

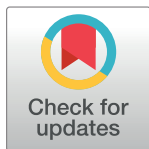
RESEARCH ARTICLE

Differential proteomic analysis under pesticides stress and normal conditions in *Bacillus cereus* 2D

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OPEN ACCESS

Citation: Gangola S, Joshi S, Kumar S, Sharma B, Sharma A (2021) Differential proteomic analysis under pesticides stress and normal conditions in *Bacillus cereus* 2D. PLoS ONE 16(8): e0253106. <https://doi.org/10.1371/journal.pone.0253106>

Editor: Pankaj Bhatt, South China Agricultural University, CHINA

Received: May 28, 2021

Accepted: July 25, 2021

Published: August 13, 2021

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Data Availability Statement: The 16S rDNA sequence of bacterial strain 2D was submitted to NCBI genbank. The accession number of the bacterial strain *Bacillus cereus* 2D is MH341691 provided from NCBI. Other relevant data is within the manuscript and its [Supporting Information](#) files.

Funding: The author(s) received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Abstract

A potential pesticide degrading bacterial isolate (2D), showing maximum tolerance (450 mg·L⁻¹) for cypermethrin, fipronil, imidacloprid and sulfosulfuron was recovered from a pesticide contaminated agricultural field. The isolate degraded cypermethrin, imidacloprid, fipronil and sulfosulfuron in minimal salt medium with 94, 91, 89 and 86% respectively as revealed by high performance liquid chromatography (HPLC) and gas chromatography (GC) analysis after 15 days of incubation. Presence of cyclobutane, pyrrolidine, chloroacetic acid, formic acid and decyl ester as major intermediate metabolites of cypermethrin biodegradation was observed in gas chromatography mass spectrometry (GC-MS) analysis. Results based on 16S rDNA sequencing, and phylogenetic analysis showed maximum similarity of 2D with *Bacillus cereus* (Accession ID: MH341691). Stress responsive and catabolic/pesticide degrading proteins were over expressed in the presence of cypermethrin in bacteria. Enzymatic kinetics of laccase was deduced in the test isolate under normal and pesticide stress conditions which suggested that the production of enzyme was induced significantly in pesticide stress (163 μg·μL⁻¹) as compare to normal conditions (29 μg·μL⁻¹) while the K_m value was decreased in pesticides stress condition ($K_m = 10.57$ mM) and increases in normal condition ($K_m = 14.33$ mM). Amplification of laccase gene showed a major band of 1200bp. The present study highlights on the potential of 2D bacterial strain i.e., high tolerance level of pesticide, effective biodegradation rate, and presence of laccase gene in bacterial strain 2D, could become a potential biological agent for large-scale treatment of mixture of pesticide (cypermethrin, fipronil, imidacloprid and sulfosulfuron) in natural environment (soil and water).

Introduction

From the last 20th century the overall global production of grain has increased from 500 million tons to 700 million tons [1]. However India alone produces 250 million tons of grain but it also losses 11 to 15% of total produced grains per year due to the pests attack and by some other causes [2]. To prevent such losses and to achieve the food targets, farmers have adopted to use hybrid seeds, systematic irrigation and application of chemical fertilizers and pesticides [3,4]. Cypermethrin, carbendazim, fipronil, sulfosulfuron, imidacloprid are some common pesticides used widely to control insects in agricultural practices and in health programs globally [5–7]. Sulfosulfuron, a new sulfonylurea herbicide is used to control weeds in wheat and other agricultural crops [8].

Pesticides are developed under strict vigilance of regulatory authorities which regulate the production of pesticides and set guidelines for their use so to put least hazardous impact on human health and on their surrounding environment. Besides these preventive measures, several health hazards can arise by their direct exposure during production and their contamination in food and water. Many environmental concern of pesticide has been already reported by researchers such as reducing biodiversity, nitrate leaching, decreased soil fertility, resistance in weed species towards common weedicides, increased cost of prevention and treatment endangering human health and acidification [9,10].

Several physical and chemical methods for degradation of the xenobiotic compounds are investigated but these methods are very costly and are not adequate enough [11,12]. Hence, the biological methods for degradation of xenobiotic compounds from the polluted sites by exploiting the microbial metabolism would be the best approach for the remediation of environment. The advantages of using this alternate remedial strategy (bioremediation) are, method is very cost effective, very economical, least hazardous, versatile and ecofriendly in nature [13,14]. Bacteria in nature degrade pesticides, and several toxic pollutants without any cost and do not cause secondary pollution. Consequently, researchers have conducted fine studies on biodegradation of toxic contaminants with clear understanding of their degradation mechanisms. Isolation of numerous bacteria and fungi efficient in degradation of pesticides into simple and non toxic forms has been done by various researchers [15–17]. Among the microbes used for biodegradation purposes, bacteria are most preferred as they could easily be developed into mutant strains with variety of biochemical pathways in adaptive environment [18,19]. Additionally, formulations of novel microbial consortia efficiently degrade xenobiotic compounds for their source of energy by using the co-metabolism process. The most promising and efficient technique for various biological purpose is the formation of proficient recombinant microbial strain which provides new opportunity to different biological fields [20].

Degradation of pesticides depends upon concentration, structure, solubility, soil types, soil moisture, temperature, pH, soil organic matter (SOM) and soil microbial biomass [12,21]. Discovery of bacteria with high metabolic potential and wide range of substrate activity could be a novel approach for bioremediation. Recently, for degradation of ametoctradin (fungicide) a packed-bed microbial bioreactor was used and reported higher proliferation of microbial communities (consortium) and their genes enhances the degradation rate [22]. Comparative microbial communities analysis in between ex vivo soil and in vitro soil correlated with bioreactor showed increases population of *Burkholderia* which is a potent biodegrader of xenobiotic compounds. Most recent techniques such as gene editing tools, ZFNs, TALEN, CRISPR-Cas can be used to target the potential pesticide degrading genes [23,24]. Here we are trying to fill this knowledge gap by studying effect of pesticide stress on different category of proteins and their expression. On the basis of above reference the current study plans to the following objectives;

- i. Isolation and characterization of Pesticide degrading bacteria
- ii. *In vitro* pesticide biodegradation in minimal medium
- iii. Identification of intermediate metabolites of biodegraded pesticide using GC-MS
- iv. Estimation of pesticide degrading enzyme and their enzymatic kinetics
- v. Proteomic analysis of pesticide degrading bacterial isolate in stress and normal condition
- vi. Amplification of pesticide degrading gene

Materials and methods

Chemicals and media used for experiment

Standard pesticides of highest purity namely cypermethrin, fipronil, imidacloprid and sulfo-sulfuron (Sigma-Aldrich 99% pure) were provided by Department of Chemistry, GBPUAT Pantnagar. Stock solutions (1mg.mL^{-1}) of the pesticides were prepared by dissolving standard pesticide in acetonitrile (for non volatile compound or HPLC) or hexane (for volatile compounds or GC), then sterilized with the help of bacterial filter and stored in colored bottles at 4°C in refrigerator till use. Isolation and characterization of bacterial isolates was done using Nutrient Agar (NA) and Mineral Salt Medium (MSM).

