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CONTAMINATED SITES 2022

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The activity has been implemented within the framework of national project
Information and providing advice on improving the quality of environment in Slovakia.
The project is cofinanced by Cohesion Fund of the EU under Operational programme Quality of Environment.

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HEAVY METAL TOLERANCE AND PESTICIDE DEGRADATION BY TWO SOIL BACTERIA ISOLATED FROM AGRICULTURAL FIELD OF WEST BENGAL, INDIA- A BIOREMEDIATION APPROACH

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INTRODUCTION

- Agricultural and Industrial revolutions are utmost resources for human civilization.
- Speedy industrialization and indiscriminate use of agrochemicals resulted severe environmental pollution.
- These pollutants cause serious damage to all the living beings including human.
- Some of these recalcitrant chemicals like pesticides and heavy metals not only kill the harmful pests but also different beneficial microbes like PGPR, PGPF etc.

Contd…

- These chemical pesticides are bio-accumulated and showed toxicity to human after bio-magnification.
- Physico-chemical based methods of remediation are costly, causes disposal problem.
- It also generate new secondary recalcitrant pollutants.
- Implementation of bioremediation method is necessary for environmental sustainability.

Bioremediation

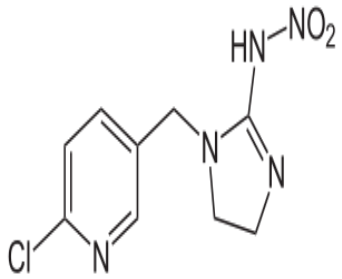
- This is the method for clean up of our environ- ment by degradation of toxic pollutants into non-toxic or less-toxic substances by some living organisms.
- Various bacterial species such as *Bacillus subtilis, B. amyloliquefaciens, B. pasteurii, B. cereus, B. pumilus, B. mycoides and B. sphaericus , B. pumilus, B. pasteurii, B. mycoides, B. sphaericus, Pseudomonas. polymyxa, P. azotofixans, p. putida* etc.
- *Some fungal species like Pleurotus, Trichoderma, Phanerochaete etc. are also help in bioremediation*

Aims and Objectives

- Isolation and characterization of pesticide and heavy metal resistance/ degrading soil bacteria.
- Study of degradation of some common pesticides
- Study of metal tolerance
- Role of plasmid in resistance/ degradation of pesticides or heavy metals

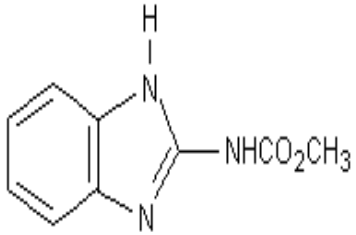
IMIDACLOPRID

- Imidacloprid is a **neonicotinoid Insecticides.**
- Neonicotinoids are synthetic forms of nicotine, and act on the nicotinic receptors of the **nervous system** by causing nerves to fire continually until they **fail**.



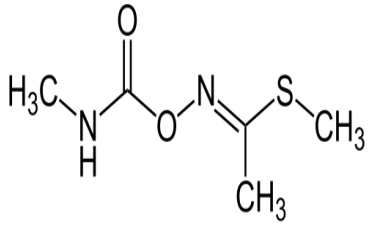
CARBENDAZIM

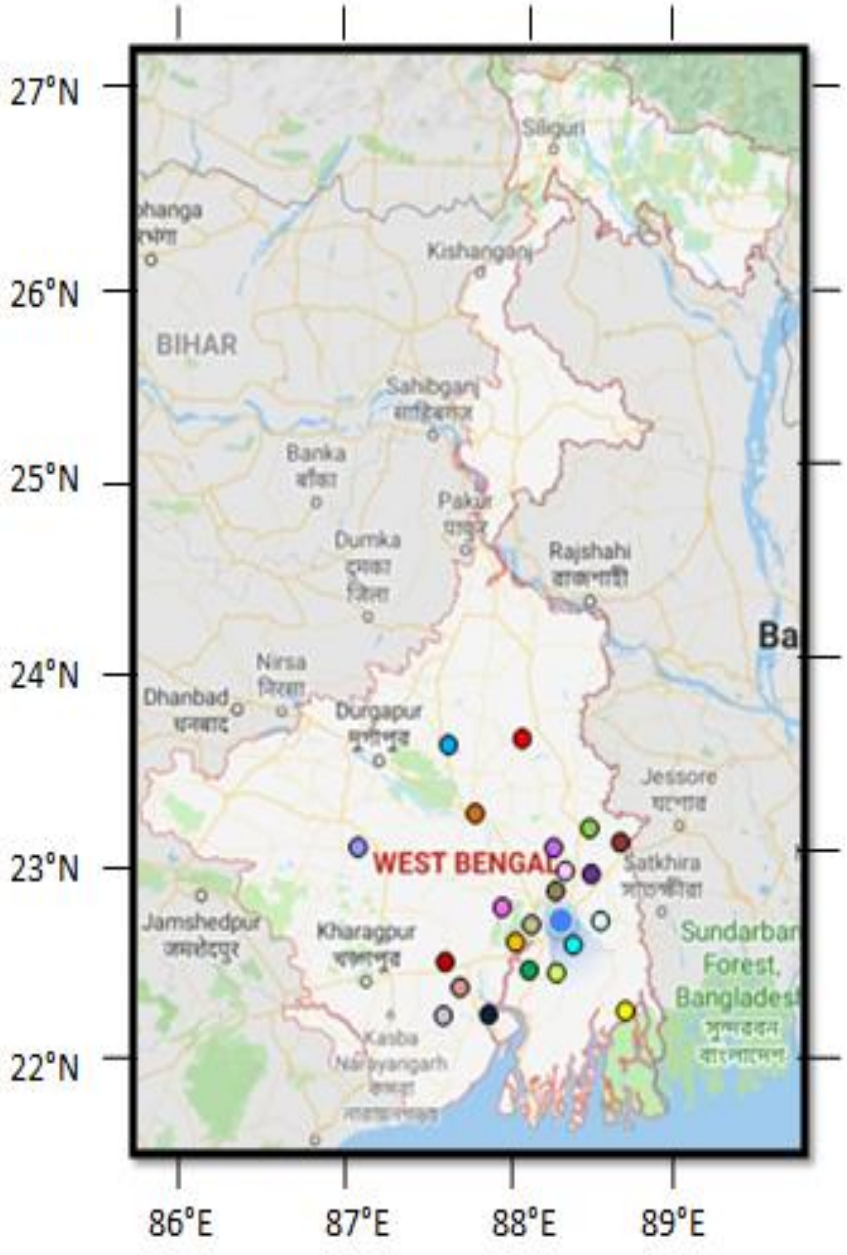
- Carbendazim is a broad-spectrum **benzimidazole fungicide** with potential antimitotic and antineoplastic activities.
- This results in **cell cycle arrest** at the G2/M phase and an induction of apoptosis.



METHOMYL

- Methomyl is a **Carbamate** type of **insecticide**
- Methomyl is potentially a highly poisonous compound for humans.
- It is highly toxic as it inhibits cholinesterase, an essential nervous system enzyme.





- Ramrajatala, Howrah
- Amta, Howrah
- Bauria, Howrah
- Katwa, Bardwan
- Naihati, North 24 parganas
- Kasba, East Midnapur
- Bolpur, Birbhum
- Kalyani, Nadia
- Chakdaha, Nadia
- Bardwan
- Moyna, East Midnapur
- Bankura
- Boyalghata, North 24 parganas
- Baruipur, South 24 parganas
- Bongaon, North 24 parganas
- Pakhiralay, South 24 parganas
- Mahishadal, East Midnapur
- Koalipota, North 24 parganas
- Rajarhat, North 24 parganas
- Amdanga, North 24 parganas
- Panskura, West Midnapur
- Bar kasimpur, East Midnapur

SAMPLE	LOCATION	CROP	CARBON CONTENT (%)	ORGANIC MATTER (%)	CHLORIDE CONTENT (%)	MOISTURE CONTENT (%)	MOISTURE HOLDING CAPACITY (%)	Na (mg/gm)	K (mg/gm)	Ca (mg/gm)	pH
1	BHAGABANPUR, MIDNAPUR (E)	*Abelmoscus esculentus* OKRA	0.26	0.44	21.3	21.21	50	0.632	0.48	1.24	6.8
2	PANSKURA, MIDNAPUR (E)	MULTICROP	0.34	0.58	18.10	13.63	78	0.116	3.430	2.01	6.4
3	AMDANGA, NORTH 24 PARGANA	*Lens culinaris* LENTIL	0.32	0.55	7.45	29.87	65	0.607	0.172	0.998	6.5
4	AMDANGA, NORTH 24 PARGANA	*Abelmoschus esculentus* OKRA	0.16	0.27	38.34	23.45	17.50	0.774	1.24	1.84	6.5
5	RAJARHAT, NORTH 24 PARGANA	MULTICROP	0.26	0.44	3.19	20.96.	37.50	0.369	0.432	0.711	6.5
6	CHANDIIPUR, MIDNAPUR (E)	*Abelmoschus esculentus* OKRA	0.32	0.55	13.48	25	63	0.527	2.615	1.488	6
7	MOYNA, MIDNAPUR (E)	MULTICROP	0.10	0.17	10.65	17.64	32.5	0.658	0.36	1.098	6.5
8	ASOKNAGAR NORTH 24 PARGANA	*Oryza sativa* PADDY	0.22	0.37	7.47	20.48	40	0.369	0.434	0.711	7
9	ASOKNAGAR NORTH 24 PARGANA	MULTICROP	0.22	0.37	80.94	33.03	40	0.687	0.429	1.534	6.5

SAMPLE	LOCATION	CROP	CARBON CONTENT (%)	ORGANIC MATTER (%)	CHLORIDE CONTENT (%)	MOISTURE CONTENT (%)	MOISTURE HOLDING CAPACITY (%)	Na (mg/gm)	K (mg/gm)	Ca (mg/gm)	pH
10	Bashirhat North 24 parganas	*Capsicum annuum* CHILLI	0.30	0.51	10.65	13.20	40	0.258	7.901	1.428	6.5
11	RAMRAJATALA HOWRAH	*Brassica oleracea* CABBAGE	0.37	0.63	10.65	91.30	22.5	0.115	3.810	1.057	6.8
12	RAMRAJATALA HOWRAH	MULTICROP	0.26	0.44	17.04	2.04	27.5	0.092	7.091	1.366	6.2
13	NAIHATI NORTH 24 PARGANA	*Daucus carota* CARROT	0.24	0.41	28.75	21.58	40	0.369	0.434	0.711	6.2
14	NAIHATI NORTH 24 PARGANA	*Daucus sp.* CARROT	0.22	0.37	35.14	23.07	30	0.59	2.043	1.179	6.5
15	NAIHATI NORTH 24 PARGANA	*Abelmoschus esculentus* OKRA	0.18	1.13	13.84	20.84	30	0.473	2.726	0.279	6.3
16	HOWRAH	*Mangifera indica* MANGO	0.40	0.68	7.45	77.70	34.5	0.721	7.897	2.060	6.4
17	AMTA HOWRAH	POTATO	0.20	0.34	46.84	12.67	45	1.223	7.774	1.552	6.7
18	BAURIA HOWRAH	MULTICROP	0.34	0.58	10.65	47.60	50	0.115	1.428	1.057	6.2

SAMPLE	LOCATION	CROP	CARBON CONTENT (%)	ORGANIC MATTER (%)	CHLORIDE CONTENT (%)	MOISTURE CONTENT (%)	MOISTURE HOLDING CAPACITY (%)	Na (mg/gm)	K (mg/gm)	Ca (mg/gm)	pH
19	KATOA BURDWAN	*Solanum tuberosum* BRINJAL	0.26	0.44	42.60	0.50	27.5	0.258	1.901	1.428	6
20	NAIHATI NORTH 24PARGANA	MULTICROP	0.30	0.51	10.65	15.27	30	0.258	7.901	1.428	6.2
21	KALYANI NADIA	*Solanum melongena*	0.34	0.58	24.49	9.28	27.5	0.230	10.79	2.331	6.2
22	KALYANI. NADIA	MULTICROP	0.30	0.51	45.79	2.04	20	0.537	3.203	0.270	6.4
23	KALYANI. NADIA	MULTICROP	0.42	0.72	21.30	8.10	40	0.594	2.726	0.279	6.8
24	MAHISHADAL MIDNAPUR (E)	*Oryza sativa* PADDY	0.32	0.55	10.65	8.10	45	2.34	3.026	1.877	6
25	BOLPUR BIRBHUM	MULTICROP	0.36	0.62	7.45	4.98	42	0.527	2.615	1.488	6.4
26	CHAKDAH, NADIA	*Musa sp.* BANANA	0.30	0.51	42.60	12.99	26	0.311	2.292	5.30	6.2
27	CHAKDAH, NADIA	*Bunium persicum* Kalojire	0.26	0.44	42.60	2.56	27.5	0.481	2.067	1.450	6.8

