Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2016, 8 (1):281-295
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Prevalence of pesticide degrading bacteria in Paddy crop field

Anitha Palanimanickam and Umamaheswari Sepperumal*

**P.G and Research Department of Zoology, Periyar EVR College, Tiruchirappalli, Tamil Nadu, India*

ABSTRACT

The application of pesticides in paddy crop field soil to enhance paddy production could cause adverse impact on the soil bacteria. Hence, the present study aimed to isolate bacteria prevalent in pesticide (Profenofos and Lambdacyhalothrin) exposed soil and also to determine whether the pesticide resistance and degradation trait exhibited by bacteria were plasmid or chromosomal mediated. The dominant bacteria isolated from paddy crop field soil Bacillus cereus and Aneurinibacillus migulanus were plasmid cured and exposed to Profenofos and Lambdacyhalothrin. The observations registered in this study revealed that Profenofos resistant and degradation trait of Bacillus cereus and Aneurinibacillus migulanus were plasmid mediated, whereas, Lambdacyhalothrin resistant and degradation trait of Bacillus cereus and Aneurinibacillus migulanus were chromosomal mediated.

Key words: Profenofos, Lambdacyhalothrin, *Bacillus cereus*, *Aneurinibacillus migulanus*, GCMS, plasmid.

INTRODUCTION

Some microbial strains possess genetic determinants that confer the resistance to pesticides. In bacteria, these determinants are often found on plasmids [1]. Bacteria isolated from toxic chemicals wastes more frequently contain plasmid DNA and demonstrate antimicrobial resistance than do bacterial isolates from domestic sewage-impacted waters or from uncontaminated open ocean sites [2]. A higher incidence of plasmids was found among *Pseudomonas* –like organisms in an industrially polluted river (18%) than in a non-polluted upstream area (7%) [3]. It is well known that plasmids can endow bacterial species with the ability to degrade various man-made organic compounds [4]. Catabolic plasmids have been thought to play an important role in the evolution of pesticide-degrading ability in microorganisms [4, 5]. A plasmid encoding the gene for hydrolysis of parathion to 4-nitrophenol has been found in *Pseudomonas diminuta* [6] and *Flavobacterium* sp. [7]. However, there have been only a few studies of plasmid-associated organophosphorus insecticide degradation.

Studies of two of the plasmids, pPDL2 from *Flavobacterium* sp. strain ATCC 27551 and pCMS1 from *Pseudomonas diminuata* strain MG, by restriction analysis and hybridisation experiments indicated that the *opd* genes were located in a highly conserved region that was estimated to extend approximately 2.6 Kb upstream and 1.7 Kb downstream of *opd* [7,8]. This study aims to determine whether the pesticide resistance exhibited by *Bacillus cereus* and *Aneurinibacillus migulanus* are plasmid borne or chromosomal mediated.

MATERIALS AND METHODS

Isolation of pesticide resistant bacteria from paddy crop field soil

1 gm of pesticide exposed paddy crop field soil were aseptically inoculated in 100 ml of sterile minimal salt media (MSM) into cotton plugged flasks in triplicates. Conical flasks were under continuous shaking at room temperature for one week. Minimal salt media containing the following salts: $CaCl_2$ – 0.002 g , MgCl – 0.02 g, K_2HPO_4 – 0.1 g, KH_2PO_4 – 0.1 g, NH_4NO_3 - 0.1 g and FeCl trace amount in distilled water (pH 7.2 – 7.4) upto 1L were used for inoculation of soil sample. Total heterotrophic bacteria were isolated and identified following Bergeys manual of Determinative Biology [9]. The dominant bacteria *Bacillus cereus* and *Aneurinibacillus migulanus* were selected for pesticide resistant studies.

Plasmid DNA analysis

Plasmid DNA were extracted from Profenofos and Lambdacyhalothrin resistant *Bacillus cereus* and *Aneurinibacillus migulanus* by alkaline lysis method [10] and analysed by gel electrophoresis on 1.2 % (w/v) agarose gels stained with ethidium bromide and visualised under UV light.

Plasmid curing

Plasmid curing was attempted by adding acridine orange at sub-inhibitory concentrations [11]. 5 ml Luria broth to which 0.1 ml of 12 hours of *Bacillus cereus* inoculum was added. After inoculation of 18 hours at 37 ºC, the plasmid cured culture was tested for pesticide degradation ability. Same procedure was followed for *Aneurinibacillus migulanus.*

GCMS analysis of Profenfos degradation metabolites of plasmid cured bacteria

24 hours of plasmid cured *Bacillus cereus* was inoculated in 100 ml of autoclaved MSM containing 500 ppm of Profenofos in 250 ml conical flask and kept at 37 ºC in rotatory shaker at 150 rpm for 10 days.

Extraction of Profenofos residues:

At the end of the 10^{th} day the samples were subjected to GC-MS analysis. Pofenofos were extracted from MSM inoculated with Plasmid cured *Bacillus cereus* for GCMS analysis based on the method of Malghani *et al.*, [12] with minor modifications. The pesticides (aliquots of liquid culture) were extracted using organic solvent extraction three times with acetone and hexane (1:1) mixture, then the extract was concentrated using rotary vacuum evaporator (Buchi R-210, Surkzer) and cleaned up in silica gel column (1:3 cm diameter X 243 cm length) . The pesticide extract was eluted with n-hexane collected in a glass vial and subjected to Gas Chromatograph-Mass Spectrometer (GC-MS) analysis. Same procedure was followed for *Aneurinibacillus migulanus.*

GCMS analysis of Lambdacyhalothrin degradation of plasmid cured bacteria

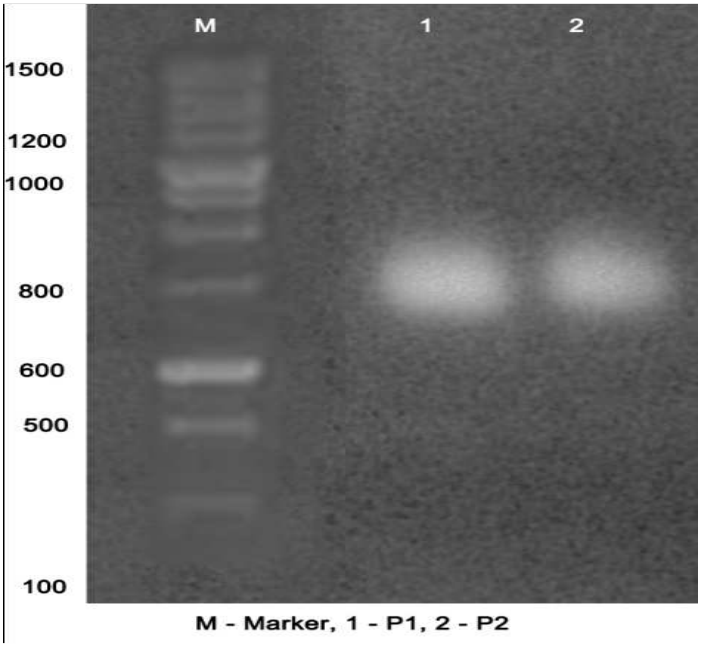
24 hours of plasmid cured *Bacillus cereus* was inoculated in 100 ml of autoclaved MSM containing 50 ppm of Lambdacyhalothrin in 250 ml conical flask and kept at 37 ºC in rotatory shaker at 150 rpm for 10 days.