Isolation of pesticide degrading bacteria through enrichment method

Pesticides polluted agricultural soil samples were collected from Gularbhoj, Udham Singh Nagar, Uttarakhand, India. Standard methods (Methods of Soil Microbiology and Biochemistry) were used to collect the soil samples [12]. For the collection of soil sample the handheld corer of stainless steel was used. During the sampling discarded the first two cores then third cores (5 cm), taken over an area of 100 m^2 were pooled to form one composite sample. All the samples were packed in aluminium foil and sealed in sterilised plastic sampling bags to reduce the possibility of contamination. After proper labelling, samples were kept at -20°C until use. Isolation of pesticide degrading indigenous soil microorganisms was done by using enrichment technique followed by dilution method. For residual analysis of pesticides present in soil, the samples were prepared and performed according to Phartyal [25] and Negi et al. [26]. Extract of pesticides were analyzed by HPLC/GC. On the basis of the occurrence of residual pesticides in the soil sample, cypermethrin, fipronil, imidacloprid and sulfosulfuron were selected for the further study.

Screening of pesticide degrading bacterial isolates

For the screening of cypermethrin, fipronil, imidacloprid and sulfosulfuron degrading bacteria, minimal salt medium was supplemented with different concentrations of the pesticide(s). Petri plates containing 20 ml of minimal agar supplemented with pesticide(s) (10 to 450 ppm from the stock solution (1mg.mL^{-1})) and left for solidification of medium. Plates were streaked with active bacterial cultures (isolated from the soil samples) and incubated for 72 h at 30°C . The bacterial growth was observed after 2–4 days. On the basis of maximum tolerance level of the pesticides and growth in the minimal medium, bacterial isolates were selected, purified and maintained for further studies [12].

Molecular characterization of pesticide degrading bacterial isolate

Selected bacterial isolate (2D) was characterized at morphological and molecular level. Genomic DNA was extracted from the bacterial isolate [27]. Amplification of 16S rDNA gene

was done using universal primers (27f: 5'AGAGTTTGATCMTGGCTCAG3' and 1492r: 5'TACGGYTACCTTGTACGACTT-3'). After that the amplicon was processed for agarose gel electrophoresis and further sent for the 16S rDNA sequencing to Biotech Centre, South Campus, Delhi University. The contig of 16s rDNA gene was formed and check the percent similarity by comparing the sequences with NCBI gene database using BLAST programme. On the basis of maximum homology among the sequences the phylogeny of the organism was deduced with the help of MEGA 7.0 software [28].

***In vitro* pesticide biodegradation in minimal medium**

Experiment on biodegradation of cypermethrin, fipronil, imidacloprid and sulfosulfuron using selected bacterial isolates was performed in an *in vitro* study conducted under laboratory condition in liquid medium. 50 mL MSM, supplemented with 20 mg.L⁻¹ (1 ml from 1000 mg.L⁻¹ pesticide stock) of pesticide individually was inoculated with 1 mL of active bacterial culture (OD = 0.8) and incubated at 30°C. Further, 2 mL of broth was withdrawn separately from all the flasks at different days interval (on 5, 10 and 15th day) and centrifuged for 10 min at 10,000 rpm. After centrifugation, 1 mL of supernatant was added with 1 gm of sodium sulphate and 1 mL of hexane/acetonitrile. Two separate layers were formed in the separating funnel. Further the upper layer was collected and evaporated at room temperature with the help of evaporator. The dried pesticide in round bottom flask was next to mix properly with 2 mL of hexane/acetonitrile. After filtration extracted pesticide solution was collected and further analyzed with the help of GC/HPLC [12,29].

Identification of intermediate metabolites of biodegraded pesticide using GC-MS

On the basis of residual analysis of the pesticides in the MSM medium using HPLC/GC, percent biodegradation of cypermethrin was maximum among all the pesticides when treated with bacterial isolate (2D). Therefore the intermediate metabolites of cypermethrin biodegradation were analyzed with the help of GC-MS [30]. Degradation products of cypermethrin were extracted (50 mL of Mineral salt medium supplemented with 20 mg.L⁻¹ of pesticide in 250 mL flask containing) and analysed by GC-MS [12]. Uninoculated flask spiked with pesticide was used as control.

Estimation of laccase enzyme in bacterial strain 2D

One ml of bacterial culture (12–14h old) inoculated into TYE broth (Tryptone yeast extract: tryptone, 2%; yeast extract, 2%; pH, 7.2) was incubated at 37°C at 150 rpm for 5 days with (20 mg.L⁻¹) and without pesticide. Further, centrifugation at 6,000g was performed for 20 min at 4°C. Before the sonication (at 20 MHz; five times, each for 45 s and 30 s gap between each step), 10 mM of PMSF (Phenylmethylsulfonyl fluoride) was added in 0.1 M phosphate buffer with pH 6.5 for washing cell pellets and inhibit protease activity in the supernatant. Again centrifugation at 14,000 g for 20 min at 4°C was done to obtain cell extract which contains crude intracellular laccase enzyme. Enzyme activity can be measured at 465 nm using guaiacol as it forms reddish brown color in presence of laccase [31].

Kinetics of laccase by Lineweaver-Burk model

Lineweaver-Burke plots were used to derive kinetic parameters of the laccase enzyme [12,32,33]. Eq 1 indicates Michaelis-Menton equation (where V_{max} is maximum rate of reaction, r is substrate degradation in mM/min, K_m is growth rate constant, S is initial substrate

concentration).

$$r = V_{max}[S]/K_m + [S] \quad (1)$$

The inverse of Eq 1 is used in the Lineweaver-Burke plot:

$$1/r = K_m/V_{max} \times 1/[S] + 1/V_{max} \quad (2)$$

Proteomic analysis of pesticide degrading bacterial isolate

To perform whole cell proteome analysis, bacterial strain was grown in minimal medium supplemented with 0.1% glucose in the presence/absence of the pesticide. 20 mL of log phase bacterial culture, grown in minimal medium was used for the extraction of extracellular proteins. Bacterial culture was centrifuged at 12,000 rpm for 10 min at 4°C. Pellets were discarded and supernatant was used for further examination. Ammonium sulphate was added to precipitate the extracellular proteins. Suspension was kept at 4°C overnight. Precipitated protein was concentrated by using dialysis. Concentration of protein was estimated [34]. Lyophilized protein samples were analysed using 2D gel electrophoresis. *In silico*, analysis of protein spots separated on 2D gel was carried with ExPasy (TagIdent database) based on their molecular weight and isoelectric point (pI) [35].

Amplification of laccase gene

Presence of laccase, a major pesticide degrading gene was targeted in the bacterial isolate and checked by using the primers [36]. The primers used for laccase amplification were Cu1AF-5'ACMWCBGTYCAYTGGCAYGG3' and Cu4R-5'TGCTCVAGBAKRTGGCAGTG-3'. Reaction mixture constitutes: 20 pm.μL⁻¹ Primers (forward and reverse), 10 mM dNTPs mix, 10x Assay buffer with MgCl₂, 3.0 U.μL⁻¹ TaqDNA polymerase, 10 mg.mL⁻¹ BSA 3 and 50 ng.μL⁻¹ Template DNA. Reaction conditions maintained were: Initial denaturation at 94 °C for 3min followed by denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec, and from second phase repeat of 35 cycles, followed by extension at 72 °C for 1min and final extension at 72 °C for 5 min.

Results

Selection of pesticides degrading bacterial isolate

Four pesticide utilizing bacterial isolates were recovered from pesticide contaminated agricultural fields using plate assay. Three bacterial isolates (2A, 2B and 2C) were able to grow at 300 mg.L⁻¹ of the selected pesticides but only bacterial isolate (2D) was able to grow upto 450 mg.L⁻¹ of the pesticides. Performance of 2D bacteria under laboratory condition (grew on cypermethrin, fipronil, imidacloprid and sulfosulfuron at 450 mg.L⁻¹) enabled us to investigate the biodegradation potential of the bacteria.