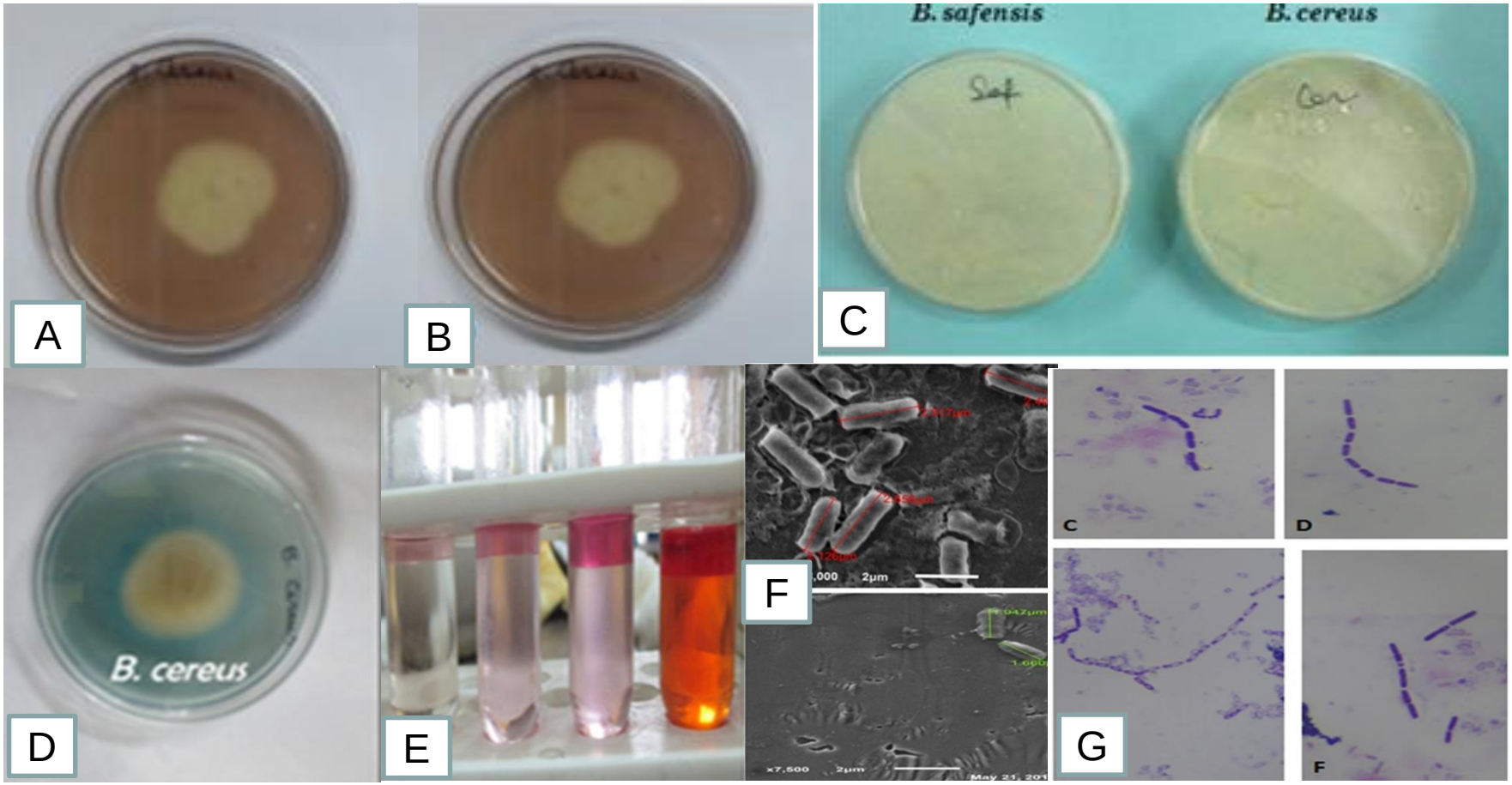
SAMPLE	LOCATION	CROP	CARBON CONTENT (%)	ORGANIC MATTER (%)	CHLORIDE CONTENT (%)	MOISTURE CONTENT (%)	MOISTURE HOLDING CAPACITY (%)	Na (mg/gm)	K (mg/gm)	Ca (mg/gm)	pH
28	CHAKDAH, NADIA	*Coccinia sp.* KUDRI	0.28	0.48	10.65	5.26	46	0.304	2.154	3.489	6.6
29	CHAKDAH, NADIA	*Trichosanthes dioic* Pointed gourd	0.34	0.58	7.45	4.98	55	0.837	3.203	0.270	6.8
30	BURDWAN	*Sesamum indicum* SESAME	0.30	0.51	10.65	1.01	57	0.230	0.537	0.270	6.5
31	BARUIPUR 24 PARGANAS (S)	*Solanum melongena*	0.30	0.51	13.84	18	35	0.607	0.360	0.711	6.6
32	BONGAON NORTH 24PARGANA	*Solanum tuberosum* POTATO	0.28	0.48	7.45	10.19	29	0.092	2.043	1.179	6.4
33	PAKHIRALOY 24 PARGANAS (S)	VEGETABLES	0.16	0.28	20.30	39.86	40	0.521	3.428	0.462	6.2

Isolation of pesticide tolerant bacterial strains

Sl no.	Isolates	Soil sample	Methomyl	Imidaclorprid	Carbendazim
1	Tn-1	2	–	–	+
2	Tn-2	3	+	–	+
3	Tn-3	4	+	–	–
4	Tn-4	2	+	+	+
5	Tn-5	2	+	–	–
6	Tn-6	12	+	–	–
7	Tn-7	15	+	–	–
8	Tn-8	15	+	–	–
9	Tn-9	17	+	–	–
10	D-1	21	+	–	–
11	D-2	21	–	–	+
12	D-3	26	–	–	+
13	D-4	27	+	–	–
14	D-5	28	+	+	+
15	D-6	28	+	–	–
16	Disha A	31	+	+	+
17	Disha B	31	+	+	+

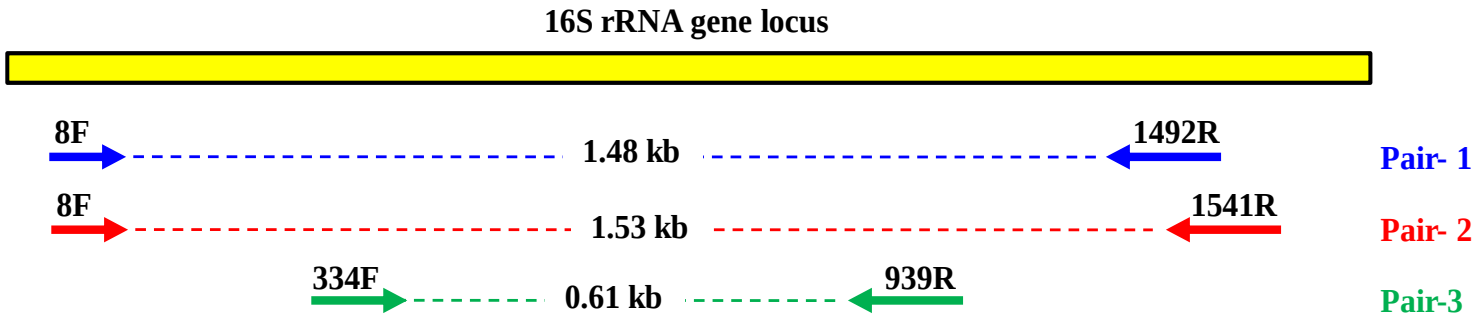
Some Biochemical Tests of *Disha –A and Disha-B*

Sl. No.	Character (s)	Disha-A	Disha-B
1	Gram Staining	Gram positive	Gram positive
2	Endospore	present	present
3	Sugar (lactose, glucose, sucrose, maltose) fermentation	positive	positive
4	Sugar mannitol fermentatrion	positive	positive
5	Indole production from tryptophan	positive	positive
6	Methyl red and Voges-Proskauer test	MR positive VP negative	MR positive VP negative
7	Citric acid utilization	Positive	negative
8	Catalase activity	positive	positive
9	Gelatin hydrolysis	positive	Negative
10	Starch hydrolysis	positive	positive
11	Lysine-decarboxylase activity	positive	Negative
12	Degradation of sulphur containing amino acids for H_2S production	negative	negative
13	Urease production	positive	positive
14	Phenylalanine deaminase production	negative	negative

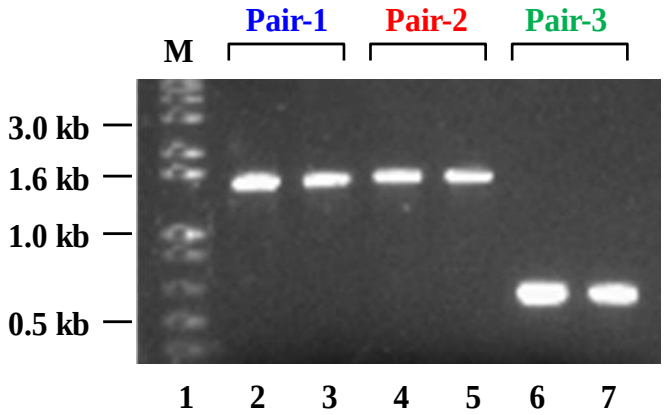


Some biochemical traits exhibited by bacterial isolatess: A) Chitinase (*Disha A*), B) Chitinase (*Disha-B*), C) HCN, D) Siderophore, E) IAA, F) SEM G) Gram staining

A.

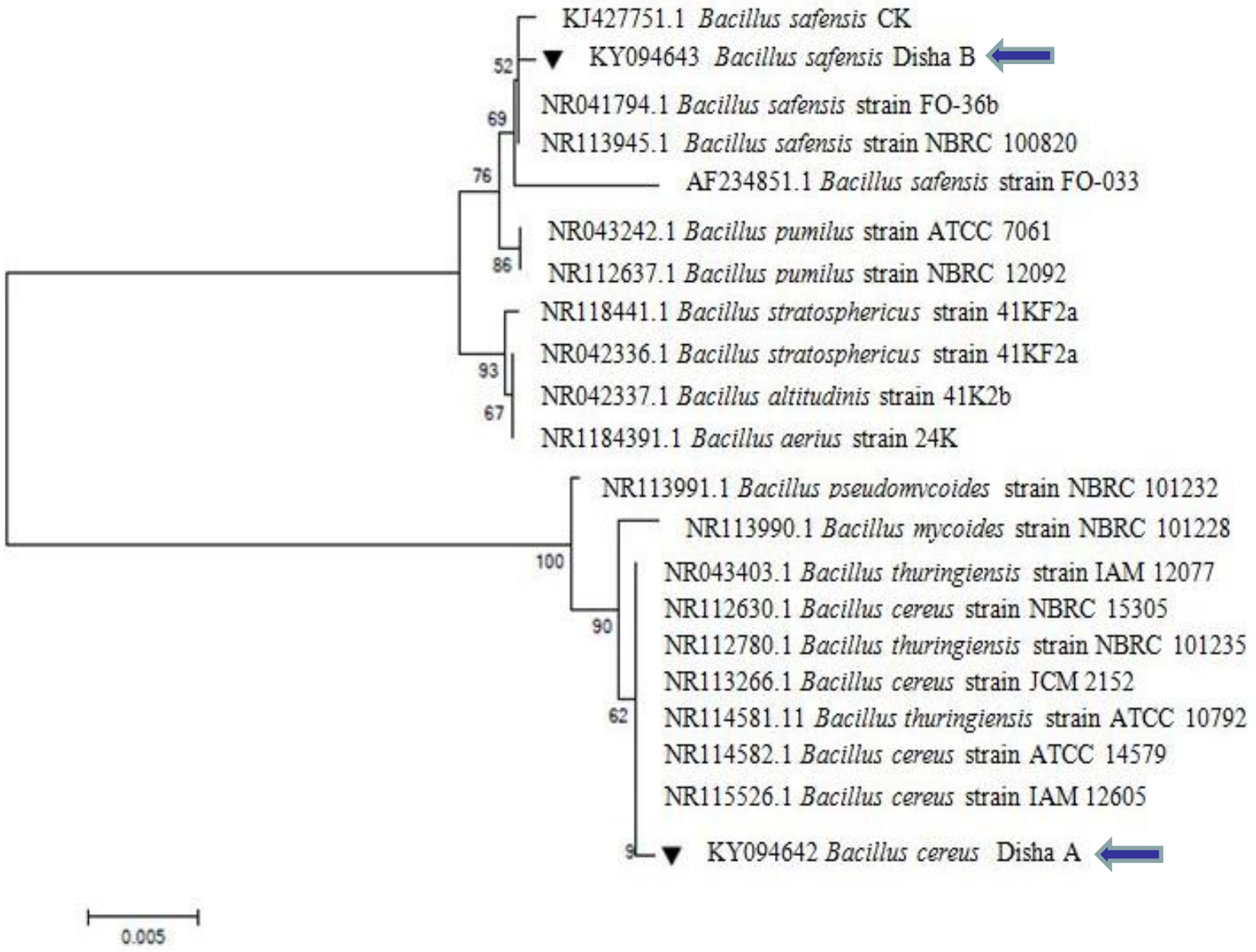


B.