Extraction of Profenofos residues:

At the end of the 10^{th} day the samples were subjected to GC-MS analysis. The plasmid cured *Bacillus cereus* containing Lambdacyhalothrin was extracted from MSM inoculated with *Bacillus cereus* for GCMS analysis based on the method of Malghani *et al.*, [12] with minor modifications. The pesticides in the treatment (aliquots of liquid culture) were extracted using organic solvent extraction three times with acetone and hexane (1:1) mixture, then the extract was concentrated using rotary vacuum evaporator (Buchi R-210, Surkzer) and cleaned up silica gel column (1:3 cm dimeter X 243 cm length) . The pesticide extract was eluted with n-hexane collected in a glass vial and subjected to Gas Chromatograph- Mass Spectrometer (GC-MS) analysis. Same procedure was followed for *Aneurinibacillus migulanus.*

RESULTS

Fig 1: Plasmid profile of *Bacillus cereus* and *Aneurinibacillus migulanus*



P1- plasmid DNA of *Bacillus cereus*
P2- plasmid DNA of *Aneurinibacillus migulanus*

Fig. 2
Gas Chromatogram of 500 ppm of Profenofos (control) degraded by Bacillus cereus

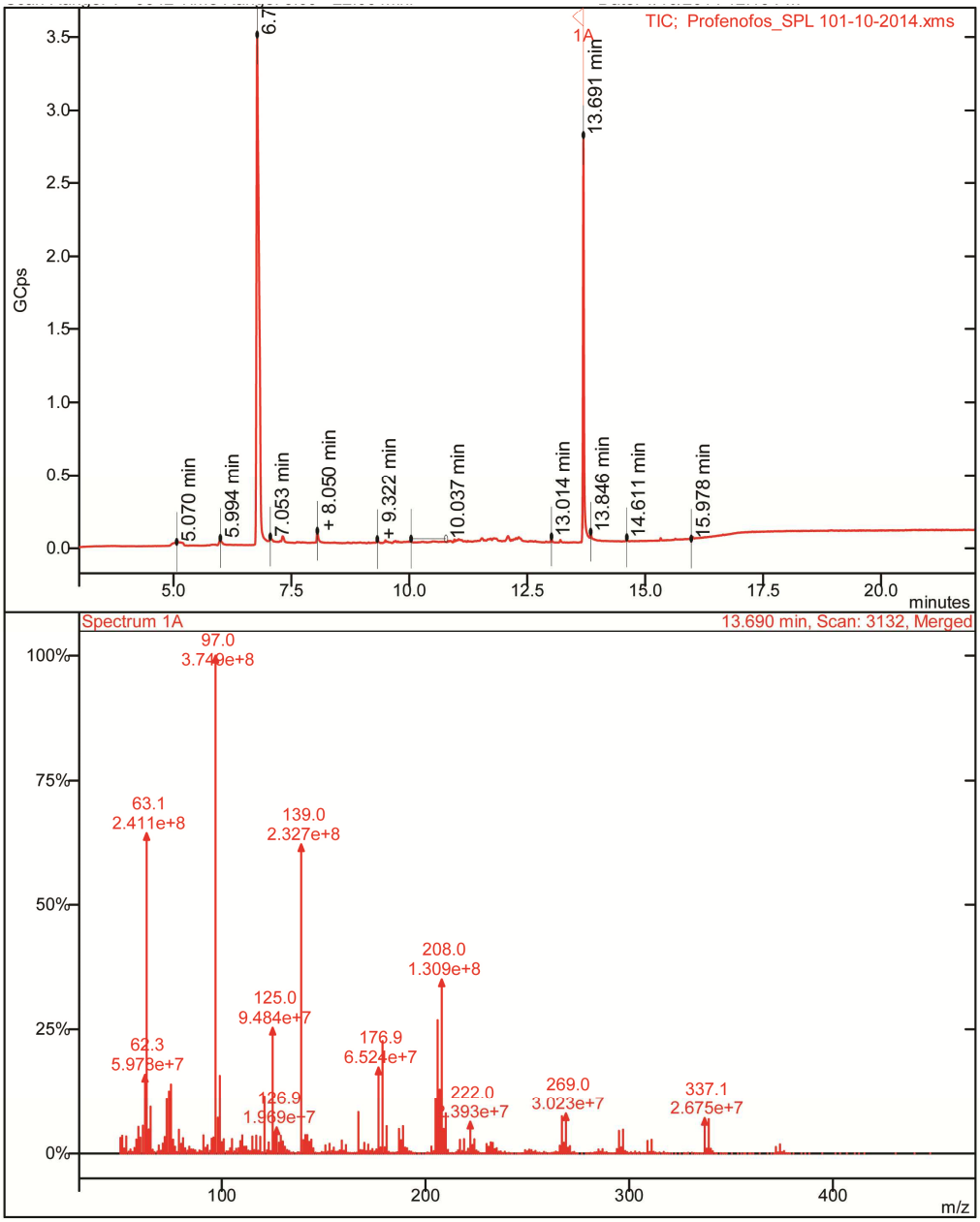


Fig. 3
Gas Chromatogram of 500 ppm of Profenofos degraded by plasmid cured *Bacillus cereus*