In vitro biodegradation of pesticides in minimal medium

Gas chromatography (GC) results of standard of imidacloprid, fipronil and sulfosulfuron revealed the presence of major single peak of each pesticide at 4.3, 4.9, and 6.3 min (retention time) respectively under optimized conditions of the instrument (S1A–S1C Fig). On the other hand cypermethrin showed four independent major peaks during a retention time of 16–17.65. These major peaks indicated at different retention time includes at 16.13 min indicate cis α, 17.00 min indicate cis β, 17.40 min indicate trans α and 17.62 min indicate trans β

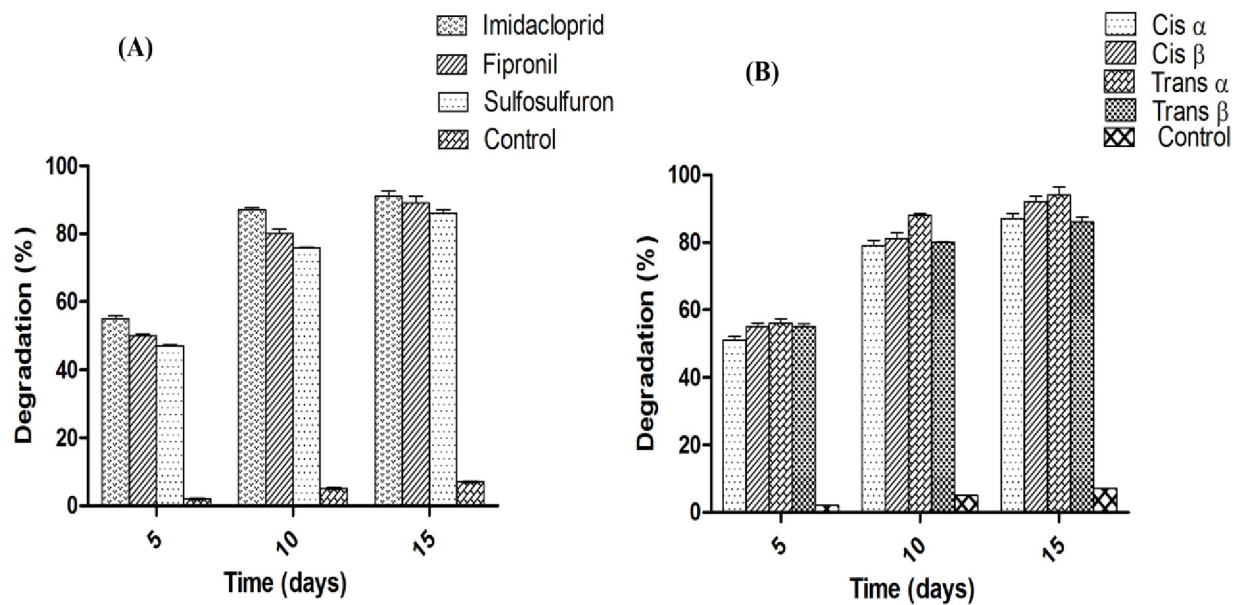


Fig 1. Biodegradation of imidacloprid, fipronil, sulfosulfuron and cypermethrin in minimal medium by 2D bacterial isolate. The graph denotes % degradation (Y axis) versus times in days (X-axis), where the numbers 5, 10 & 15 represent the withdrawal of sample(s) at different time interval. (A) Represents the biodegradation of imidacloprid, fipronil, sulfosulfuron while (B) represents the biodegradation of different isomers of cypermethrin.

<https://doi.org/10.1371/journal.pone.0253106.g001>

isomers of cypermethrin, (S1D Fig). Isolate 2D showed maximum degradation of cypermethrin (94%) followed by imidacloprid (91%), fipronil (89%) and sulfosulfuron (86%) after 15 days of incubation in minimal medium (Fig 1).

The intermediate metabolites of the cypermethrin were analyzed. Retention timings of different biodegraded products of cypermethrin under the treatment of bacterial isolate 2D were: 4.005, 25.018, 26.311, 28.286, 29.024 and 29.025 min which corresponded to 3-penten-1-ol and 1-[(1-oxo-2-propenyl) oxy]-2,5-pyrrolidin, 3-buten-2-one, 4,4-bis (dimethylamino) 4-quinolinol, 1-ethyl-4-ethynyldecahydro, cyclobutane, pyrrolidine, formic acid, decyl ester, 2h-pyrido[1,2-f][1,6] diazacyclooctadecin, acetic acid, n-[4-cyclooctylaminobutyl] aziridine, chloroacetic acid and octahydropyrrolo [1,2-a] pyrazine respectively (S2 Fig).

Characterization of the bacterial isolate

Phylogenetic analysis of amplified 16S rDNA gene sequences revealed 99% homology with *Bacillus cereus* of bacterial strain 2D (S3 and S4 Figs). Gene sequences were submitted to GenBank and the organism was provided with an accession numbers (Accession No. MH341691).

Enzyme kinetics

Laccase enzyme activity of test bacterial isolate was examined quantitatively and qualitatively for its possible role in degradation of cypermethrin. Biodegradation of pesticides is an enzyme-catalyzed transformation of organic compounds into their simpler products. Specificity of laccase enzyme make it crucial for degradation of huge variety of toxic environmental pollutants.

Michaelis-Menten equation was solved in best way by plotting the data as $1/r$ vs $1/S$ to get a straight-line equation [32,37,38] (Fig 2). In the presence and absence of cypermethrin,

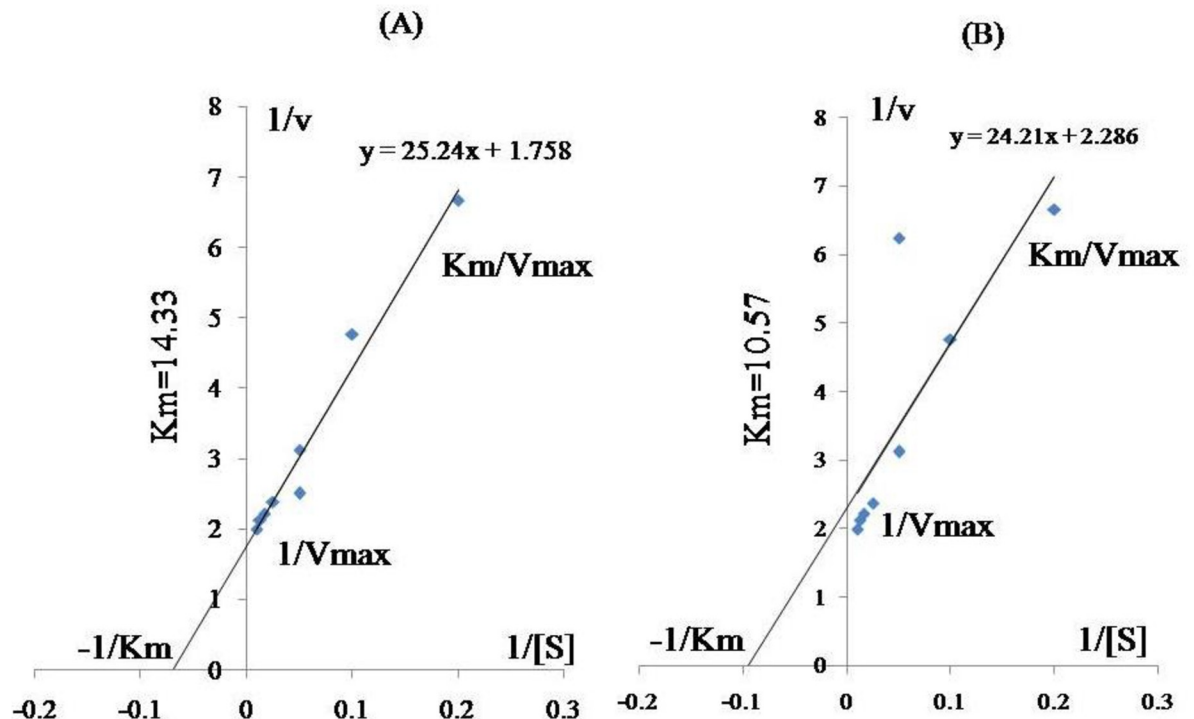


Fig 2. Lineweaver-Burke plot of laccase in bacterial isolate 2D (plot A and B). Plot A shows laccase activity under normal condition while plot B shows laccase activity in minimal medium with pesticide.