Lanes 2, 4 and 6: Disha A

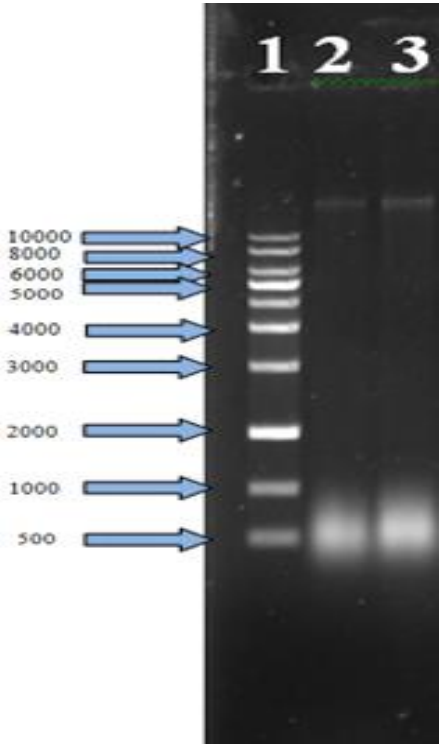
Lanes 3, 5 and 7: Disha B



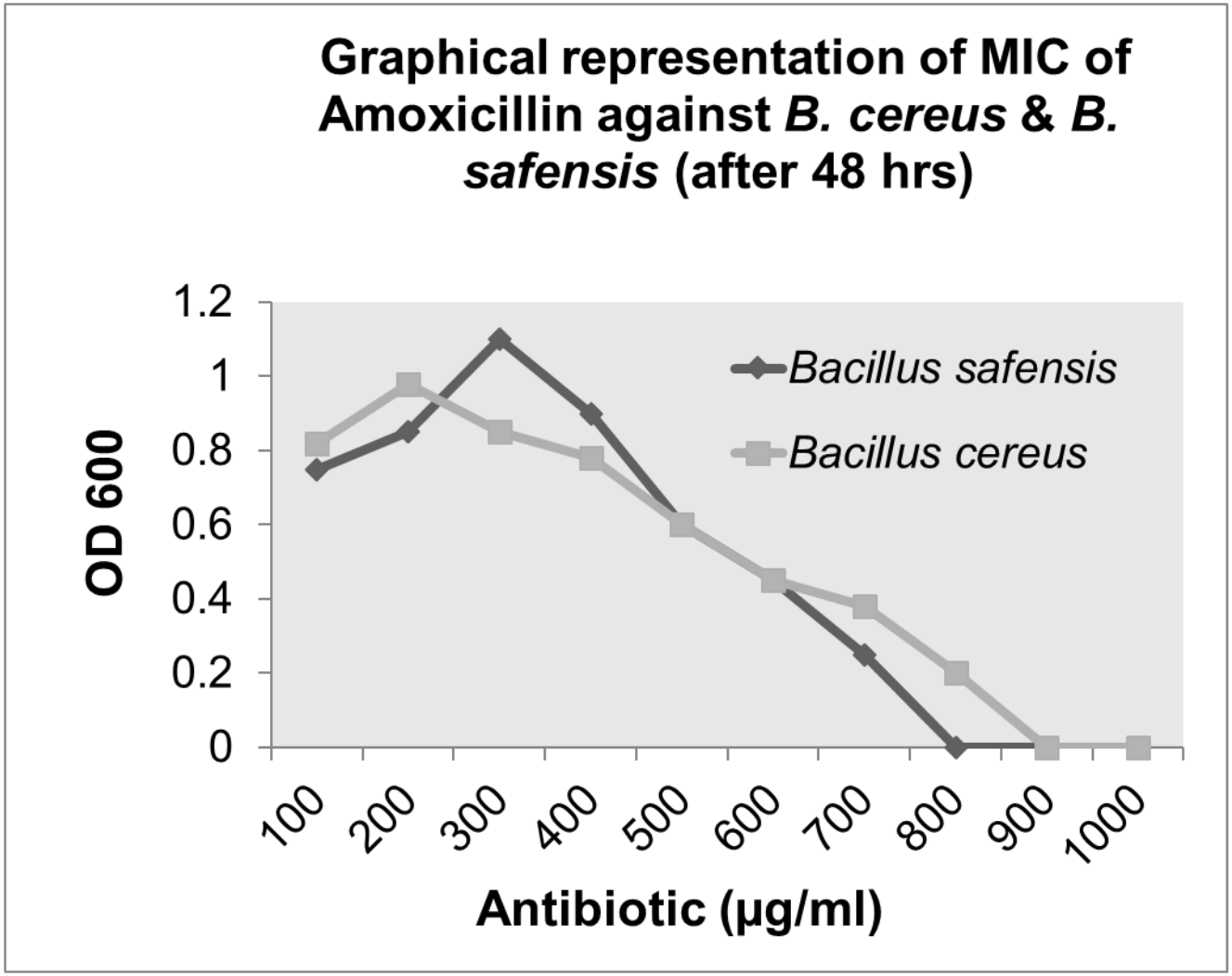
The phylogenetic tree conducted using the Neighbour Joining method among the isolates of *B. cereus* and *B. safensis* with other ex-type strains obtained from NCBI GeneBank database by MEGA6 software.

Comparative study of plant growth promoting traits exhibited by *Bacillus cereus* and *Bacillus safensis* in presence of pesticides.

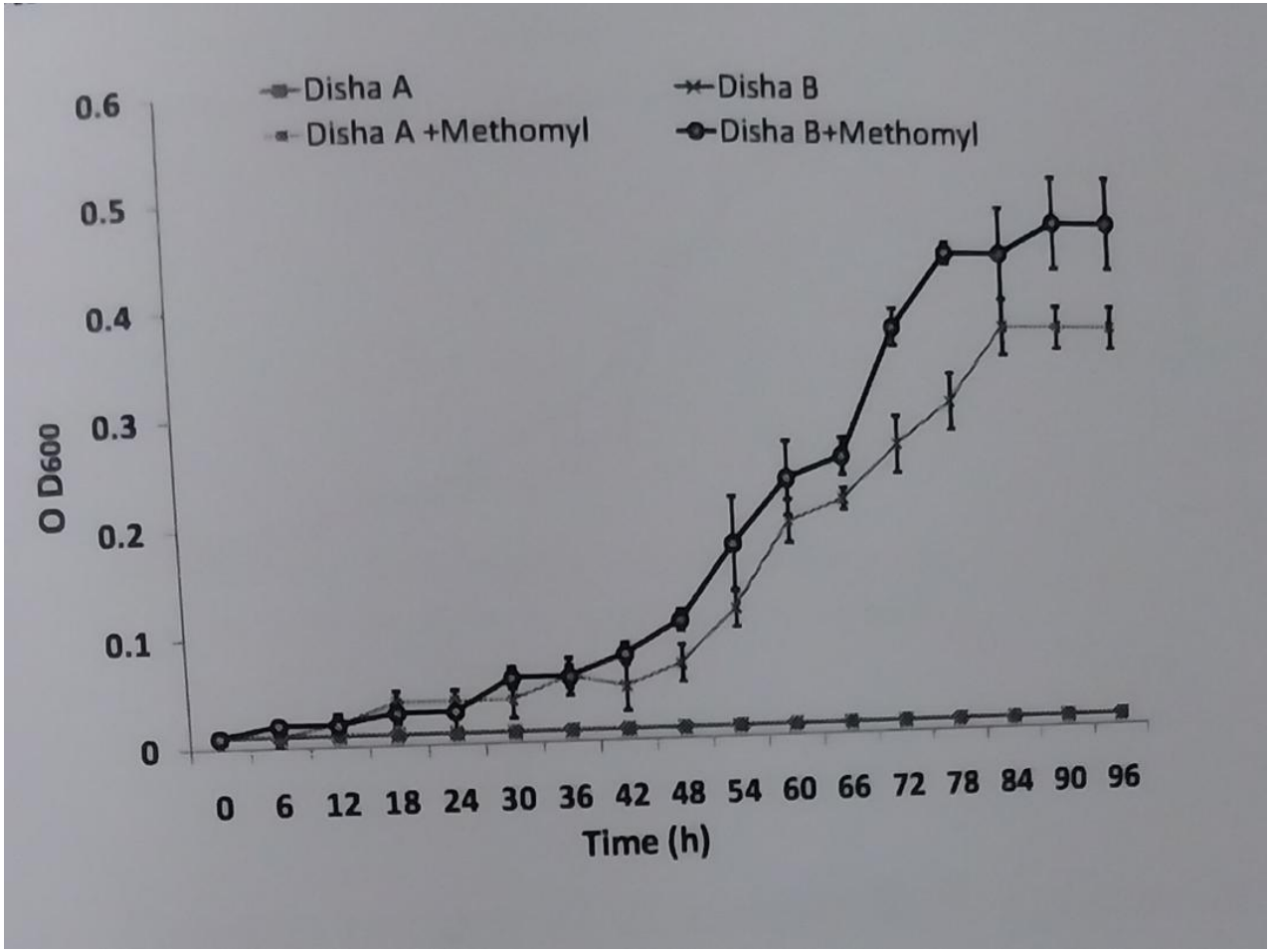
Bacteria	Condition		IAA (mM)	Soluble phosphate (µg/ml)	Chitinase U /ml
B. cereus	With Pesticide	Methomyl	0.12±0.03	193 ±1.52	202
		Carbendazim	0.127±0.03	50±0.50	257
		Imidacloprid	0.13±0.02	120±1.72	162.2
	Without pesticide		0.23 ± 0.11	103±3.21	107
B. safensis	With Pesticide	Methomyl	0.13 ± 0.07	203±3.60	245
		Carbendazim	0.132±0.01	16±0.33	193
		Imidacloprid	0.176±0.06	63±0.12	109
	Without pesticide		0.25 ± 0.02	113±3.60	115



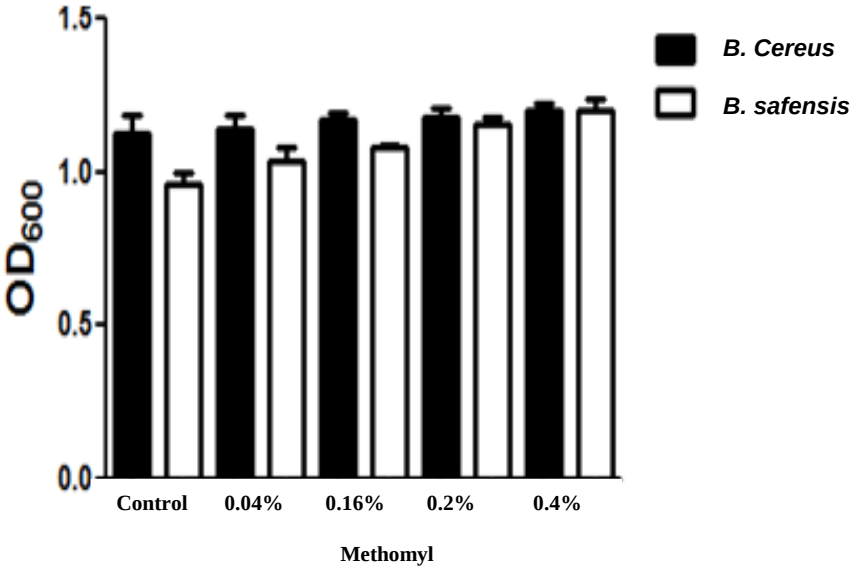
Plasmid profile of selected bacterial isolates; 1) DNA ladder 2) *B. cereus* wild 3) *B. safensis wild* .