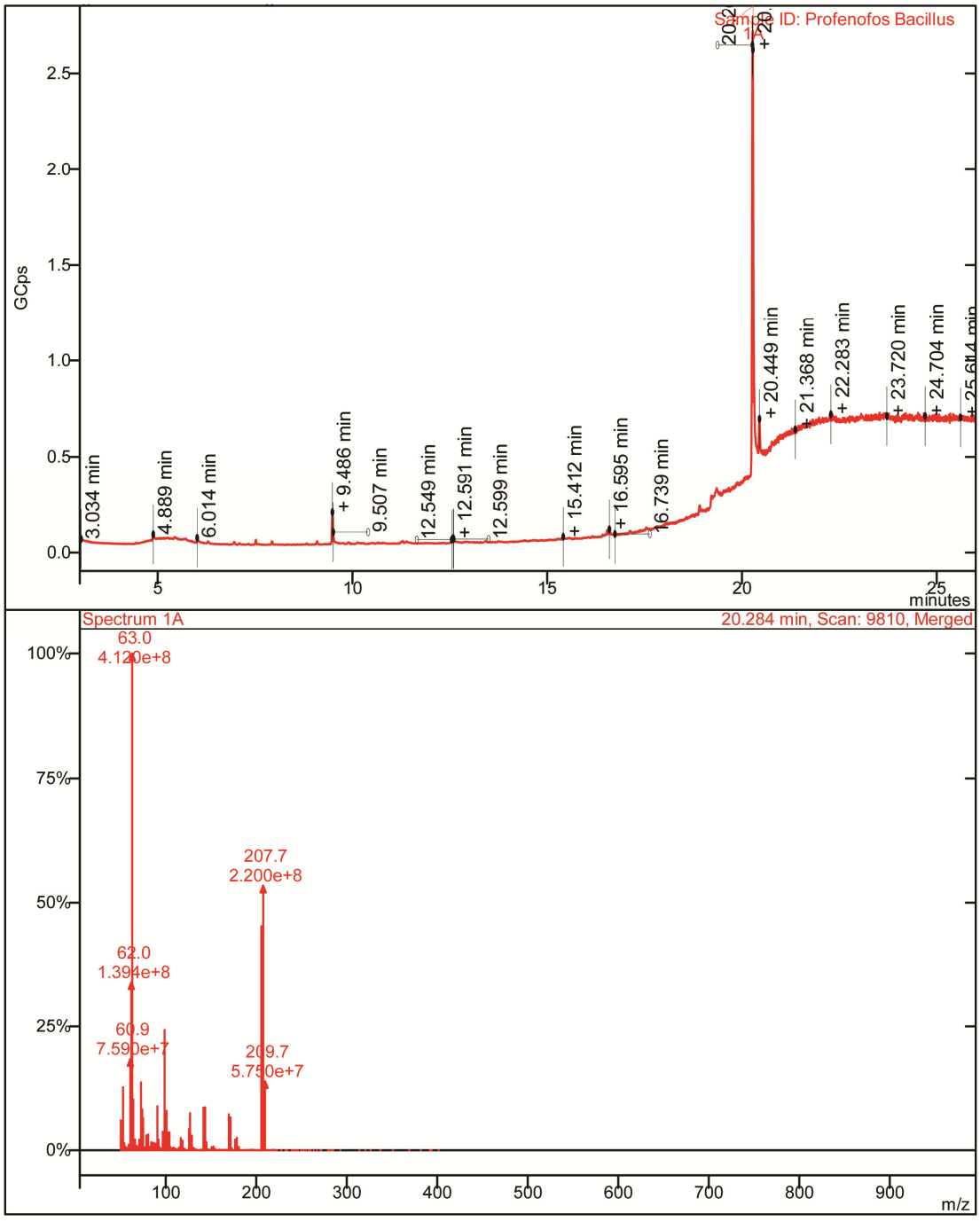


Fig. 4
Gas Chromatogram of 500 ppm Profenofos (control) degraded by *Aneurinibacillus migulanus*

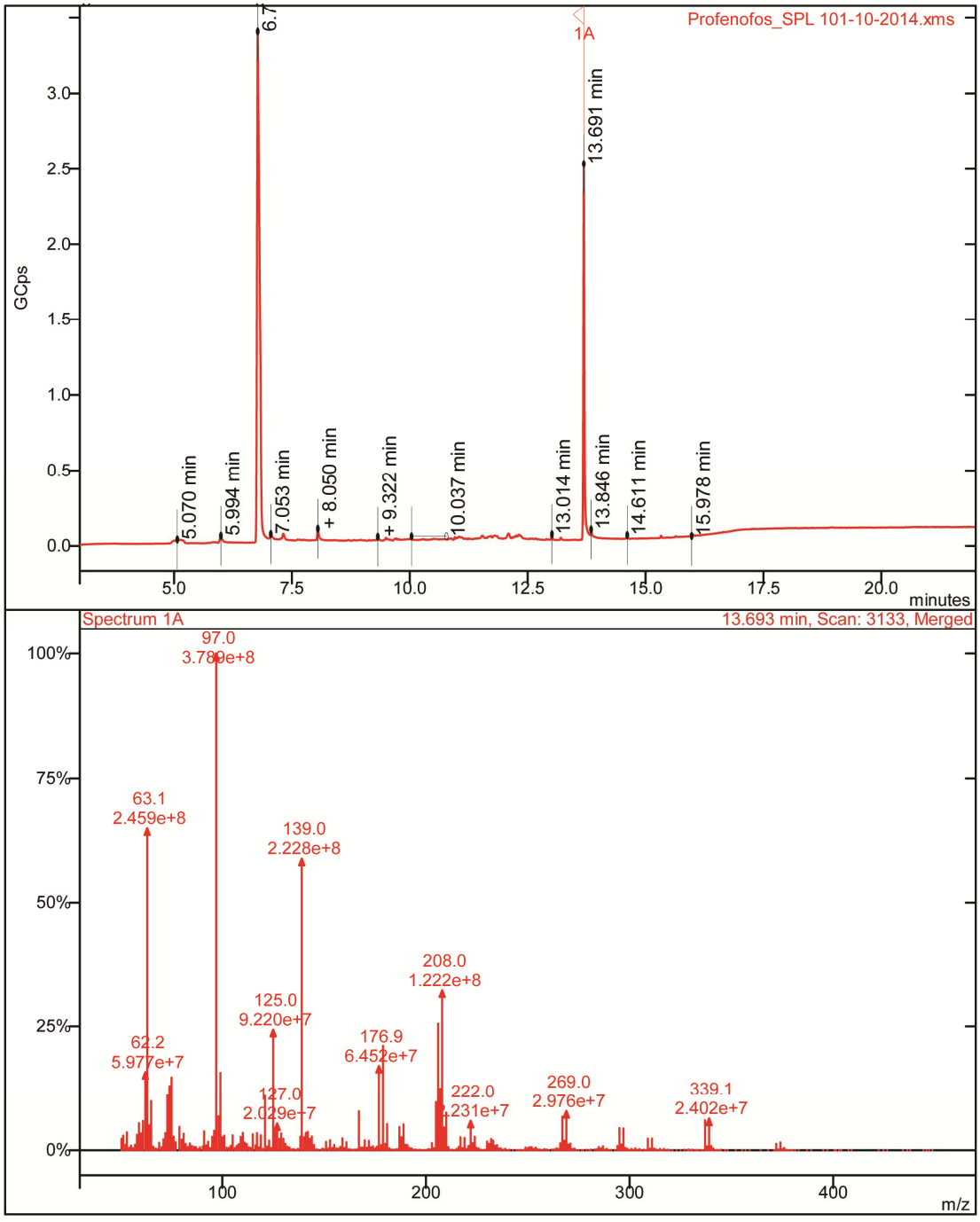


Fig. 5
Gas Chromatogram of 500 ppm Profenofos degraded by plasmid cured *Aneurinibacillus migulanus*