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bacterial isolate (2D) produced 163 and 29 $\mu\text{g}\cdot\mu\text{L}^{-1}$ laccase respectively. Significant difference in K_m values was observed with and without pesticide for laccase enzyme. K_m values for laccase in 2D under stress and normal condition were 10.57 and 14.33 mM respectively.

Laccase gene amplification

Amplicon of laccase gene was run in gel electrophoresis. Laccase amplification was found positive in test bacterial isolate and the size of amplicon for laccase was approximately 1200 bp (Fig 3).

Comparative analysis of the pesticide induced proteins in bacterial strain 2D under stress and normal conditions

SDS PAGE was performed to analyse the expression of pesticide induced proteins in bacterial culture (2D) in the presence of cypermethrin ($50 \text{ mg}\cdot\text{L}^{-1}$). Size of different proteins bands were obtained ranged from 10 to 202 KD (S5 Fig). Ten different protein bands were observed under stress condition as compared to normal condition. Further two-dimensional electrophoresis (2D electrophoresis) of the proteins was carried out in the absence and presence of pesticides (S6 and S7 Figs) and major proteins were categorized in five classes namely: uncharacterized proteins, stress response proteins, catabolic proteins, energy production/chemotaxis, gene regulation/transcription and protein synthesis/modification. Expression of protein by 2D was different under stress and normal conditions which could be differentiated on the basis of number of protein spots. Approximately 60 to 80 protein spots were found in the gel. We have characterized only 50 protein spots from each gel which were related to our experiment. Bacterial strain (2D) expressed 14% proteins for stress response, 32% proteins involved in protein

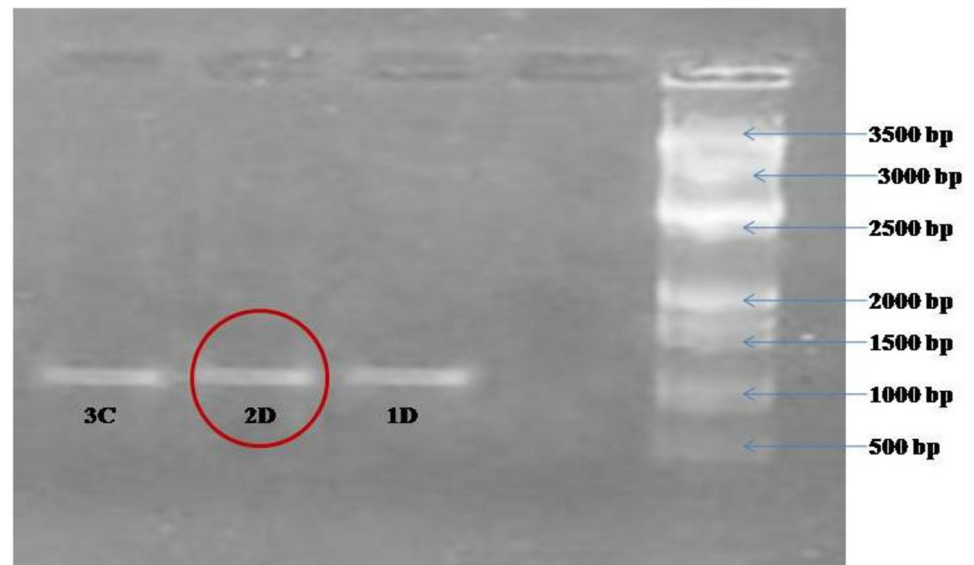


Fig 3. Amplification of Laccase gene from bacterial isolate 2D.

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synthesis and modification, 14% in gene regulation, 20% in energy production, 18% catabolic/pesticide degrading proteins and 2% were uncharacterized proteins under normal condition. Under stress, percent expression of protein was different and the order was: 30% stress response proteins, 22% of proteins involved in protein synthesis and modification, 12% in gene regulation, 14% in energy production, 22% catabolic/pesticide degrading protein and 2% uncharacterized proteins (Fig 4).

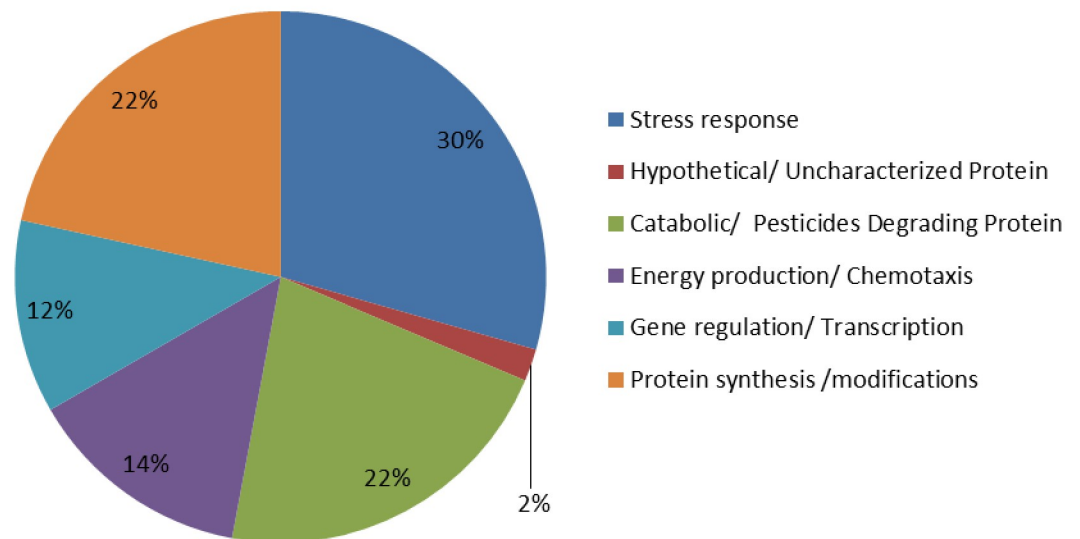
Discussion

In the present study, interactions with the local farmers, survey of the nearby areas and the literature citations led to the findings that imidacloprid, cypermethrin, fipronil and sulfosulfuron are some common pesticides which are being used by the farmers in different cropping systems [39]. Presence of imidacloprid ($0.42\mu\text{g.g}^{-1}$), cypermethrin ($0.35\mu\text{g.g}^{-1}$) and fipronil ($0.26\mu\text{g.g}^{-1}$) and sulfosulfuron ($0.18\mu\text{g.g}^{-1}$) was found in soil samples. Degree of contamination in soil varies from less polluted (less than $\leq 0.5\text{ mg.kg}^{-1}$) to moderately polluted (in between 0.5 to 1 mg.kg^{-1}) to heavily polluted (more than $\geq 1\text{ mg.kg}^{-1}$) based on environmental quality standards [40]. Hence four pesticides (cypermethrin, fipronil, imidacloprid and sulfosulfuron) were selected in the present study for microbial biodegradation. The similar residual analysis of pesticides (carbendazim, endosulfan and imidacloprid) from the agricultural soil was done by the several researchers [25,26]. Our pesticide utilizing/tolerant bacterial isolates were recovered from pesticide polluted agricultural soil using plate assay.