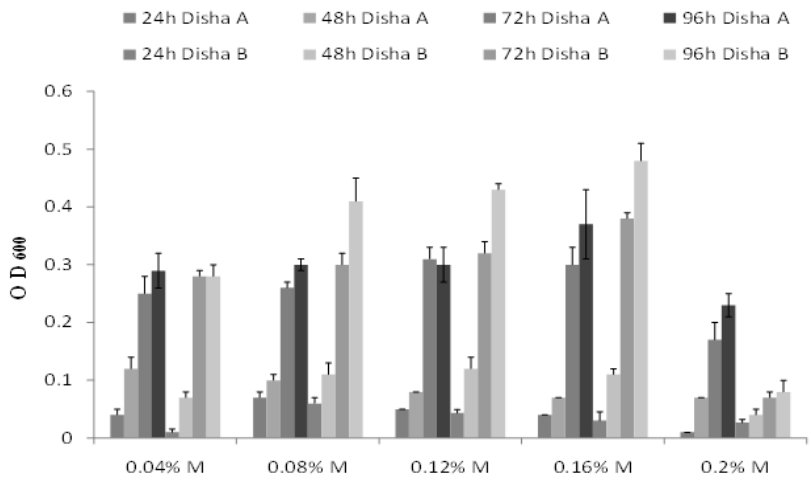
Growth curve of Disha A and Disha B in presence and absence of pesticide (methomyl) in MSM



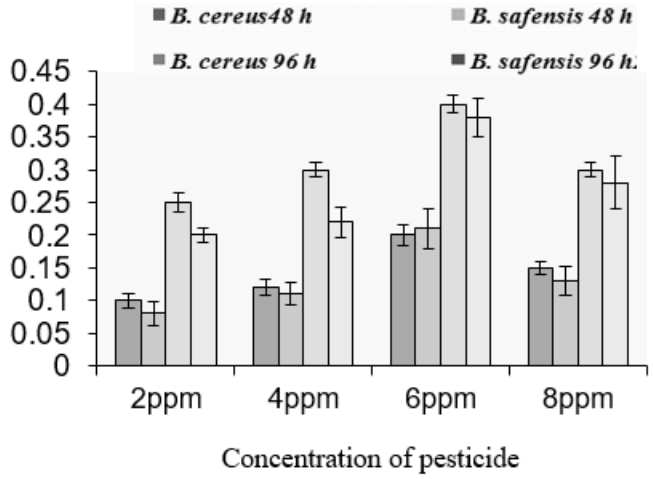
Growth pattern of *B. cereus* (Disha A)and *B. safensis* (Disha B) in NB medium in different concentrations of pesticides



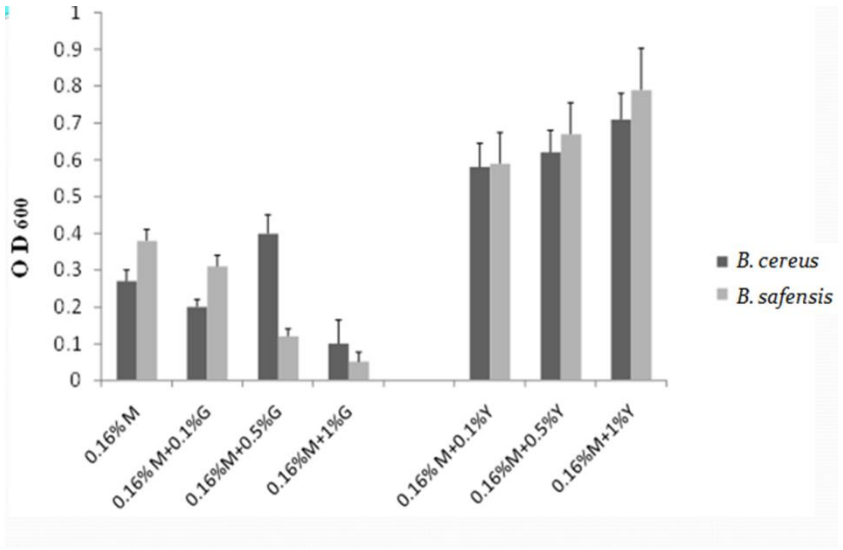
Growth pattern of *B. cereus* (Disha A)and *B. safensis* (Disha B) in MS medium in different concentrations of methomyl

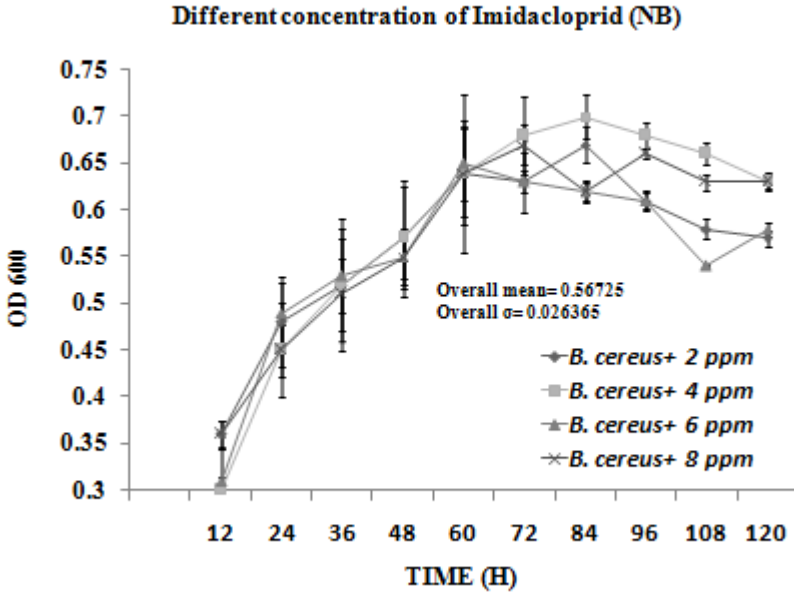


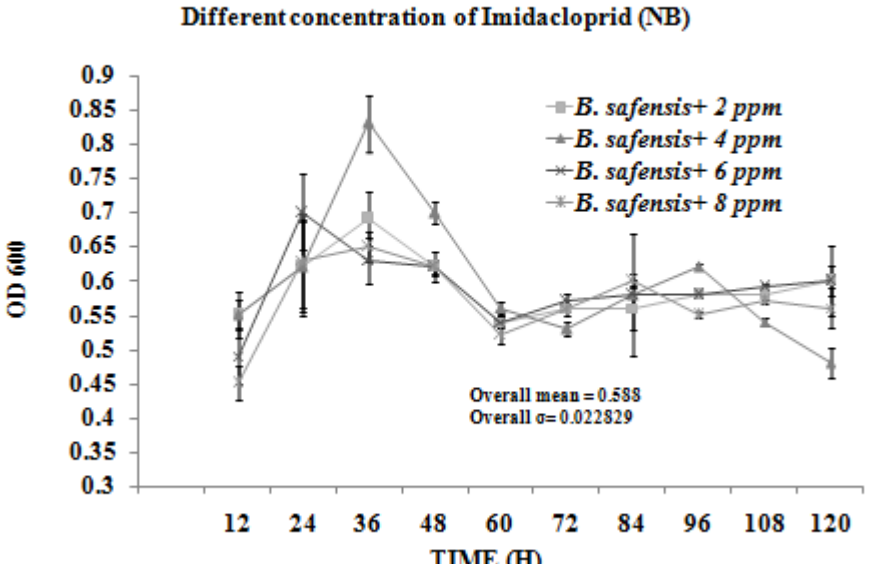
Growth of *B. cereus* and *B. safensis* in different concentration of methomyl (MSM)

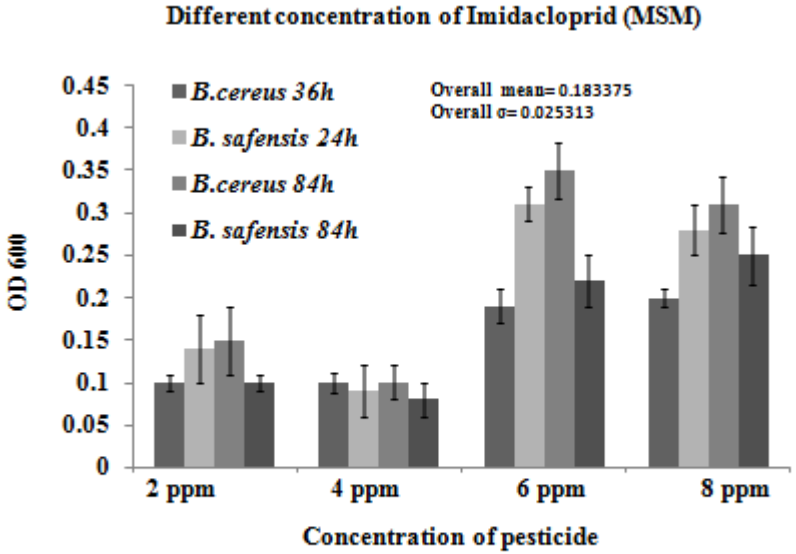


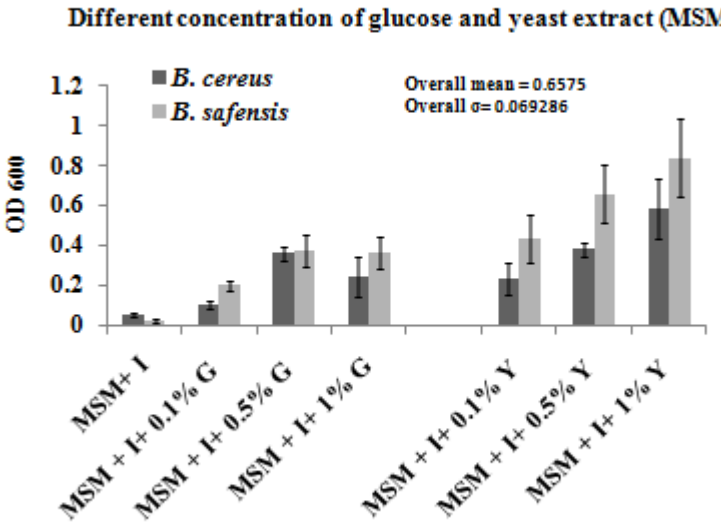
Different concentrations of glucose and yeast

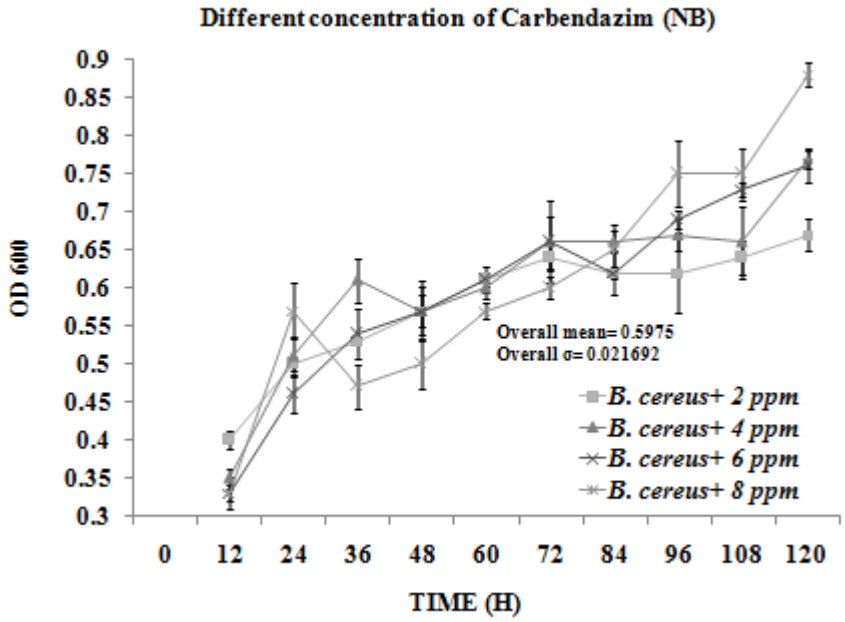


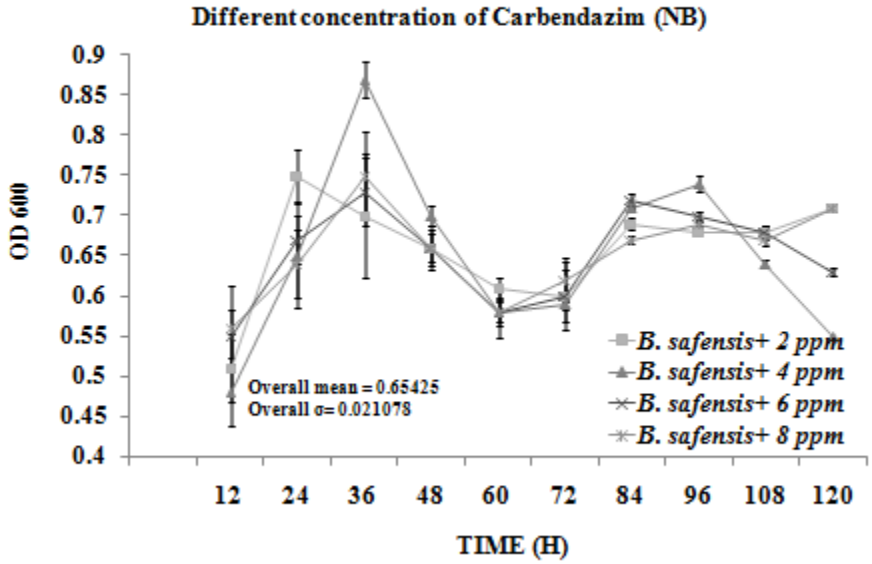

Different concentration of Imidacloprid (NB)
OD 600
TIME (H)
Overall mean= 0.56725
Overall σ= 0.026365
B. cereus+ 2 ppm
B. cereus+ 4 ppm
B. cereus+ 6 ppm
B. cereus+ 8 ppm