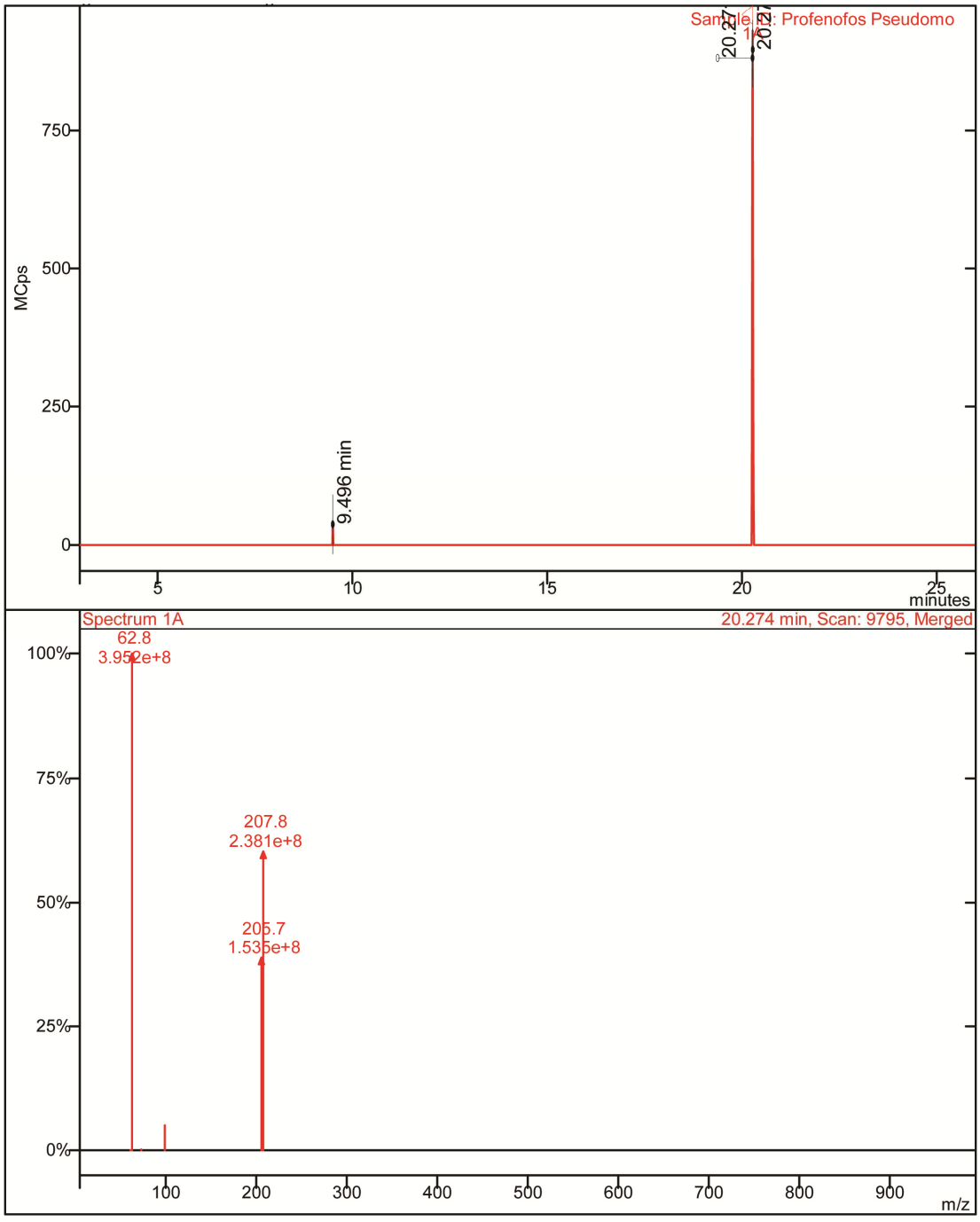


Fig. 6
Gas Chromatogram of 50 ppm Lambdacyhalothin (control) degraded by *Bacillus cereus*

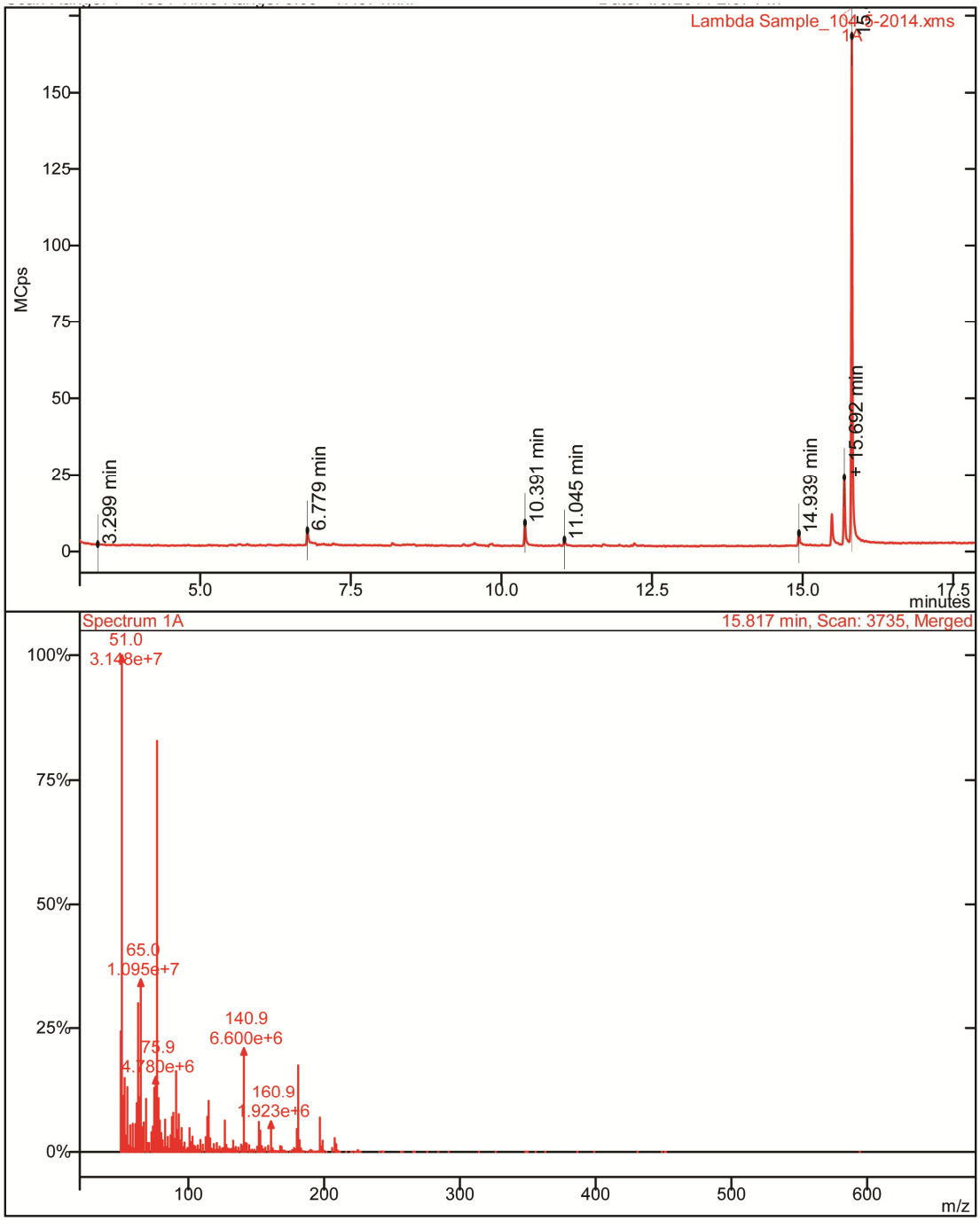


Fig. 7
Gas Chromatogram of 50 ppm Lambdacyhalothrin degraded by plasmid cured *Bacillus cereus*