Performance of 2D bacteria under laboratory condition (grew on cypermethrin, fipronil, imidacloprid and sulfosulfuron at 450 mg.L^{-1}) enabled us to investigate the biodegradation potential of the bacteria. Cypermethrin, endosulfan, imidacloprid, fipronil and sulfosulfuron degrading bacterial and fungal strains were also isolated from the rhizospheric fields by several authors [12,41–43].

Bacterial strain (2D) tolerated 450 mg.L^{-1} of all the four pesticides and degraded upto 20 mg.L^{-1} of the same in MSM medium. This indicates ability of bacterial isolate for pesticide degradation in different environments, as they can survive and degrade the toxic compounds on

2DS



2DN

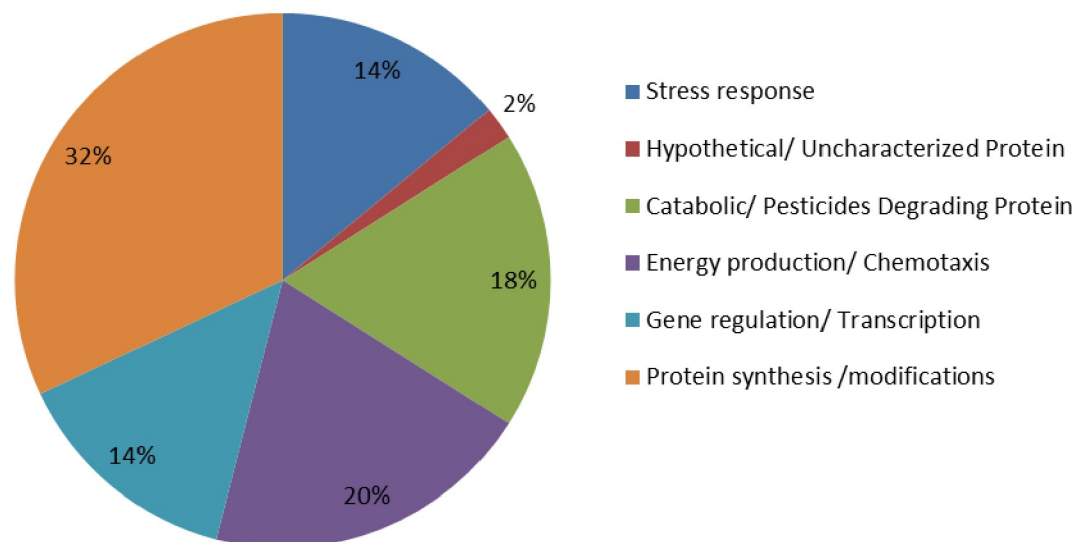


Fig 4. Expressed protein in normal (without pesticide) (2DN) and pesticide stress condition (2DS) for bacterial strain 2D.

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exposure of their higher concentrations. Recently similar study has been done in this field and found that *Bacillus spp.* tolerated carbendazim (150 mg.L^{-1}), *Microbacterium* tolerated 172 mg.L^{-1} of endosulfan *Pseudomonas spp.* tolerated 150 mg.L^{-1} of imidacloprid and *Pseudomonas* tolerated 30 mg.L^{-1} of lindane [26,44]. Therefore the bacterial isolate used in present study showed more tolerance for the pesticide(s) than those used by Nawab et al. and Negi et al. [26,44]. Bacterial strain 2D could degrade all the tested pesticides at higher concentration.

Changes of regular expression of some enzyme(s) involved in degradation of imidacloprid, fipronil, cypermethrin and sulfosulfuron at increased concentrations by 2D cannot be ignored. On the basis of our experiment on tolerance for pesticides by 2D, we presume that metabolic activity of our strain was not subjected to catabolic repression at higher concentration of the pesticides. *Bacillus cereus* (strain 2D) potential of degradation and tolerance to pesticides (higher concentration) proved it as a suitable strain for remediation of polluted sites.

Isolated bacterial strain *Bacillus cereus* 2D showed higher range of pesticide degradation potential (94–86%) within shorter period of time (15 days). Pankaj et al. and Bhatt et al. [43,45] have reported biodegradation of cypermethrin using *Bacillus* spp. and found 85% degradation after 15 days under similar conditions. *Bacillus subtilis* BSF01 has been also reported for their schematic growth linked biodegradation pathway of β -cypermethrin [46]. Microorganisms used in biodegradation need an acclimatization period to induce synthesis of enzymes for biodegradation that may account for prolonged lag phase, which was observed at higher concentrations of cypermethrin, fipronil and sulfosulfuron [47]. *Bacillus* sp. FA3 degrades fipronil (76%) within 15 days, isolated from agricultural field and effective for its removal from water and soil environment [38]. Hence our bacterial strain *Bacillus cereus* 2D is more efficient than the previously reported bacteria for the removal of mixture of pesticide from the natural environment [38].

Toxicity of metabolites produced during cypermethrin degradation was checked by inoculating it (fifteen days old broth with metabolites) with fresh bacterial culture. Results proved these metabolites as nontoxic due to no effects on bacterial growth even after complete breakdown of pesticide. In the biodegradation experiment using 2D, a common intermediate of cypermethrin i.e., 3-Phenoxy benzoic acid was absent. It can be proposed that 3-PBA may be converted into smaller compounds like cyclobutane, chloroacetic acid and acetic acid. After molecular characterization of bacterial isolate 2D, it showed maximum similarity (99%) with *Bacillus cereus*. Several researchers displayed degradation abilities of *Bacillus* species of pesticides (such as profenos and cypermethrin) and other xenobiotic compounds [12,46–48].

Supplementation of pesticide with *Bacillus cereus* 2D, growing in minimal medium, showed reduced K_m values as compared to higher K_m values obtained under normal conditions, which indicates laccase production induced by addition of pesticide. This confirms competitive inhibition as the reason behind enzyme production. Laccase shows conformational changes, efficient activity and lower K_m values in presence of pesticide. Increase in laccase activity and decreased k_m value under stress condition demonstrated that enzyme production increases under stressed conditions as compared to the normal condition. Production of laccase is high which helps bacterial isolates to overcome from the pesticide stress and make them comfortable to breakdown and utilize pesticides as carbon and energy source. Michaelis-Menten equation defines K_m as the total substrate present at half of its maximum velocity (V_{max}) [49]. Results indicate that to overcome the pesticide stress, the bacterial strain induces laccase enzyme. Oxidation of various inorganic and organic compounds (both phenolic and non-phenolic substrates) was catalysed by laccase *via* reduction of molecular oxygen to water [50]. Several biotechnological applications of laccase includes plastic degradation, xenobiotics bioremediation, bioleaching, decolourization of textile dyes, biosensors, lignin degradation, food industry, and biofuels production etc. [51–53]. Gangola and co-workers [12], reported efficient detoxification and degradation of cypermethrin by soil inhabitant *Bacillus subtilis* 1D with esterase and laccase activity. Laccase concentration in presence of cypermethrin was $62 \mu\text{g}\cdot\mu\text{L}^{-1}$ with K_m value 61.57 mM and in its absence was $42 \mu\text{g}\cdot\mu\text{L}^{-1}$ with K_m value 83 mM after 15 days [12]. *Pseudomonas putida* MTCC 7525 was isolated from the soil sample containing sawdust and dairy effluent showed optimum laccase production (94.10 U/ml) at pH 8 and incubation at 30 °C with 1mM sodium nitrate as N source and 10% skim milk [54].