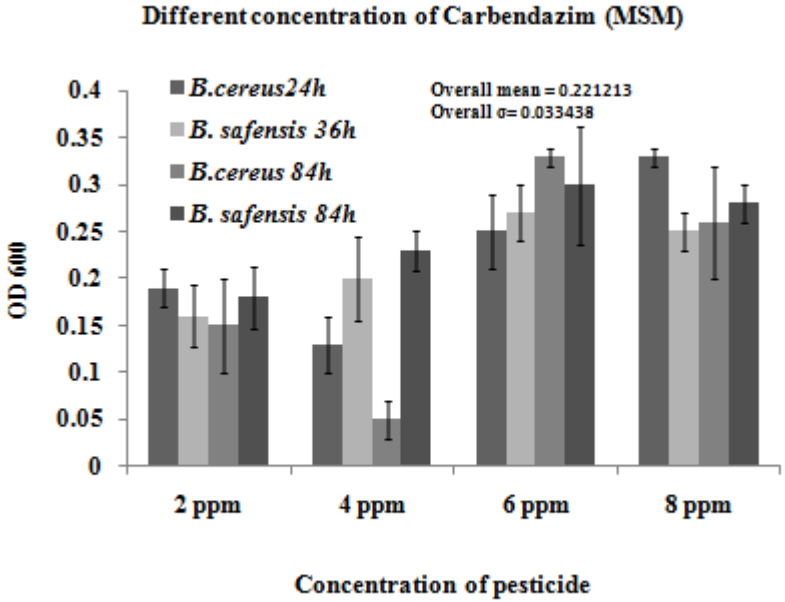

Different concentration of Imidacloprid (NB)
OD 600
TIME (H)
Overall mean = 0.588
Overall σ= 0.022829
B. safensis+ 2 ppm
B. safensis+ 4 ppm
B. safensis+ 6 ppm
B. safensis+ 8 ppm

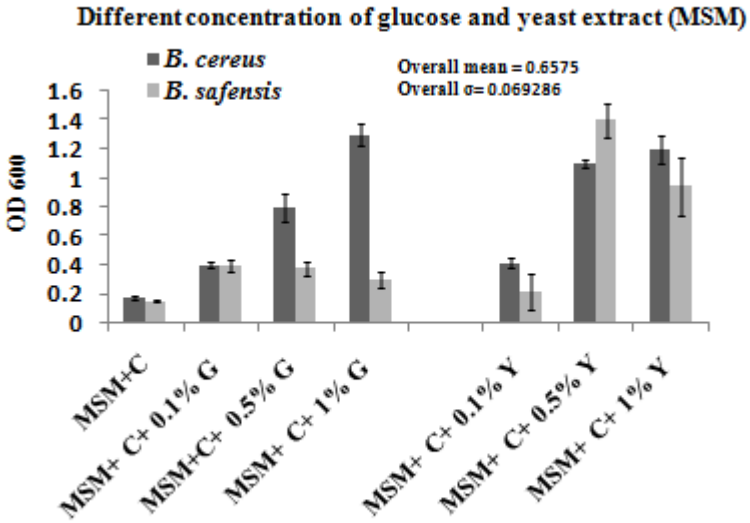

Different concentration of Imidacloprid (MSM)
OD 600
Concentration of pesticide
Overall mean= 0.183375
Overall σ= 0.025313
B.cereus 36h
B. safensis 24h
B.cereus 84h
B. safensis 84h
2 ppm
4 ppm
6 ppm
8 ppm


Different concentration of glucose and yeast extract (MSM)
OD 600
Overall mean = 0.6575
Overall σ= 0.069286
B. cereus
B. safensis
MSM+ I
MSM + I+ 0.1% G
MSM + I+ 0.5% G
MSM + I+ 1% G
MSM + I+ 0.1% Y
MSM + I+ 0.5% Y
MSM + I+ 1% Y

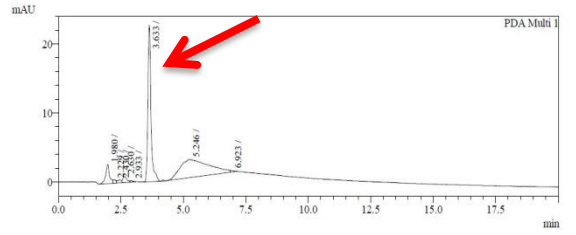

Different concentration of Carbendazim (NB)
OD 600
TIME (H)
Overall mean= 0.5975
Overall σ= 0.021692
B. cereus+ 2 ppm
B. cereus+ 4 ppm
B. cereus+ 6 ppm
B. cereus+ 8 ppm


Different concentration of Carbendazim (NB)
OD 600
TIME (H)
Overall mean = 0.65425
Overall σ= 0.021078
B. safensis+ 2 ppm
B. safensis+ 4 ppm
B. safensis+ 6 ppm
B. safensis+ 8 ppm

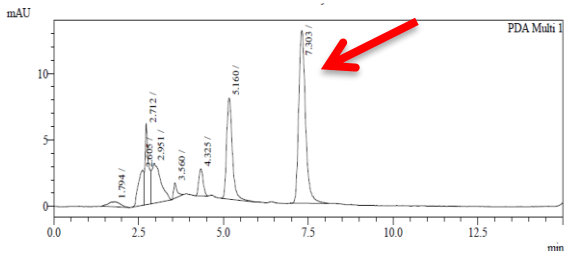

Different concentration of Carbendazim (MSM)
OD 600
Concentration of pesticide
Overall mean = 0.221213
Overall σ= 0.033438
B.cereus24h
B. safensis 36h
B.cereus 84h
B. safensis 84h
2 ppm
4 ppm
6 ppm
8 ppm


Different concentration of glucose and yeast extract (MSM)
OD 600
Overall mean = 0.6575
Overall σ= 0.069286
B. cereus
B. safensis
MSM+C
MSM+ C+ 0.1% G
MSM+C+ 0.5% G
MSM+ C+ 1% G
MSM+ C+ 0.1% Y
MSM+ C+ 0.5% Y
MSM+ C+ 1% Y

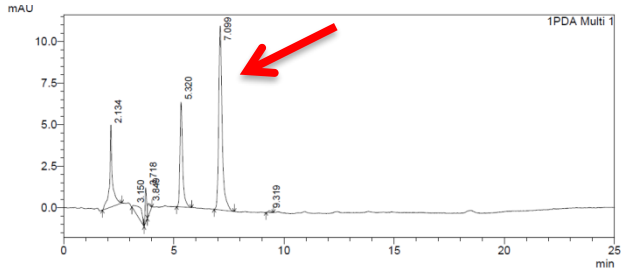
A1 **IMIDACLOPRID**



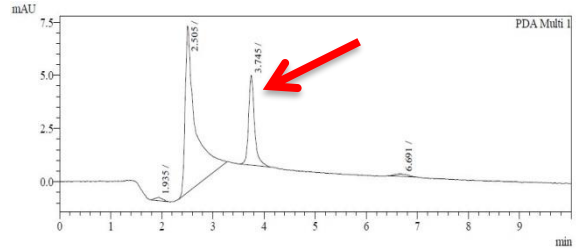
A2 **CARBENDAZIM**



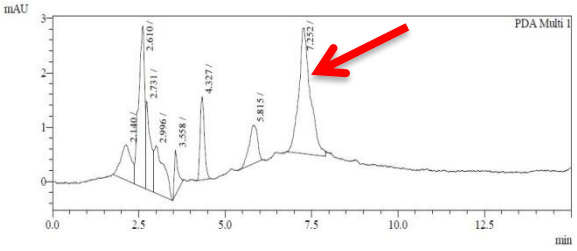
A3 **METHOMYL**



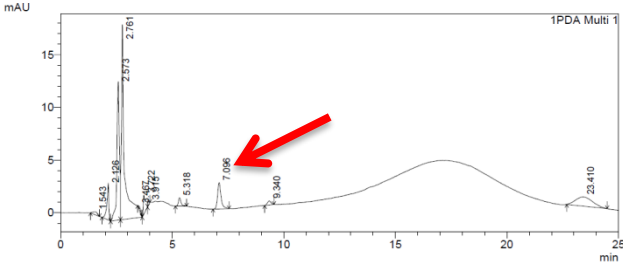
B1



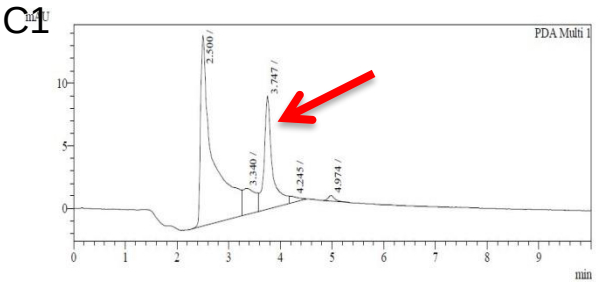
B2



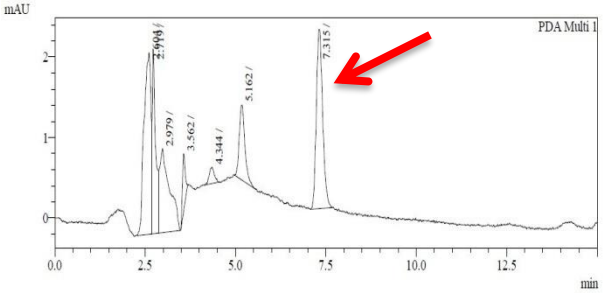
B3



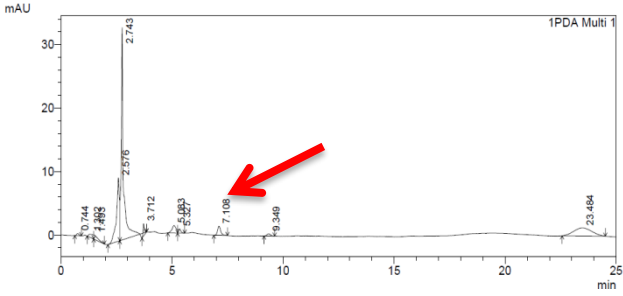
C1



C2



C3



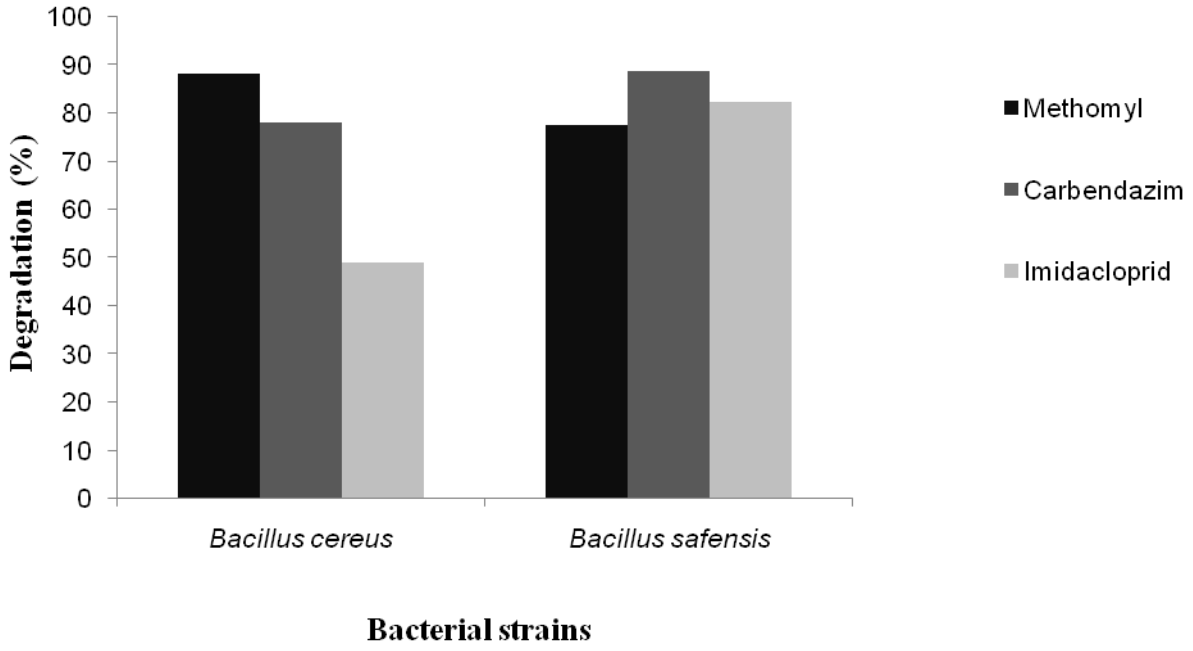
HPLC of imidacloprid **A1** standard imidacloprid solution (10 ppm). **B1** Culture filtrate of *B. cereus*. **C1** Culture filtrate of *B. safensis*, (Column size = 250 × 4.6 mm, Eluent = 80% Acetonitrile, and 20% Water, Flow rate = 1 ml/min, Type = Gradient, Detector = UV-272 nm, Injection volume = 10 µl)

