	30 mcg	S	S	R
Clindamycin	2 mcg	S	R	S
Augmentin	30 mcg	R	S	R
Amikacin	30 mcg	S	S	S
Cifixime	5 mcg	S	S	R
Tetracycline	30 mcg	S	R	R

S = Sensitive, R= Resistance

Table 3: HPLC Analysis of Bioremediation of Glyphosate and Monocrotophos

Sample	Concentration of Glyphosate (mM)	Retention Time (mins)	Area Under Curve	Glyphosate Degraded (%)
Standard	15	1.498	89750.59	Nil
SR 01	30	1.490	110027.05	38.70
SR I	30	1.498	106227.82	40.82
SR G	30	1.453	100521.66	43.99
Sample	**Concentration of Monocrotophos (mM)**	**Retention Time (mins)**	**Area Under Curve**	**Monocrotophos Degraded (%)**
Standard	10	6.148	285118.18	Nil
SR 01	20	6.355	380579.26	33.25
SR I	20	6.570	416306.82	26.99
SR G	20	6.977	371839.01	34.79

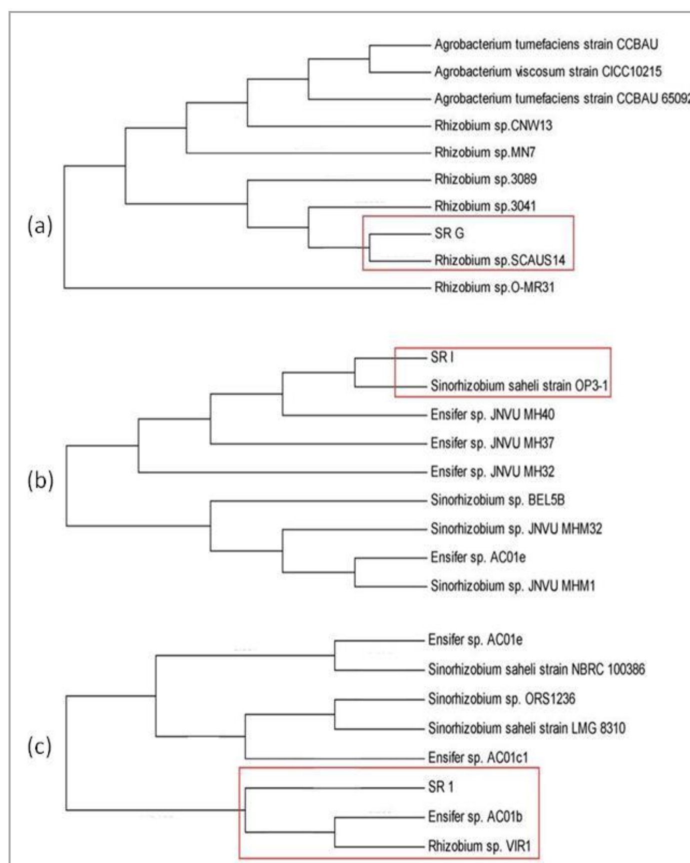

Figure 1: Dendrograms Constructed by Neighbour Joining Method using MEGA5 Showing Relationships of Isolates SR G (a), SR I (b) And SR 01 (c) With Those in the Gen Bank

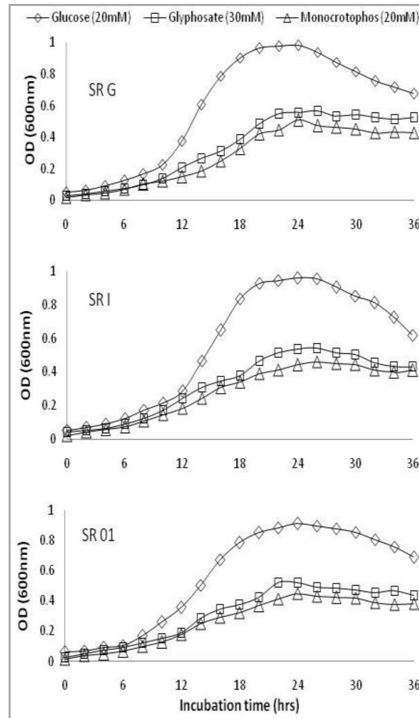

Figure 2: Growth Curve of Isolates Grown in MS1 Medium Supplemented with Glucose (20mM), (b) Glyphosate (30mM), and (c) Monocrotophos (20mM)

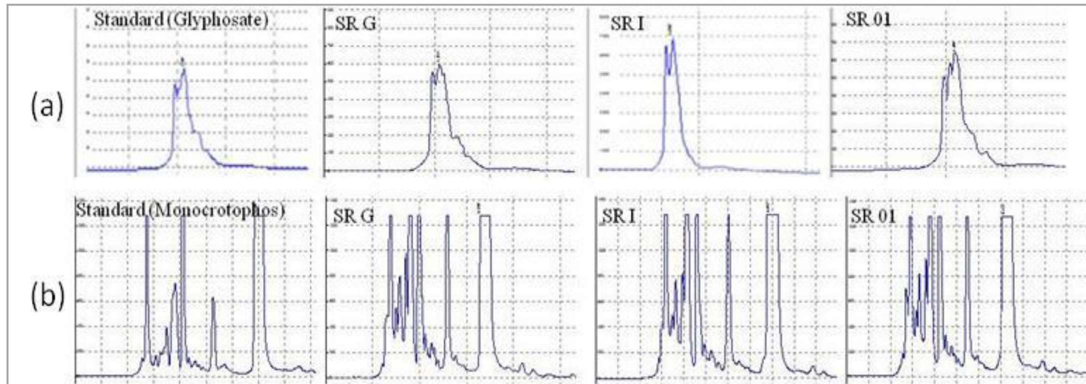

Figure 3: HPLC Profile of (a) Glyphosate and (b) Monocrotophos