Presence of laccase gene was found in *Bacillus cereus* 2D and the size of amplicon for laccase was approximately 1200 bp (Fig 3). Our results were similar to findings of Ausec et al. [36] as amplicon size of 1200 bp in *B. cereus* 2D was observed. By using primers it was confirmed that laccase encoding genes exists in bacterial genome and may get activated in stressed conditions in presence of pesticides). This gene is major regulatory gene and found responsible for the degradation of imidacloprid, fipronil, sulfosulfuron and cypermethrin. These pesticide have some common chemical bonds in their structures hence could be degraded by similar laccase. Presence of laccase gene has been already reported in *Azospirillum* sp. [55], *Pseudomonas syringae* [56] and *B. subtilis* [12]. Fungal laccase enzyme for degradation of liluron, chlorpyrifos and metribuzin was reported by Gouma, [57]. The release of extracellular laccase from *Bacillus cereus* allows degradation and decolorization of dye [58]. More studies were available on pesticide degradation by fungal laccase as compared to bacterial laccase. So, bacterial species with laccase activity makes them prominent candidate for degradation of variety of pollutants. The presence of laccase in *Bacillus cereus* (2D) is the first report in context of pesticide degradation.

Proteomic study revealed that under stress condition, percentage of stress responsive proteins and catabolic/pesticide degrading proteins was high, which make the organism more comfortable under such environment. There is direct involvement of proteins in pesticide degradation. Expression of such protein encoding genes have essential role in breakdown of toxic organic pollutant [59]. Proteomics explored degradation of PCB (polychlorinated biphenyl) by microbes at molecular level [60]. Moreover, Pankaj and co-workers [61], reported expression of 250 proteins under stressed conditions (cypermethrin) and 223 proteins under normal conditions in *Bacillus* spp. These unique proteins were further classified into different groups based on their functionality, viz., translational proteins, stress proteins and catabolic enzymes etc. Up-regulation of proteins responsible for substrate transport and energy metabolism during pesticide degradation was reported in different microorganisms [7,45,62].

Conclusion

Present study recommends the application of indigenous microorganisms in biodegradation of common agriculture pesticides. Because in stress conditions bacteria could change their genetic profile and easily induce mutant strains, which can adopt in different environmental condition by activating their vast range of biochemical metabolism diversity, hence need to indepth study in this regard. The use of proteomics tools for the purpose of environmental bioremediation gives detailed information of microbial cells protein and composition. Hence it offers a valuable approach to decipher the mechanisms involved in bioremediation at molecular level. Diversity, composition and metabolic potential of soil microbiome is of crucial importance in bioremediation. In addition soil microbial communities also regulate biogeochemical cycling. Hence, detailed analysis of microbial communities at functional and structural level will guide to monitor and assess the effect of pesticides on soil health and their biological status respectively. Therefore, based on the biodegradation potential and wide range of activity for different substrates, the *Bacillus cereus* 2D could be a strong recommendation for the biodegradation of pesticides. For future prospects the activity of bacterial isolate could be check against the other xenobiotic compounds and the mechanism of gene regulation.

Supporting information

S1 Fig. Standard peaks of imidacloprid (a), fipronil(b), sulfosulfuron(c) and cypermethrin(d) at 10 ppm concentration.
(TIF)

S2 Fig. GC-MS chromatogram of cypermethrin intermediates under the treatment of bacterial isolate 2D after 15 days.

(TIF)

S3 Fig. 16S rDNA amplification of bacterial isolate (2D) denoted by red encircled.

(TIF)

S4 Fig. Phylogenetic analysis of *Bacillus cereus* 2D by using neighbor joining method.

(TIF)

S5 Fig. Analysis of expression of extracellular proteins under normal and stress condition (presence of pesticides) in bacterial strain 2D. Bacterial strains designated as 2D in lane number 5 and 2DN in lane number 4 respectively under stress and normal condition. The bacterial proteins expressed in stress conditions are denoted by highlighted encircled.

(TIF)

S6 Fig. 2D- electrophoresis for strain 2D in normal condition (2DN).

(TIF)

S7 Fig. 2D- electrophoresis for strain 2D in pesticide stress condition (2D).

(TIF)

S1 File. Clarification regarding images.

(DOCX)

Acknowledgments

Authors are acknowledging the Department of Microbiology and Department of Chemistry, G.B. Pant University of Agriculture and Technology, Pantnagar for providing all the laboratory facility during the study.

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References

1. Food and Agriculture Organization of the United Nations. Available online: <http://www.fao.org/faostat/en/#data/QC> (accessed on 19 August 2018).
2. Walter GH, Chandrasekaran S, Collins PJ, Jagadeesan R, Mohankumar S, Alagusundaram K, et al. The grand challenge of food security: general lessons from a comprehensive approach to protecting