HPLC of carbendazim **A2** standard carbendazim solution (10 ppm). **B2** Culture filtrate of *B. cereus* (10 ppm). **C2** Culture filtrate of *B. safensis*, (10 ppm), (Column size = 250 × 4.6 mm, Eluent = 70% Acetonitrile, and 30% Water, Flow rate = 1 ml/min, Type = Gradient, Detector = UV-233 nm, Injection volume = 10 µl)

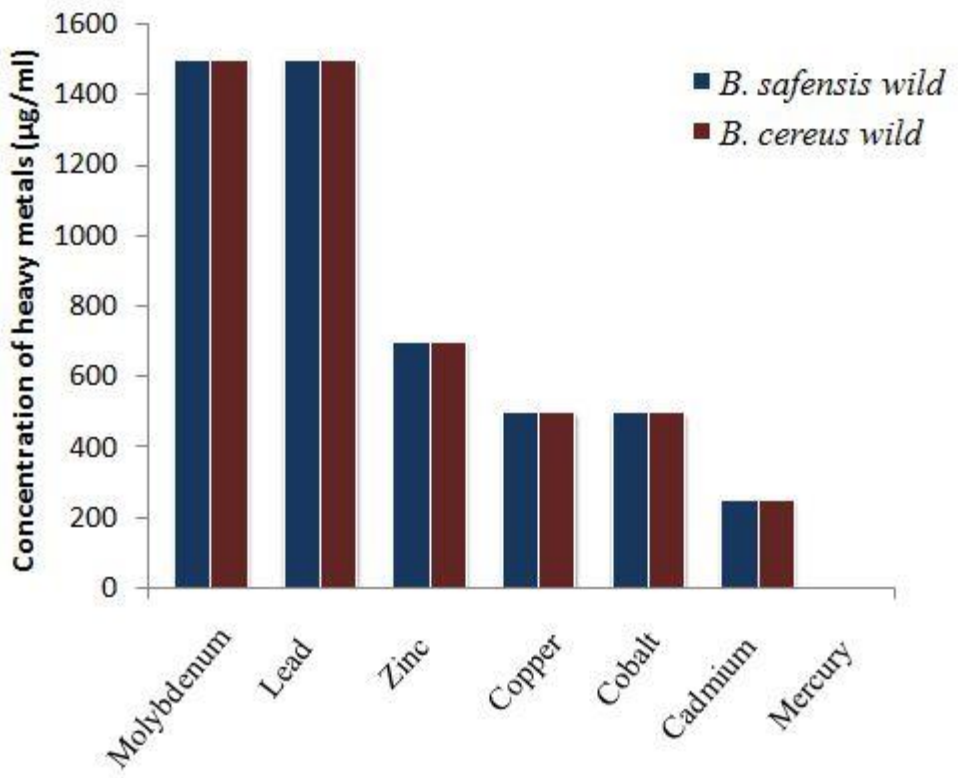
HPLC of Methomyl **A3** 6 ppm in minimal media (control), **B3** *B. safensis* culture filtrate in methomyl containing MSM, **C3** *B. cereus* culture filtrate in methomyl containing MSM (Column size = 250X4.6 mm, Eluent= 20% Acetonitrile and 80% Water, Flow rate= 1ml/min, Type= Isocratic, Detector=UV-233 nm, Injection volume=10µl).

.

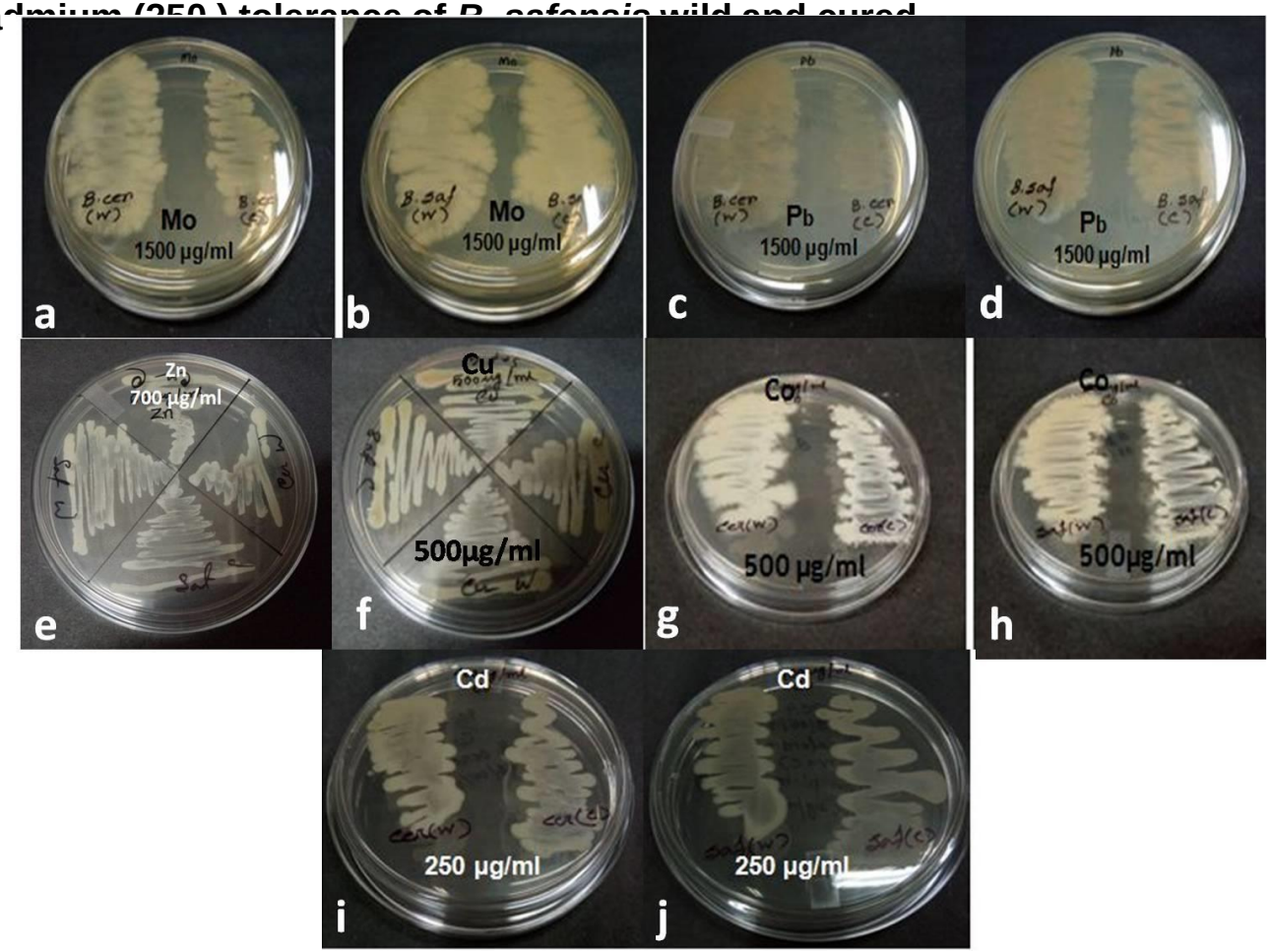
Comparative degradation of different pesticides as evidenced by HPLC by *B. cereus* and *B. safensis.*



Metal tolerances of *B. cereus* and *B. safensis* at different concentration in μg ml^{-1}

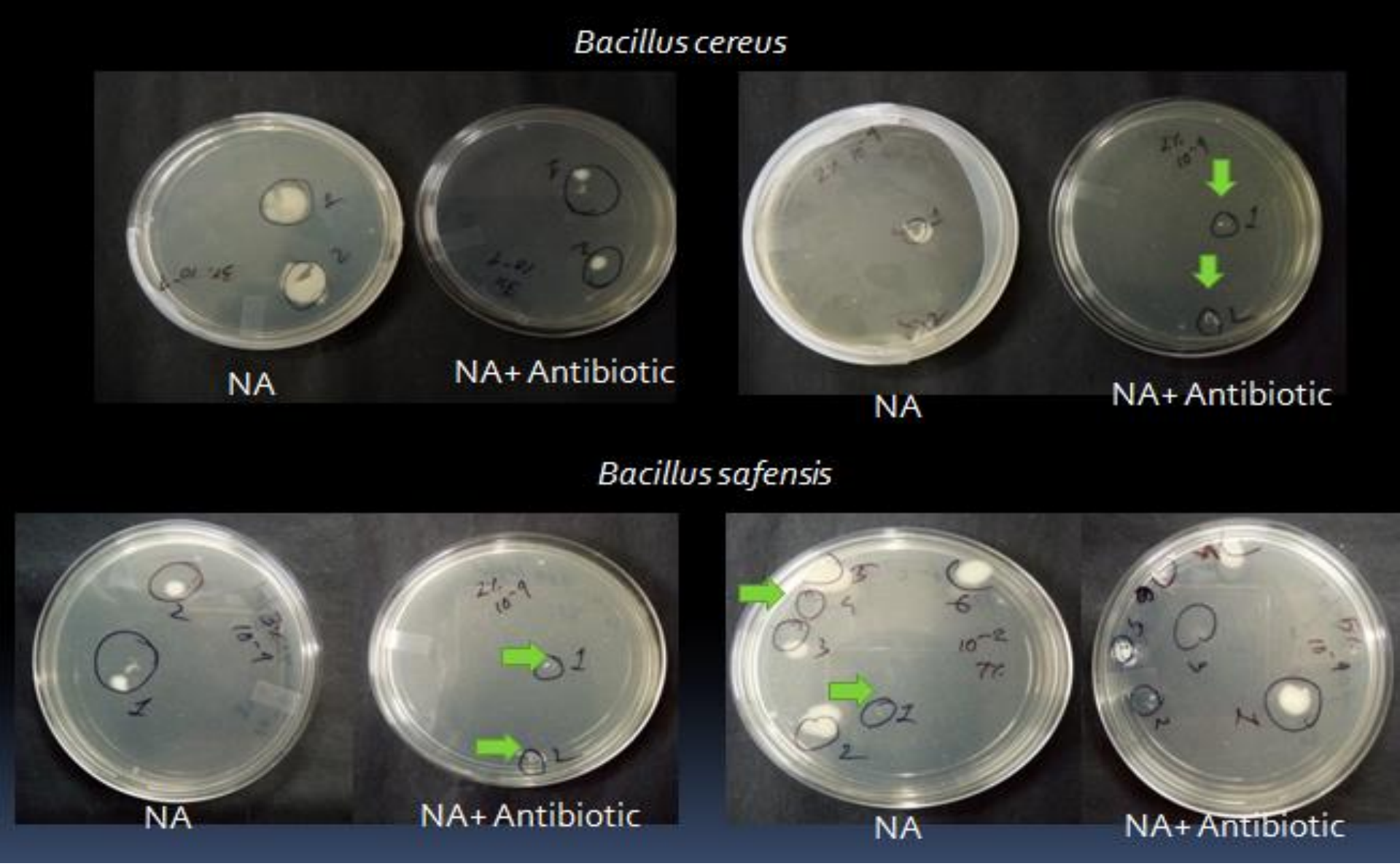


Heavy metal tolerance of wild (w) and cured (c) *B. cereus* (cer) and *B. safensis* (saf) strains at different concentrations; a) Molybdenum(1500) tolerance of *B. cereus* wild and cured, b) Molybdenum(1500) tolerance of *B. safensis* wild and cured, c) Lead (1500 tolerance of *B. cereus* wild and cured, d) Lead (1500) tolerance of *B. safensis* wild and cured, e) Zinc (700) tolerance of *B. cereus* & *B. safensis* wild and cured, f) Copper (500) tolerance of *B. cereus* & *B. safensis* wild and cured, g) Cobalt (500) tolerance of *B. cereus* wild and cured, h) Cobalt (500) tolerance of *B. safensis* wild and cured, i) Cadmium (250) tolerance of *B. cereus* wild and cured, j) Cadmium (250) tolerance of *B. safensis* wild and cured