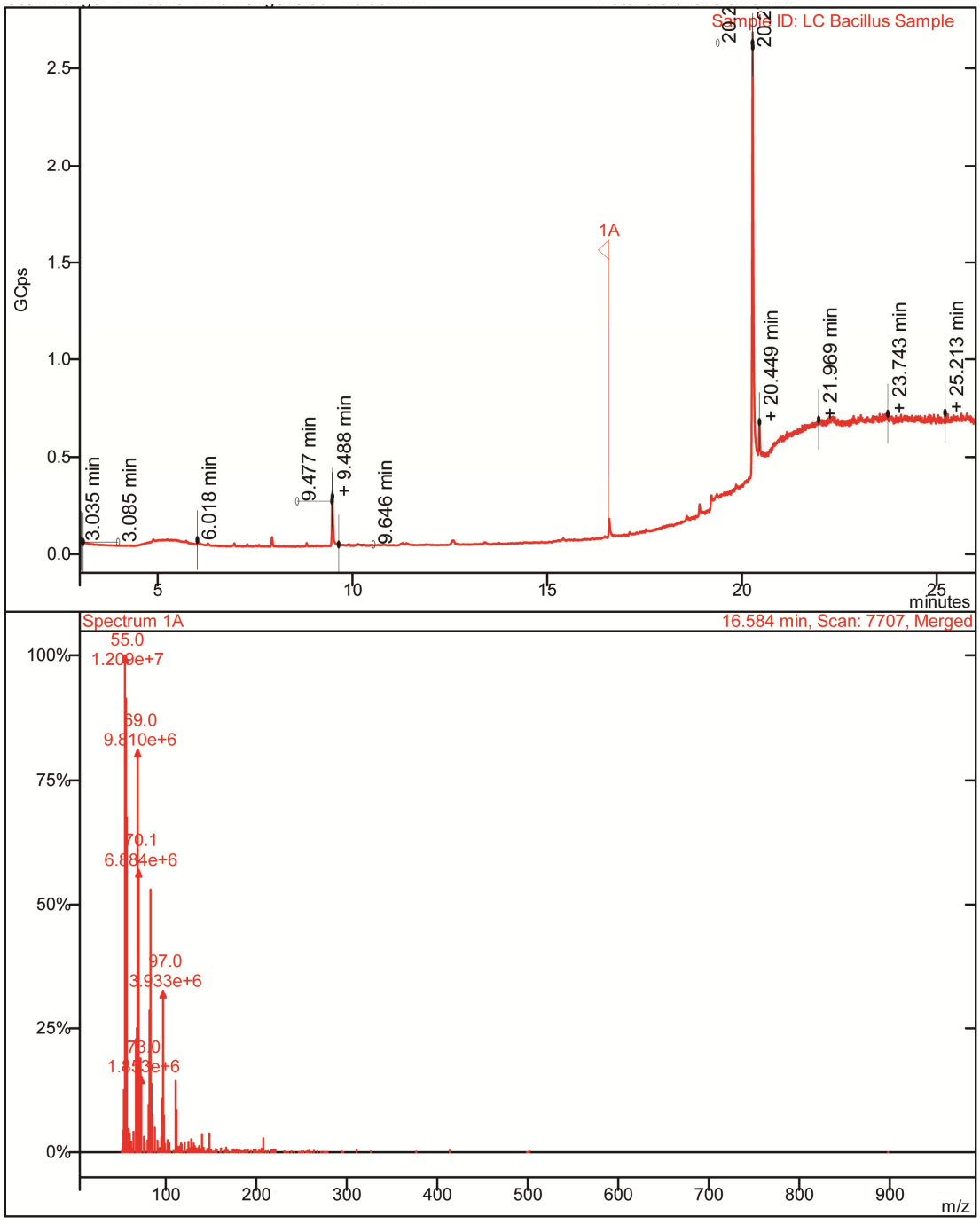


Fig. 8
Gas Chromatogram of 50 ppm Lambdacyhalothin (control) degraded by *Aneurinibacillus migulanus*