- stored grain from insect pests in Australia and India. *Indian J Entomol.* 2016; 78:7–16. <https://doi.org/10.5958/0974-8172.2016.00020.1>
3. Singh BK, Walker A. Microbial degradation of organophosphorus compounds. *FEMS Microbiol Rev.* 2006; 30:428–471. <https://doi.org/10.1111/j.1574-6976.2006.00018.x> PMID: 16594965
 4. Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, et al. Solutions for a cultivated planet. *Nature.* 2011; 478(7369):337. <https://doi.org/10.1038/nature10452> PMID: 21993620
 5. Uniyal S, Paliwal R, Verma M, Sharma RK, Rai JPN. Isolation and characterization of fipronil degrading *Acinetobacter calcoaceticus* and *Acinetobacter oleivorans* from rhizospheric zone of *Zea mays*. *Bull Environ Contam Toxicol.* 2016; 96(6):833–838. <https://doi.org/10.1007/s00128-016-1795-6> PMID: 27084098
 6. Rajmohan KS, Chandrasekaran R, Varjani S. A review on occurrence of pesticides in environment and current technologies for their remediation and management. *Indian J Microbiol.* 2020;1–4. <https://doi.org/10.1007/s12088-019-00841-x> PMID: 32255845
 7. Bhatt P, Bhatt K, Huang Y, Lin Z, Chen S. Esterase is a powerful tool for the biodegradation of pyrethroid insecticides. *Chemosphere.* 2020; 244:125507. <https://doi.org/10.1016/j.chemosphere.2019.125507> PMID: 31835049
 8. Gangola S. Impact assessment of pesticides on soil health through conventional and metagenomic approaches and their biodegradation using indigenous microbes. (Doctoral dissertation, GB Pant University of Agriculture and Technology, Pantnagar-263145 (Uttarakhand)). 2019.
 9. Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. *Nature.* 2002; 418(6898):671–677. <https://doi.org/10.1038/nature01014> PMID: 12167873
 10. Verma P, Verma P, Sagar R. Variations in N mineralization and herbaceous species diversity due to sites, seasons, and N treatments in a seasonally dry tropical environment of India. *For Ecol Manag.* 2013; 297:15–26. <https://doi.org/10.1016/j.foreco.2013.02.006>
 11. Wang WD, Niu JL, Cui ZJ. Biodegradation of Pesticides: A review. *J Heilongjiang August First Land Reclamation University.* 2005; 2.
 12. Gangola S, Sharma A, Bhatt P, Khatri P, Chaudhary P. Presence of esterase and laccase in *Bacillus subtilis* facilitates biodegradation and detoxification of cypermethrin. *Sci Rep.* 2018; 8(1):12755. <https://doi.org/10.1038/s41598-018-31082-5> PMID: 30143738
 13. Porto AM, Melgar GZ, Kasemodel MC, Nitschke M. Bio-degradation of pesticides, pesticides in modern world-pesticides use and management. ISBN. 2011;407–438.
 14. Zang H, Wang H, Miao L, Cheng Y, Zhang Y, Liu Y. Carboxylesterase, a de-esterification enzyme, catalyzes the degradation of chlorimuron-ethyl in *Rhodococcus erythropolis* D310-1. *J Hazard Mater.* 2020; 387:121684. <https://doi.org/10.1016/j.jhazmat.2019.121684> PMID: 31784128
 15. Akbar S, Sultan S. Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. *Braz J Microbiol.* 2016; 47(3):563–570. <https://doi.org/10.1016/j.bjm.2016.04.009> PMID: 27266625
 16. Jabeen H, Iqbal S, Anwar S. Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a novel rhizobial strain *Mesorhizobium* sp. HN3. *Water Environ J.* 2015; 29(1):151–160. <https://doi.org/10.1111/wej.12081>
 17. Ramya SL, Venkatesan T, Murthy KS, Jalali SK, Varghese A. Degradation of acephate by *Enterobacter asburiae*, *Bacillus cereus* and *Pantoea agglomerans* isolated from diamondback moth *Plutella maculipennis* (L), a pest of cruciferous crops. *J Environ Biol.* 2016; 37(4):611. PMID: 27498509
 18. Racke KD, Skidmore M, Hamilton DJ, Unsworth JB, Miyamoto J, Cohen SZ. Pesticide fate in tropical soils. *Pest Manag Sci.* 2015; 55:219–220. [https://doi.org/10.1002/\(SICI\)1096-9063\(199902\)55:2<219::AID-PS821>3.0.CO;2-Y](https://doi.org/10.1002/(SICI)1096-9063(199902)55:2<219::AID-PS821>3.0.CO;2-Y)
 19. Oliveira BR, Penetra A, Cardoso VV, Benoliel MJ, Crespo MB, Samson RA, et al. Biodegradation of pesticides using fungi species found in the aquatic environment. *Environ Sci Pollut Res.* 2015; 22(15):11781–11791. <https://doi.org/10.1007/s11356-015-4472-0> PMID: 25860553
 20. Bhatt P, Gangola S, Bhandari G, Zhang W, Maithani D, Mishra S, et al. New insights into the degradation of synthetic pollutants in contaminated environments. *Chemosphere.* 2020;128827. <https://doi.org/10.1016/j.chemosphere.2020.128827> PMID: 33162154
 21. Huang Y, Xiao L, Li F, Xiao M, Lin D, Long X, et al. Microbial degradation of pesticide residues and an emphasis on the degradation of cypermethrin and 3-phenoxy benzoic acid: a review. *Molecules.* 2018; 23(9):2313. <https://doi.org/10.3390/molecules23092313> PMID: 30208572
 22. Whittington HD, Singh M, Ta C, Azcárate-Peril MA, Bruno-Bárcena JM. Accelerated biodegradation of the agrochemical ametoctradin by soil-derived microbial consortia. *Front Microbiol.* 2020; 11:1898. <https://doi.org/10.3389/fmicb.2020.01898> PMID: 32982997

23. Jaiswal S, Shukla P. Alternative strategies for microbial remediation of pollutants via synthetic biology. *Front Microbiol.* 2020; 11:808. <https://doi.org/10.3389/fmicb.2020.00808> PMID: 32508759
24. Huang X, Lu G. Bioremediation of chemical pesticides polluted soil. *Front Microbiol.* 2021; 12. <https://doi.org/10.3389/fmicb.2021.682343> PMID: 34093509
25. Phartyal S. Characterization of lindane degrading Bacteria from tarai region of Uttarakhand and their potential assessment in bioremediation. Ph.D Thesis, GBPUA&T, Pantnagar, India. 2012.
26. Negi G, Pankaj, Shrivastawa A, Sharma A. *In situ* biodegradation of endosulfan, imidacloprid, and carbandazim using indigenous bacterial cultures of agriculture fields of Uttarakhand, India. *Int J Biol Food Vat Agric Eng.* 2014; 8(9):935–943.
27. Bazzicalupo M, Fani R. The use of RAPD for generating specific DNA probes for microorganisms. In *Species Diagnostics Protocols.* Humana Press. 1996;155–175. <https://doi.org/10.1385/0-89603-323-6:155>
28. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007; 24(8):1596–1599. <https://doi.org/10.1093/molbev/msm092> PMID: 17488738
29. Pankaj, Negi G, Gangola S, Khati P, Srivastava A, Sharma A. Optimization of sulfosulfuron biodegradation through response surface methodology using indigenous bacterial strain isolated from contaminated agriculture field. *Int J Curr Microbiol App Sci.* 2015; 4:105–112.
30. Kataoka R, Takagi K, Sakakibara F. A new endosulfan-degrading fungus, *Mortierella* species, isolated from a soil contaminated with organochlorine pesticides. *J Pestic Sci.* 2010;1003160118–1003160118. <https://doi.org/10.1584/jpestics.G10-10>
31. Kaur S, Khatri M, Singh G. Isolation and biochemical characterization of prokaryotic laccase. *Int J Adv Technol Eng Sci.* 2017; 5:637–640.
32. Chauhan A, Pandey G, Sharma NK, Paul D, Pandey J, Jain RK. p-Nitrophenol degradation via 4-nitro-catechol in *Burkholderia* sp. SJ98 and cloning of some of the lower pathway genes. *Environ Sci Technol.* 2010; 44(9):3435–3441. <https://doi.org/10.1021/es9024172> PMID: 20359211
33. Bueno PR, Watanabe AM, Faria RC, Santos ML, Riccardi CS. Electrogravimetric real-time and in situ Michaelis–Menten enzymatic kinetics: progress curve of acetylcholinesterase hydrolysis. *J Phys Chem B.* 2010; 114(49):16605–16610. <https://doi.org/10.1021/jp106274m> PMID: 21082796
34. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72(1–2):248–254. <https://doi.org/10.1006/abio.1976.9999> PMID: 942051
35. Jain A, Bacolla A, Chakraborty P, Grosse F, Vasquez KM. Human DHX9 helicase unwinds triple-helical DNA structures. *Biochem.* 2010; 49(33):6992–6999. <https://doi.org/10.1021/bi100795m> PMID: 20669935
36. Ausec L, Zakrzewski M, Goesmann A, Schlüter A, Mandic-Mulec I. Bioinformatic analysis reveals high diversity of bacterial genes for laccase-like enzymes. *PLoS One.* 2011; 6(10): e25724. <https://doi.org/10.1371/journal.pone.0025724> PMID: 22022440
37. Kovárová-Kovar K, Egli T. Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. *Microbiol Mol Biol Rev.* 1998; 62(3):646–666. <https://doi.org/10.1128/MMBR.62.3.646-666.1998> PMID: 9729604
38. Bhatt P, Sharma A, Rene ER, Kumar AJ, Zhang W, Chen S. Bioremediation of fipronil using *Bacillus* sp. FA3: Mechanism, kinetics and resource recovery potential from contaminated environments. *J Water Process Eng.* 2020;101712. <https://doi.org/10.1016/j.jwpe.2020.101712>
39. Moghaddam NS, Zakaria MP, Omar D, Sijam K. Extraction efficiency and HPLC determination of imidacloprid in soil. *Soil Sediment Contam: An Int J.* 2012; 21(8):985–995. <https://doi.org/10.1080/15320383.2012.712071>
40. Xiaofei W, Dianzhong W, Xiaofei Q, Xiaobai X. Residues of organochlorine pesticides in surface soils from college school yards in Beijing, China. *J Environ Sci.* 2008; 20(9):1090–1096. [https://doi.org/10.1016/S1001-0742\(08\)62154-3](https://doi.org/10.1016/S1001-0742(08)62154-3)
41. Gangola S, Khati P, Sharma A. Mycoremediation of imidaclopridin the presence of different soil amendments using *Trichoderma longibrachiatum* and *Aspergillus oryzae* isolated from pesticide contaminated agricultural fields of Uttarakhand. *J Bioremediat Biodegr.* 2015; 6(5):1. <https://doi.org/10.4172/2155-6199.1000310>
42. Gangola S, Pankaj, Negi G, Srivastava A, Sharma A. Enhanced biodegradation of endosulfan by *Aspergillus* and *Trichoderma* spp. isolated from an agricultural field of tarai region of Uttarakhand. *Pestic Res J.* 2015; 27(2):223–230.