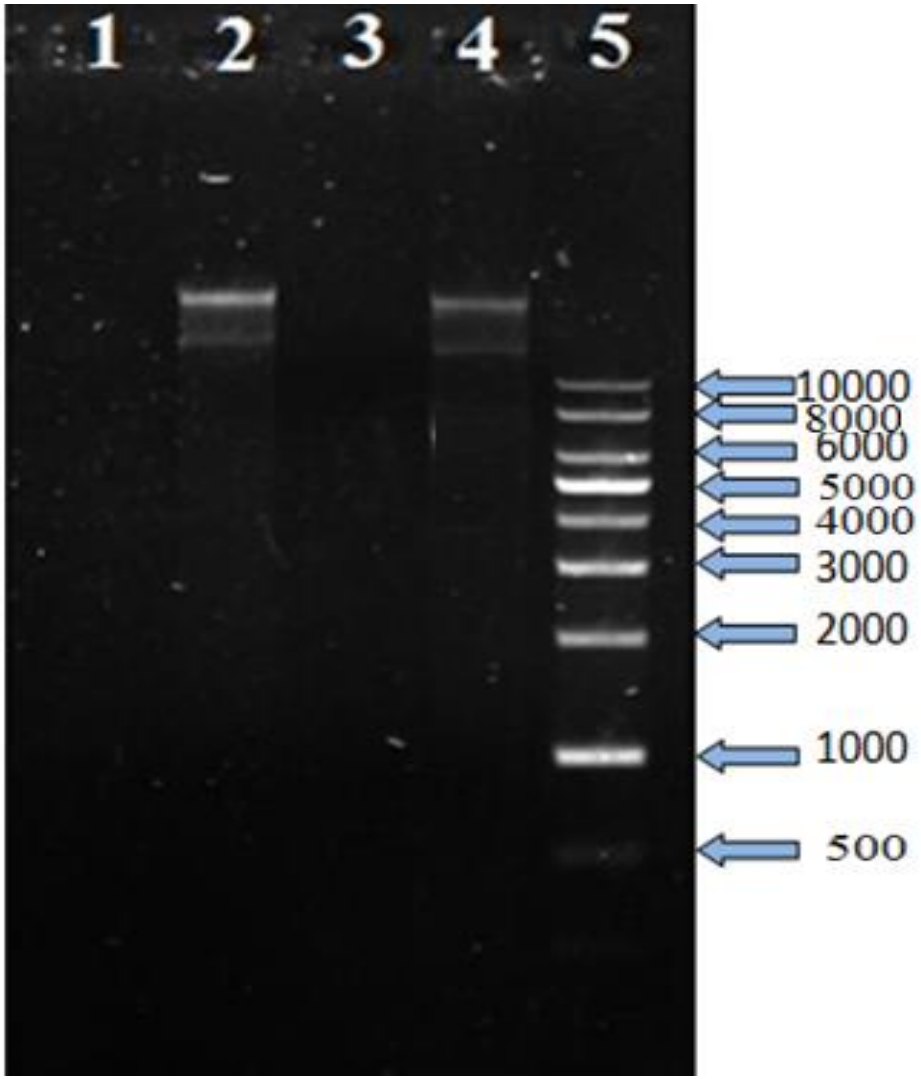


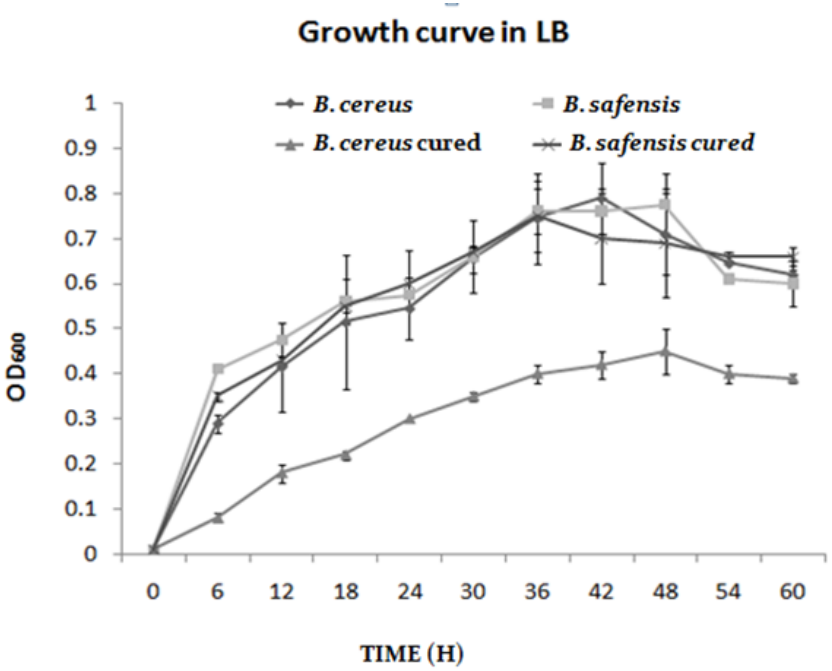
Percent of cured colony in different concentration of tested curing agents

Bacteria	Curing agent					
	Sodium Dodecyl Sulphate (SDS)		Acridine Orange (AO)		Crystal violet (CV)	
	2%	5%	250µg/ml	500µg/ml	250µg/ml	500µg/ml
B. safensis	0%	0%	14.81%	70%	63%	0%
B. cereus	22.14%	28%	36.36%	80%	72%	0%



Selection of cured colony

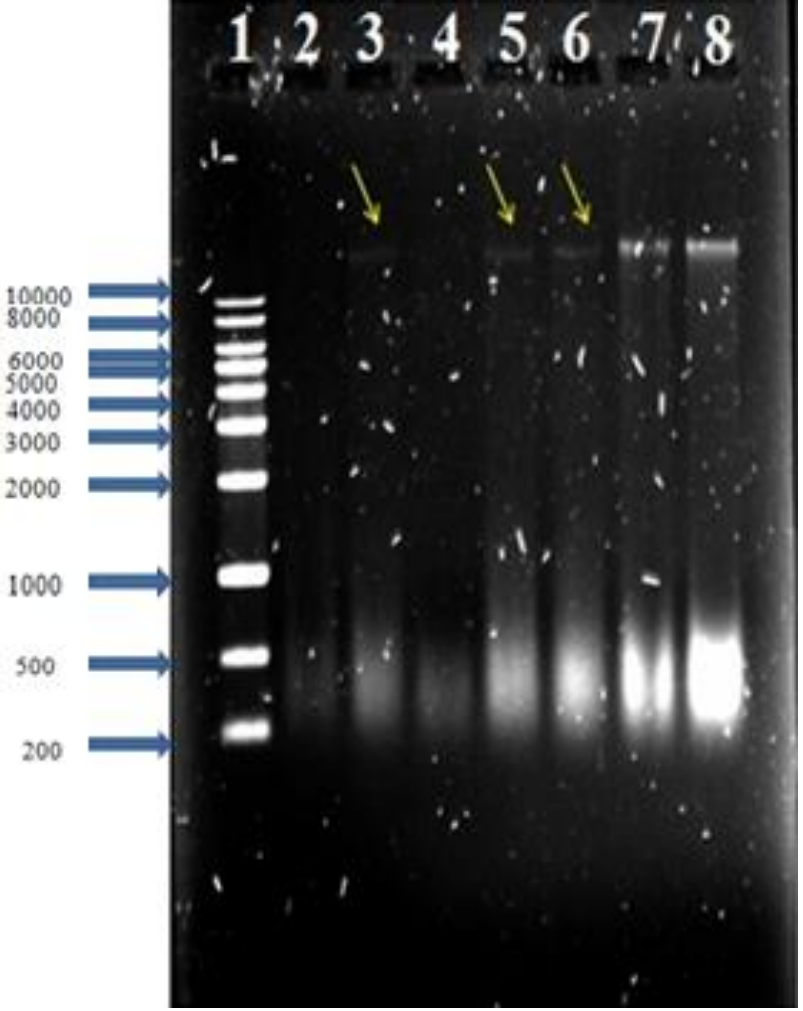




Isolation of plasmid from cured and wild strain, B. 1) *B. safensis* cured 2) *B. safensis wild* 3) *B. cereus* cured 4) *B. cereus* wild 5) DNA ladder

Heavy metal and antibiotic resistance pattern of wild and cured cells

Bacterial strains	Antibiotic Amoxicillin (500µg ml^{-1})	Mo (1500 µg ml^{-1})	Pb (1500 µg ml^{-1})	Zn (700 µg ml^{-1})	Cu (500 µg ml^{-1})	Co (500 µg ml^{-1})	Cd (250 µg ml^{-1})	Hg (100 µg ml^{-1})
B. cereus **(wild)**	+	+	+	+	+	+	+	-
B. cereus **(cured)**	-	+	+	+	+	+	+	-
B. safensis **(wild)**	+	+	+	+	+	+	+	-
B. safensis **(cured)**	-	+	+	+	+	+	+	-



The plasmids of *Bacillus cereus* transformed into the wild strain of *E. coli* by heat shock method. Lane 1-Marker DNA, Lane 2 - wild strain of *E. coli*, Lane 3, 4 5, 6 showed the transformed *E.coli*, Lane 7 & 8 - plasmid of *Bacillus cereus & Bacillus safensis* respectively.

Summary

- Two pesticide and heavy metal resistance soil bacteria, Disha A (*Bacillus cereus*) and Disha B (*Bacillus safensis*),were isolated.
- Both of these soil isolates could not be grown in mineral salt medium (MSM) but proficiently grown in MSM supplemented with pesticide.
- The HPLC studies indicated the efficient degradation of these pesticides by both bacteria.
- *B. safensis* degraded more amount of imidacloprid (82.48%) and carbendazim (88.93%) than *B. cereus*, 49.12% and 78.07%, respectively, whereas *B. cereus* exhibited slightly better degradation (88.25%) of methomyl in comparison to *B.safensis* (77.5%).

Contd…

- Both bacteria tolerated various concentrations of heavy metals, viz. cadmium, cobalt, copper, lead, molybdenum and zinc but not mercury.
- The highest tolerance level (1500 μg ml^{-1}) shown against lead and molybdenum.
- Both bacteria contained a single plasmid.
- The plasmid-cured *B. cereus* strains did not tolerate any tested pesticide, whereas the wild and cured *B. safensis* strains tolerated all the tested pesticides.
- So, possibly the pesticide tolerance gene(s) of *B. cereus* are plasmid-dependent but genomic in *B. safensis*.
- Plasmid curing did not influence the heavy metal tolerance potential of both the bacteria.

Contd…

- The transformation experiments also confirmed the genomic nature of heavy metal tolerance genes in both the isolates, whereas pesticide resistance genes are plasmid-dependent in *B. cereus* but genomic in *B. safensis*.

- These bacteria can be used in heavy metal and pesticide bioremediaton in addition to sustainable agricultural practices.

Isolation, Characterization, and Identification of Two Methomyl-Degrading Bacteria from a Pesticide-Treated Crop Field in West Bengal, India[1]

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**e-mail: nirmalendus@yahoo.co.uk*

Received January 28, 2017

Abstract—Two methomyl-degrading bacteria (initially named Disha A and Disha B) were isolated from a pesticide-treated crop field in Baruipur, 24 Parganas (South), West Bengal, India. Both strains could not grow in mineral salt (MS) medium but showed efficient growth in the presence of methomyl. The highest growth was observed in the MS medium containing 0.16% methomyl. When methomyl was supplemented with glucose, no further enhancement of growth was observed, whereas supplementation with yeast extract had a positive effect on growth of both strains, indicating that methomyl could be utilized as the sole source of carbon but not that of nitrogen. In Nutrient Broth and Luria Bertani medium, these strains could tolerate 0.4% methomyl. Optimum pH and temperature for growth of both bacteria in the methomyl-containing MS medium were 7.0 and 30°C, respectively. Protein concentration in the cell-free extracts of bacterial cultures was directly proportional to methomyl concentration in the medium. Disha A was more resistant to the antibiotics amoxicillin and penicillin, as indicated by minimum inhibitory concentration (600 and 500 μg/mL, respectively), which were higher than those obtained for Disha B (350 and 300 μg/mL, respectively). Both Disha A and Disha B were plasmid-bearing, gram-positive, endospore-producing, rod-shaped bacteria. Biochemical studies, 16S rDNA sequencing, and phylogenetic analysis indicated maximum similarity of Disha A to *Bacillus cereus* ATCC 14579, whereas Disha B showed maximum similarity to *Bacillus safensis* F0-36b ATCC BAA-1126. The HPLC analysis clearly indicated that *B. cereus* and *B. safensis* showed 88.25 and 77.5% of methomyl (Sigma) degradation, respectively within 96 h of growth. This is the first report of *Bacillus* species that can degrade the carbamate pesticide methomyl and thrive in presence of its high concentrations.

Keywords: Bacillus sp., carbamate, methomyl, pesticide, 16S rRNA

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Bio-effective disease control and plant growth promotion in lentil by two pesticide degrading strains of *Bacillus* sp.

Tina Roy[a,c], Anuradha Bandopadhyay[a], Parshuram J. Sonawane[b], Sukanta Majumdar[c], Nitish R. Mahapatra[b], Shariful Alam[d], Nirmalendu Das[a,*]

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ARTICLE INFO

Keywords:
Alternaria sp.
Bacillus sp.
Bio-control agent
Lentil
Pesticide tolerance
Plant growth promoting rhizobacteria

ABSTRACT

Antagonistic bacteria are common soil inhabitants with potential to control several soil-borne diseases of various crops. In this study, two methomyl degrading *Bacillus* sp. were screened for their antagonistic potential against soil borne pathogen identified as *Alternaria* sp. which causes leaf spot and blight disease in lentil. Both the strains produced non-volatile and volatile organic compounds, extracellular enzymes, siderophore, indole acetic acid and solubilized phosphate which ascribed to the mechanism of bio-control and plant growth promotion. These bacterial strains produced indole acetic acid, chitinase and solubilized phosphate even in presence of pesticides (namely methomyl, carbendazim and imidacloprid). The production of chitinase increased by 51–140% in presence of different tested pesticides by the bacterial strains. However, phosphate solublization was only increased up to 79% in *B. cereus* and 87% in *B. safensis* in presence of methomyl. Both strains promoted plant growth and suppressed leaf spot and the incidence of blight in lentil plants under controlled conditions in green house. Application of *B. cereus* and *B. safensis* isolates to sterile rhizospheric soil increased the dry weight of plants by 40.8% and 43.2%, respectively as compared to control. In similar set of experiments the disease incidence was reduced by 67.7–81.6% in *B. cereus* and 57.2–78.8% in *B. safensis* in sterile condition and by 51.4–76.5% and 48.6–63.4%, respectively in non-sterile condition. The present investigation shows both *B. cereus* and *B. safensis* as potential plant growth promoting rhizobacteria that can be exploited as efficient bio-control organisms against soil borne plant pathogens as well as can be applied in plant growth enhancement even in pesticide infested soil.