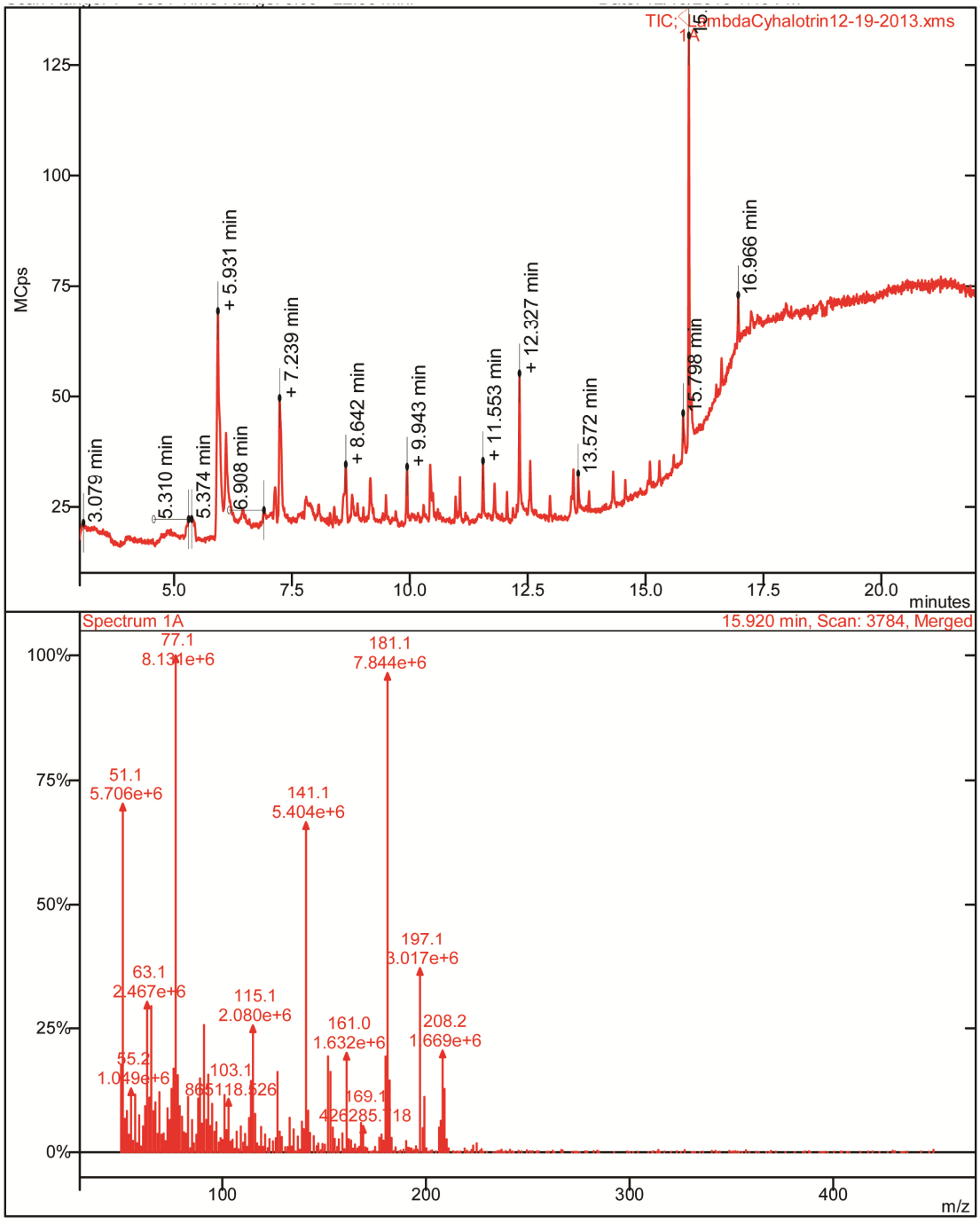


Fig. 9
Gas Chromatogram of 50 ppm Lambdacyhalothin degraded by plasmid cured
Aneurinibacillus migulanus

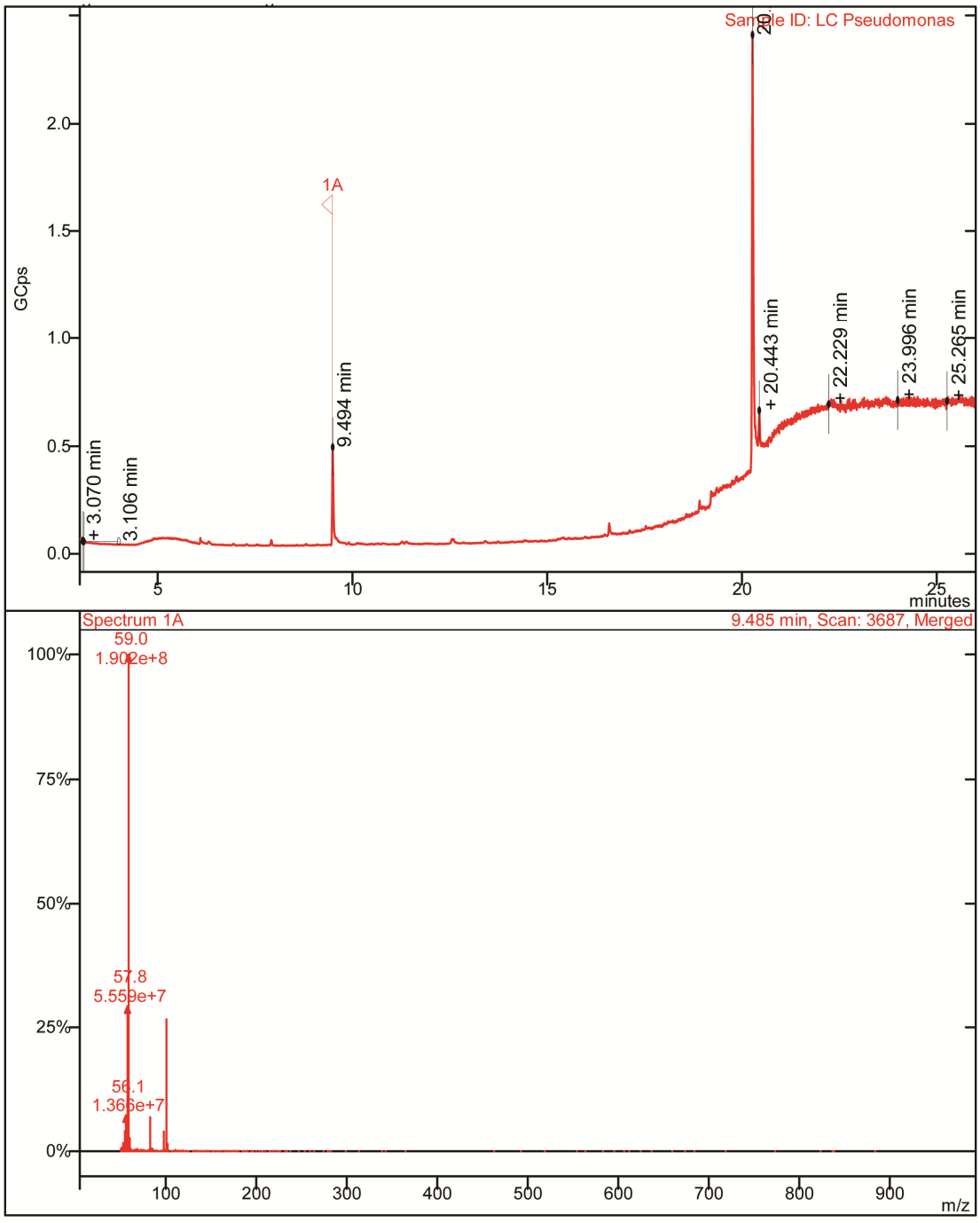


Table 1: GCMS result showing the pesticide degradation ability of Plasmid cured *Bacillus cereus* and *Aneurinibacillus migulanus* at 10th day

Treatment	RT	Remaining pesticide (ppm)	% of degraded pesticide
MSM + 500 ppm Profenofos + 100 µl *Bacillus cereus*	13.691	0.56	99.88
MSM + 500 ppm Profenfos + 100 µl plasmid cured *Bacillus cereus*	-	0	100
MSM + 500 ppm Profenfos + 100 µl *Aneurinibacillus migulanus*	13.691	0.62	99.87
MSM + 500 ppm Profenfos + 100 µl plasmid cured *Aneurinibacillus migulanus*	-	0	100
MSM + 50 ppm Lambdacyhalothrin + 100 µl *Bacillus cereus*	15.817	23.85	52.3
MSM + 50 ppm Lambdacyhalothrin + 100 µl plasmid cured *Bacillus cereus*	-	0	100
MSM + 50 ppm Lambdacyhalothrin + 100 µl *Aneurinibacillus migulanus*	15.920	12.1	75.0
MSM + 50 ppm Lambdacyhalothrin + 100 µl plasmid cured *Aneurinibacillus migulanus*	-	0	100