43. Pankaj, Sharma A, Khati P, Kumar G, Gangola S, Srivastava A. Novel pathway of cypermethrin biodegradation in a *Bacillus* Sp. strain SG2 isolated from cypermethrin contaminated agriculture field. 3-Bio-tech. 2016; 6:45. <https://doi.org/10.1007/s13205-016-0372-3> PMID: 28330116
44. Nawab A, Aleem A, Malik A. Determination of organochlorine pesticides in agricultural soil with special reference to γ -HCH degradation by *Pseudomonas* strains. Bioresour Technol. 2003; 88(1): 41–46. [https://doi.org/10.1016/s0960-8524\(02\)00263-8](https://doi.org/10.1016/s0960-8524(02)00263-8) PMID: 12573562
45. Bhatt P, Gangola S, Chaudhary P, Khati P, Kumar G, Sharma A, et al. Pesticide induced up-regulation of esterase and aldehyde dehydrogenase in indigenous *Bacillus* spp. Bioremediat J. 2019; 23(1):42–52. <https://doi.org/10.1080/10889868.2019.1569586>
46. Xiao Y, Chen S, Gao Y, Hu W, Hu M, Zhong G. Isolation of a novel beta-cypermethrin degrading strain *Bacillus subtilis* BSF01 and its biodegradation pathway. Appl Microbiol Biotechnol. 2015; 99(6):2849–2859. <https://doi.org/10.1007/s00253-014-6164-y> PMID: 25398281
47. Dubey KK, Fulekar MH. Investigation of potential rhizospheric isolate for cypermethrin degradation. 3 Biotech. 2013; 3(1):33–43. <https://doi.org/10.1007/s13205-012-0067-3> PMID: 28324344
48. Arora PK. Decolourization of 4-chloro-2-nitrophenol by a soil bacterium, *Bacillus subtilis* RKJ 700. PLoS One. 2012; 7(12):e52012. <https://doi.org/10.1371/journal.pone.0052012> PMID: 23251673
49. Salunkhe AB, Khot VM, Thorat ND, Phadatare MR, Sathish CI, Dhawale DS, et al. Polyvinyl alcohol functionalized cobalt ferrite nanoparticles for biomedical applications. Appl Surf Sci. 2013; 264:598–604. <https://doi.org/10.1016/j.apsusc.2012.10.073>
50. Jablonski MR, Shaligram S, Qureshi A, Purohit HJ, Reisel JR. Degradation kinetics of resorcinol by *Enterobacter cloacae* isolate. African J Microbiol Res. 2013; 7(28):3632–3640.
51. Sondhi S, Saini K. Response surface based optimization of laccase production from *Bacillus* sp. MSK-01 using fruit juice waste as an effective substrate. Heliyon. 2019; 5(5):e01718. <https://doi.org/10.1016/j.heliyon.2019.e01718> PMID: 31193314
52. Kudanga T, Le Roes-Hill M. Laccase applications in biofuels production: current status and future prospects. Appl Microbiol Biotechnol. 2014; 98(15):6525–6542. <https://doi.org/10.1007/s00253-014-5810-8> PMID: 24841120
53. Sondhi S, Kaur R, Kaur S, Kaur PS. Immobilization of laccase-ABTS system for the development of a continuous flow packed bed bioreactor for decolorization of textile effluent. Int. J Biol Macromol. 2018; 117:1093–1100. <https://doi.org/10.1016/j.ijbiomac.2018.06.007> PMID: 29885397
54. Kuddus M, Joseph B, Ramteke PW. Production of laccase from newly isolated *Pseudomonas putida* and its application in bioremediation of synthetic dyes and industrial effluents. Biocatal Agric Biotechnol. 2013; 2(4):333–338.
55. Givaudan A, Effosse A, Faure D, Potier P, Bouillant ML, Bally R. Polyphenol oxidase in *Azospirillum lipoferum* isolated from rice rhizosphere: evidence for laccase activity in non-motile strains of *Azospirillum lipoferum*. FEMS Microbiol Lett. 1993; 108(2):205–210. <https://doi.org/10.1111/j.1574-6968.1993.tb06100.x>
56. Mellano MA, Cooksey DA. Nucleotide sequence and organization of copper resistance genes from *Pseudomonas syringae* pv. tomato. J Bacteriol. 1988; 170(6):2879–2883. <https://doi.org/10.1128/jb.170.6.2879-2883.1988> PMID: 3372485
57. Gouma S. Biodegradation of mixtures of pesticides by bacteria and white rot fungi. 2009.
58. Allos MM, Hussein AA. Optimum conditions for laccase production by local isolate of *Bacillus Cereus* B5. J Al-Nahrain University-Sci. 2018; 18(2):133–140. <https://doi.org/10.22401/JNUS.18.2.17>
59. Colbert CL, Agar NY, Kumar P, Chakko MN, Sinha SC, Powlowski JB, et al. Structural characterization of *Pandoraea pnomenusa* B-356 biphenyl dioxygenase reveals features of potent polychlorinated biphenyl-degrading enzymes. PLoS One. 2013; 8(1):e52550. <https://doi.org/10.1371/journal.pone.0052550> PMID: 23308114
60. Kim SJ, Kweon O, Cerniglia CE. Proteomic applications to elucidate bacterial aromatic hydrocarbon metabolic pathways. Curr Opin Microbiol. 2009; 12(3):301–309. <https://doi.org/10.1016/j.mib.2009.03.006> PMID: 19414279
61. Pankaj, Negi G, Gangola S, Khati P, Kumar G, Srivastava A, et al. Differential expression and characterization of cypermethrin degrading potential proteins in *Bacillus thuringiensis* strain, SG4. 3-Biotech. 2016; 6:225. <https://doi.org/10.1007/s13205-016-0541-4> PMID: 28330297
62. Cao B, Loh KC. Catabolic pathways and cellular responses of *Pseudomonas putida* P8 during growth on benzoate with a proteomics approach. Biotechnol Bioeng. 2008; 101(6):1297–1312. <https://doi.org/10.1002/bit.21997> PMID: 18980183