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Special issue on *Environmental Toxicology & Bioremediation*

IMPACT OF PESTICIDE TOLERANT SOIL BACTERIA ON DISEASE CONTROL, PLANT GROWTH PROMOTION AND SYSTEMIC RESISTANCE IN COWPEA

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Highlights

- Methomyl, imidacloprid and carbendazim tolerant strains of *Bacillus cereus, B. safensis, Pseudomonas donghuensis* and *P. aeruginosa* have plant growth promoting and antagonistic traits.
- Pesticide tolerant rhizobacteria showed *in vitro* antagonistic activity against pathogen *Macrophomina phaseolina*.
- PGPRs controlled *Macrophomina* diseases and promoted growth in cowpea in a toxic environment.
- PGPRs triggered induced systemic resistance and enhanced enzyme activity of PAL, PO, PPO and chitinase after pathogen challenge inoculation in host plant.
- These PGPRs proved prospective for disease control, plant growth promotion and decontamination of pesticide and heavy metal contaminated soil for sustainable eco-friendly agriculture.

Abstract. Cowpea, an annual legume, suffers from several disease symptoms caused by *Macrophomina phaseolina*. Rhizobacteria isolated from pesticide infested soil, identified by blast analysis as *Bacillus cereus*, *Bacillus safensis*, *Pseudomonas donghuensis* and *Pseudomonas aeruginosa* ascertained tolerant to at least 0.1% pesticides *viz.* methomyl, imidacloprid and carbendazim. *In vitro* antagonism against pathogen exhibited maximum by *P. aeruginosa* 63%. All rhizobacteria were bestowed with attributes responsible for pathogen control and plant growth promotion. Field evaluation resulted highest 75% disease control, enhancement of length, nodule counts, biomass or yield per plant by *P. aeruginosa*. All rhizobacteria induced systemic resistance in cowpea under challenged inoculation with pathogen by augmenting defensive enzyme production. Highest Phenylalanine Ammonia Lyase activity was expressed in *P. aeruginosa* treated plants 1.02 µMoles/ml/min, Polyphenol Oxidase by *P. donghuensis* 1.39 µMoles/ml/min, Chitinase by *B. cereus* 0.745 µMoles/ml/min and 400 percent relative activity of Peroxidase by *P. aeruginosa*. The rhizobacteria were prospective for plant disease control, growth promotion and as immunity boosters in pesticide and heavy metal infested toxic environment.

Keywords: soil contamination, pesticide tolerant rhizobacteria, disease control, plant growth, systemic resistance.

Current Microbiology (2022) 79:106
https://doi.org/10.1007/s00284-022-02793-w

Role of Plasmid in Pesticide Degradation and Metal Tolerance in Two Plant Growth-Promoting Rhizobacteria *Bacillus cereus* (NCIM 5557) and *Bacillus safensis* (NCIM 5558)

Tina Roy[1,2] · Anuradha Bandopadhyay[2] · Chandana Paul[2,3] · Sukanta Majumdar[1] · Nirmalendu Das[2]

Received: 1 June 2021 / Accepted: 31 January 2022

Abstract

Disha A (*Bacillus cereus*) and Disha B (*Bacillus safensis*) were isolated from pesticide-infested agricultural field and showed tolerance against pesticides, heavy metals, and antibiotics. The isolates exhibited PGPR activities in vitro as well as in field conditions in lentil (*Lens culinaris*) and cow pea (*Vigna unguiculata*). Both the *Bacillus* species could not be grown in mineral salt medium but efficiently grown in the media supplemented with pesticide (imidacloprid/carbendazim) demonstrating the utilization of pesticide as carbon/nitrogen source. The HPLC studies exhibited the pesticide (imidacloprid/carbendazim) degradation by both the bacteria. *B. safensis* showed better degradation of carbendazim (88.93%) and imidacloprid (82.48%) than that of *B. cereus* 78.07% and 49.12%, respectively. The bacterial isolates showed high concentration of heavy metal tolerance viz. lead, molybdenum, cadmium, copper, cobalt, and zinc, except mercury. Both the bacteria possessed single plasmid. The plasmid-cured isolates of *B. cereus* did not tolerate any pesticide, whereas that of *B. safensis* tolerated all the pesticides, like wild strains. The plasmid curing experiments did not affect the heavy metal tolerance ability of both the bacteria indicating the genomic nature of heavy metal tolerance genes, whereas pesticide resistance genes are plasmid-dependent in *B. cereus* but genomic in *B. safensis*.

Environment, Development and Sustainability
https://doi.org/10.1007/s10668-022-02684-x

Influence of pesticide-tolerant soil bacteria for disease control caused by *Macrophomina phaseolina* (Tassi.) Goid and plant growth promotion in *Vigna unguiculata* (L.) Walp

A. Bandopadhyay[1] · T. Roy[1,2] · S. Alam[3] · S. Majumdar[2] · N. Das[1]

Received: 22 March 2020 / Accepted: 18 September 2022

Abstract

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important legume that suffers from several diseases caused by *Macrophomina phaseolina* (Tassi.) Goid from seedlings until harvest. The present study involved the biological management of *Macrophomina* diseases by six pesticide-tolerant non-pathogenic isolates of rhizobacteria, including species of *Bacillus* and *Pseudomonas*. All isolates were tolerant to methomyl, imidacloprid, or carbendazim at different concentrations. Antibiosis, competition, the production of volatile and non-volatile compounds, cell wall-degrading enzymes, siderophores, growth hormones, and phosphate solubilisation were attributed to the biocontrol and growth promotion mechanisms of all the selected rhizobacteria. The disease severity index (DSI) and plant health index (HI) were calculated after applying bioagents under controlled conditions in pot and field trials for three consecutive years. A *t*-test was performed on the DSI data for the different treatment groups and time periods. A formula has been devised correlating changes in DSI with time in different culture conditions, revealed a progressive increase in DSI in treatments without bacteria, hydropriming, or pathogens. Treatment with *Bacillus cereus*, *Pseudomonas donghuensis*, and *Pseudomonas aeruginosa* showed lower DSI compared to the other treatments. The HI of *Vigna* plants was found to be maximal after treatment with *P. aeruginosa*. The link between DSI and HI was inversely proportional, and a highly significant correlation was found at $p < 0.01$. There was a high pod yield in plants treated with *Pseudomonas* sp. and *B. cereus* under sterile, non-sterile, and field conditions. Thus, the selected pesticide-tolerant PGPR in this study have disease control and plant growth-promoting abilities, which may be exploited for applications in sustainable agriculture.

Keywords Biocontrol · Cowpea · Growth promotion · *Macrophomina phaseolina* · Pesticide tolerance · Rhizobacteria

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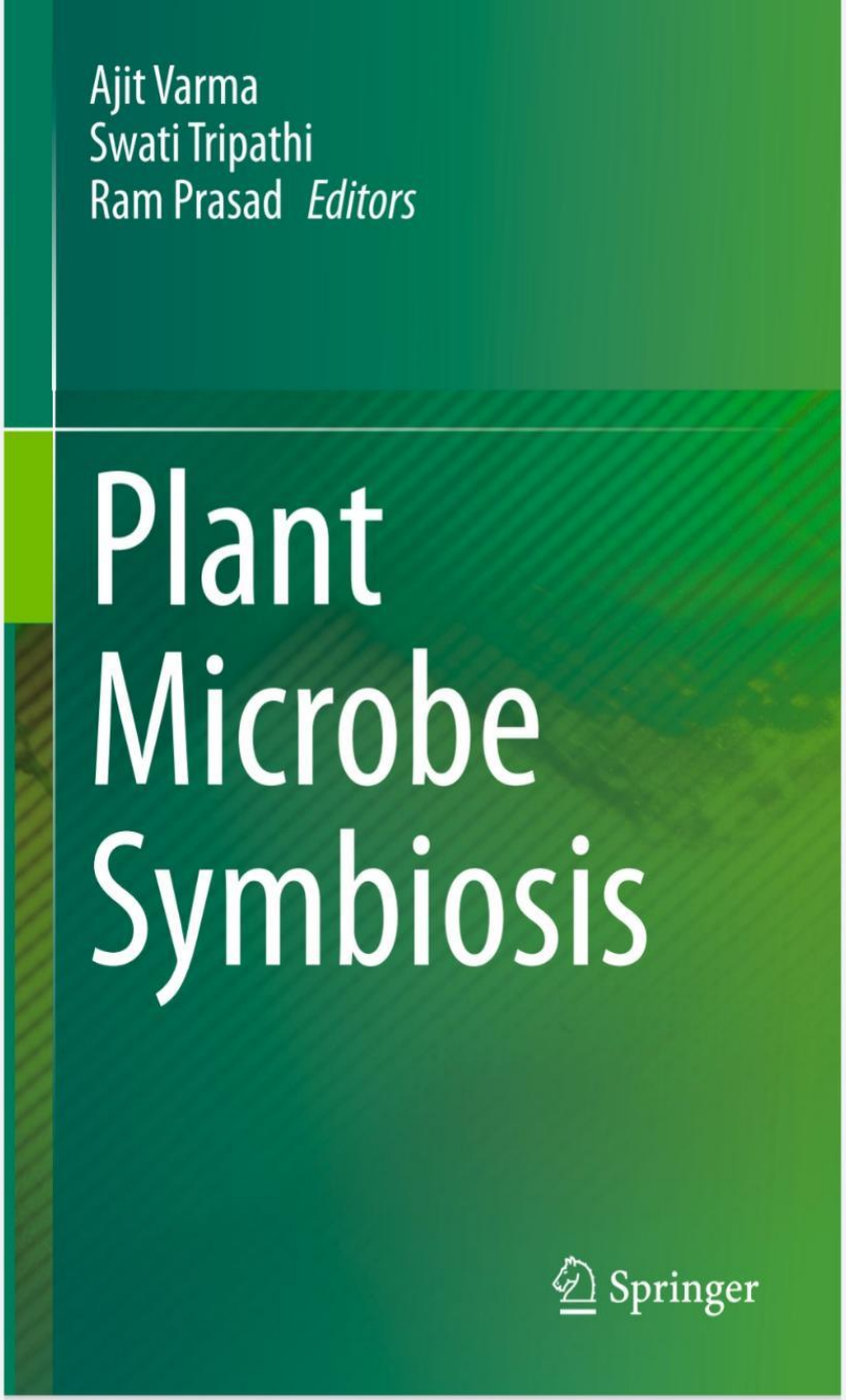

Chapter 12

Pesticide Tolerant Rhizobacteria: Paradigm of Disease Management and Plant Growth Promotion

Tina Roy, Nirmalendu Das, and Sukanta Majumdar

Abstract Plant growth-promoting rhizobacteria (PGPR) are soil bacteria, colonizing rhizospheric region of plants, which have the ability to enhance plant health and promote plant growth by increasing seed emergence, plant weight, and yields to a wide variety of crops either through direct action or via biological control of plant diseases. PGPR improve plant growth by either fixing atmospheric nitrogen; solubilizing insoluble phosphates and iron and producing plant growth regulators (PGRs) like auxins, gibberellins, cytokinins, etc.; or suppression of deleterious root-colonizing microorganisms including plant pathogens through antibiosis, i.e., production of fungitoxic compounds, competition with pathogenic microorganisms for nutrients by producing siderophores, or niche exclusion. Indiscriminate use of different chemicals in the form of fertilizers and pesticides targeting to increase the agricultural produce for ever-increasing population outburst has led to the contamination of the groundwater, soil, and sediments. Accretion of diversified range of chemicals in significant quantities has a direct impact not only on the living beings but also on the environment. The ecological balance of the soil microorganisms has been distorted which show the negative impact on their rhizospheric competence. When exogenous PGPR are applied in this pesticide-infested soil, they not only hardly show their plant growth-promoting or disease-suppressing activities but also might not survive at all. Isolation of native PGPR from the pesticide-challenged rhizospheric soils mostly shows pesticide-tolerant/degrading properties. These PGPR might show the rhizospheric competence in similar pesticide-infested soil. These strains easily acclimatize in the pesticide-contaminated microenvironment in soil and show their plant growth-promoting and pathogen-suppressive activities.

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