No peak, RT-Retention time

Table 2: Bacterial degraded metabolites of 500 ppm Profenofos detected by GC-MS

Treatment	RT	Intermediate compounds
Control (MSM + 500 ppm Profenofos + 100 µl *Bacillus cereus*)	5.994	Benzoic acid
	6.715	4-bromo-2-chloro phenol
	7.053	4-bromo-2(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisophenol
	8.050	Phosphorothioic acid,O,O,S-triethylester
	13.691	**Profenofos**
	13.846	2-bromo-4-chloro phenol
MSM + 500 ppm Profenofos + 100 µl plasmid cured *Bacillus cereus*	20.267	4-bromo-2-chloro phenol
Control (MSM + 500 ppm Profenofos +100µl *Aneurinibacillus migulanus*)	5.994	Benzoic acid
	6.775	4-bromo-2-chloro phenol
	8.050	Phosphorothioic acid, O,O,S-triethyl ester
	13.691	**Profenofos**
MSM + 500 ppm Profenofos +100µl plasmid cured *Aneurinibacillus migulanus*	20.281	4-bromo-2-chloro phenol
	20.450	2-(2-Ethoxy ethoxy carbonyl) benzoic acid

RT-Retention time

Table 3: Bacterial degraded metabolites of 50 ppm Lambdacyhalothrin detected by GC-MS

Treatment	RT	Intermediate compounds
Control (MSM + 50 ppm Lambdacyhalothrin + 100 µl *Bacillus cereus*)	10.391	2-Propenoic acid
	11.045	Phenoxy benzaldehyde
	14.939	Propanoic acid
	15.692	2-Propenoic acid, tetradecyl ester
	15.817	**Lambdacyhalothrin**
MSM + 50 ppm Lambdacyhalothrin + 100 µl plasmid cured *Bacillus cereus*	7.923	1,2,4- Trimethylbenzene
	9.499	2-Propanone, 1-(dimethyamino)-
	16.576	4-Cyclo propyl carbonyloxy dodecane
	20.448	2-Acetylbenzoic acid
Control (MSM + 50 ppm Lambdacyhalothrin +100µl *Aneurinibacillus migulanus*)	6.104	(1-[1,2-di (methoxy methoxy)-3-oxopropyl-2-propyny Benzoic acid.
	7.239	Phthalic anhydride
	8.642	4-(2,2-Dimethyl-6-methyl-enecyclohexyl) butanol
	9.943	N-trifluoro acetyl-dehydro norketamine
	11.553	Cyclopropane propionic acid, 2[(2-decyl cyclopropyl) methylene
	15.920	**Lambdacyhalothrin**
MSM + 50 ppm Lambdacyhalothrin +100µl plasmid cured *Aneurinibacillus migulanus*	7.934	1,2,4 Trimethyl benzene
	9.468	1,3-Propanediamine, N- (3-aminopropyl)-N-methyl-
	16.598	Cyclopropane

RT-Retention time

Plasmids of 800 Kb size were observed in *Bacillus cereus* and *Aneurinibacillus migulanus* (Fig 1). The Profenofos and Lambdacyhalothrin degradation potential of *Bacillus cereus* and *Aneurinibacillus migulanus* were evaluated by curing the plasmid in these isolates. The Plasmid cured bacterial isolates were subjected to degradation experiment for a period of 10 days. After 10 days the degraded products were subjected to GCMS in order to evaluate the amount of pesticide degraded. The data presented in table 1 revealed that *Bacillus cereus* and *Aneurinibacillus migulanus* mediated degradation of lambdacyhalothrin was chromosomal mediated.

100 % degradation of profenofos (500 ppm) and lambdacyhalothrin (50 ppm) was elicited by plasmid cured *Bacillus cereus* and *Aneurinibacillus migulanus*. *Bacillus cereus* mediated 99.88 % and 52.3 % profenofos (500 ppm) and lambdacyhalothrin (50 ppm) degradation, respectively. On the other hand, plasmid cured *Bacillus cereus* elicited 100 % degradation of profenofos and lambdacyhalothrin. *Aneurinibacillus migulanus* exhibited 99.87 % and 75.0 % degradation of profenofos and lambdacyhalothrin, respectively. On contrary, plasmid cured *Aneurinibacillus migulanus* caused complete (100 %) degradation of profenofos and lambdacyhalothrin.

From table 2, it is observed that Benzoic acid, 4-bromo-2-chloro phenol, 4-bromo-2(1,2,3,4-tetrahydro-6,7-dimethoxy-2-methylisophenol, Phosphorothioic acid, O,O,S-triethylester, profenofos and 2-bromo-4-chloro phenol were the degradative metabolites of profenofos (500 ppm) in MSM inoculated with *Bacillus cereus* after a period of 10 days (Fig 2). On the other hand, plasmid cured *Bacillus cereus* inoculated in MSM containing (500 ppm) profenofos resulted in the formation of a single metabolite 4- bromo-2-chloro phenol (Fig 3).

Benzoic acid, 4-bromo-2-chloro phenol, Phosphorothioic acid, O,O,S-triethyl ester and Profenofos were the metabolites detected in MSM containing 500 ppm profenofos inoculated with *Aneurinibacillus migulanus*, whereas, 4-bromo-2-chloro phenol and 2-(2-Ethoxy ethoxy carbonyl) benzoic acid were the metabolites present in MSM inoculated with plasmid cured *Aneurinibacillus migulanus* after a period of 10 days (table 2) (Fig 4,5).

Several metabolites were observed in MSM containing 50 ppm lambdacyhalothrin inoculated with *Bacillus cereus* (2-Propenoic acid, Phenoxy benzaldehyde, Propanoic acid, 2-Propenoic acid, tetradecyl ester, Lambdacyhalothrin) (Fig 6). The degradative metabolites of lambdacyhalothrin were 1,2,4- Trimethylbenzene, 2-Propanone, 1-(dimethyamino)-, 4-Cyclo propyl carbonyloxy dodecane, 2-Acetylbenzoic acid in MSM containing 50 ppm lambdacyhalothrin inoculated with plasmid cured *Bacillus cereus* after a period of 10 days (Fig 7). Inoculation of MSM containing lambdacyhalothrin (50 ppm) with *Aneurinibacillus migulanus* resulted in the formation of (1-[1,2-di (methoxy methoxy)-3-oxopropyl-2-propyny Benzoic acid, Phthalic anhydride, 4-(2,2-Dimethyl-6-methyl-enecyclohexyl) butanol, N-trifluoro acetyl-dehydro norketamine, Cyclopropane propionic acid, 2[(2-decyl cyclopropyl) methylene and Lambdacyhalothrin, whereas, plasmid cured *Aneurinibacillus migulanus* elicited the formation of 1,2,4 Trimethyl benzene, 1,3-Propanediamine, N- (3-aminopropyl)-N-methyl and Cyclopropane (Fig 8,9). These results indicate the degradation of profenofos and lambdacyhalothrin elicited by *Bacillus cereus* and *Aneurinibacillus migulanus* were plasmid and chromosomal mediated, respectively.

DISCUSSION

The present result coincides with that of Bahig *et al.*, [13] who have demonstrated the presence of plasmid in bacteria isolated from agriculture soil irrigated with waste water and canal water. They have observed that out of 771 bacterial isolates, approximately 138 bacterial isolates from site irrigated with canal water and ~ 199 isolates from site irrigated with waste water harboured plasmid. They have further reported that the plasmid sizes ranged from 25 to 200 Kb (large plasmid) and the plasmid size less than 25 Kb (small plasmid). In addition, they have noticed the incidence of large plasmids of the total population from site irrigated with waste water when compared to canal water. Our results partially agrees with that of Masahito Hayatou *et al.*, [14] who have demonstrated that two plasmid pNF1 and pNF2 was involved in Fenitrothion degradation by *Burholderia* sp. isolated from soil exposed to fenitrothion for at least 2 years. Furthermore, Mohamad [15] have demonstrated through transformation experiment (pMb was transferred to *E. coli* DH5α strain the gene responsible for methomyl degradation by *Stenotrophomonas matophilia* M1 was encoded in plasmid pMb (5 Kb). Further, *Stenotrophomonas matophilia* was known to harbour two different plamsids pMa (8 Kb) and pMb (5 Kb). Also, new gene combination could allow the degradation of related compounds, degradation via different pathways, or recombination between related gene to generate even greater metabolic diversity [16]. When this plasmid is genetically transferred to a new host it confirm the capacity to metabolise the compound.

The present study is in good accord with Dayananda siddavatta *et al.*, [17] who have isolated several bacterial strains that can use organophosphate pesticides as a source of carbon from diverse geographical regions, and these organisms synthesised parathion hydrolase, which were encoded by a gene (opd) located on a large indigenous plasmid. In addition, they have characterised (*Flavobacterium* plasmid : pPDL2; *Pseudomonas diminuta* : pCMS1). Moreover, they have demonstrated transposon like organisation of the plasmid borne organophosphate degradation (opd) gene cluster in *Flavobacterium sp.*

Fisher *et al.*, [18] have isolated and characterised pesticide-degrading plasmid pJP1 form *Alcalgenes paradoxus*. Siddavattam [19] have demonstrated that *Flavobacterium balutinum* was able to degrade methyl parathion, an organophosphorus insecticide into parnitrophenol and other metabolites isolated from agriculture soils of Anantapur district , Andrapradesh, India. Further, they have identified an indigenous plasmid of approximately 86 Kb in size in *Flavobacterium blaustinum.* They have also proved that parathion hydrolase was encoded by this plasmid as no enzyme activity was observed in plasmid cured strain. Present observation coincides with that of Kulkarni and Kaliwal [20] demonstrated that the isolated *Pseudomonas aeriginosa* possesses the ability to degrade methomyl. This strain contains a plasmid (22 Kb) carrying the gene responsible for the degradation of methomyl. This plasmid could be transferred to another bacterial strain in the environment and provide it with methomyl pesticide degradative ability and potentially a selective advantage under given environmental state.

Manisha Deb Mandal *et al.* [21] have reported that *Bacillus licheniformis* isolated from the intestine of *Labeo rohita* used dimethoate as the sole source of carbon. They have further evinced that the bacteria harboured a single plasmid of approximately 54 Kb. Further, they have demonstrated that the wild *Bacillus licheniformis* strain transformed dimethoate degradation property to *E.coli* (600 (Na^r, F^-) strain and the transconjugant also harboured a plasmid of the same molecular size (approximately 54 Kb) as that of the donor plasmid; the cured strain was plasmidless.

Deshparide *et al.*, [22] have demonstrated the ability of *Pseudomonas aeriginosa* (isolated from soil pre exposed to dimethoate for atleast 7-8 years) to degrade dimethoate. As evinced in this study they have also observed the presence of 6.6 Kbp plasmid (pDM 427) in *Pseudomonas aeriginosa* MCMB-427. Further, they have transferred this plasmid to *Escherichia coli* , Nova Blue. Subsequently, *E. coli* acquired the ability to degrade dimethoate. Further, they have also evinced that curing of the plasmid by plumbagin or ethidium bromide resulted in the less of ability of MCMB-427 to degrade dimethoate. They have concluded that the ability of *Pseudomonas aeruginosa* MCMB-427 to degrade dimethoate is plasmid mediated and transferable to other strains. Further, Masahito Mayatsu *et al.*,[23] have demonstrated the involvement of two plasmids pRC1 and pRC2 of 110 and 1720 Kbp, respectively in the degradation of carbaryl by *Arthrobacter* strin RC 100.These observations are in consistent with the present findings.

Similar findings were reported by Umamaheswari and Murali [24] who have reported that Bavistin resistant exhibited by *Pseudomonas aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus* were plasmid borne. In addition, Umamaheswari *et al.*, [25] have reported that *Bacillus ciradans* and *Acinetobacter* sp. isolated from chilly crop field soil of Chettiyakulum village, Trichy district, Tamil Nadu, India, (exposed to endosulfan for several years) were resistant to endosulfan. Further, they cured the plasmid using acridine orange and confirmed that endosulfan resistant trait were plasmid mediated.

Involvement of plasmid in parathion degradation was reported by Serdar *et al.*,[26]. *Pseudomonas diminuta* was able to hydrolyse parathion. Cells grown for 48 hours contained 3400 U of parathion hydrolase activity per liter of broth. The activity was found to be associated with plasmid pSC1 of molecular mass 44 x 10^6 daltons. The gene (opd) which encodes the broad spectrum organophophate phosphotriesterase in *Pseudomonas diminuta* was cloned into M13 mp10 and found to express parathion hydrolase under control of lac promoter in *Escherichia coli.* In *Flavobacterium* sp. strain ATCC 27551, a 43 Kb plasmid was associated with the experiments. Southern hybridization experiments demonstrated that a genetic region from the 43 Kb *Flavobacterium* sp. plasmid possessed significant homology to the opd sequence. It appeared that the two discrete bacterial plasmid from parathion hydrolysing soil bacteria possess a common but limited region of sequence homology within potentially non - homologous plasmid structures [7].

Chaudhary *et al.*, [27] described two mixed bacterial cultures utilising methylparathion and parathion. *Pseudomonas* sp. from the mixed culture could degrade the pesticides p-nitrophenol. The hybridisation data showed that the DNAs from *Pseudomonas* sp. and from the mixed culture had homology with opd (organophosphate degradation) gene from a previously reported parathion hydrolysing bacterium *Flavovobacterium* sp. The nucleotide sequence of the mpd (methyl parathion degrading) gene was determined and the gene could be expressed in *E. coli* . Somara and Siddvattam [28] described *Flavobacterium balustinum* harbouring an indigenous plasmid of approximately 86 Kb in size. The degradative enzyme parathion hydrolase, was found to be encoded by this plasmid. No enzyme activity was observed in plasmid cured strain. Plasmid mediated profenofos degradation by

bacteria observed in this study lies in parallel with the findings of Segers *et al*., [29] also reported plasmid controlled parathion degradation in *Brevundimonas diminuta* (earlier classified as *Pseudomonas diminuta).*

CONCLUSION

The observations registered in this study revealed that Profenofos resistant and degradation trait of *Bacillus cereus* and *Aneurinibacillus migulanus* were plasmid mediated, whereas, Lambdacyhalothrin resistant and degradation trait of *Bacillus cereus* and *Aneurinibacillus migulanus* were chromosomal mediated.

Acknowledgements
The authors register their thanks to Dr. D. Saravanan and Mrs. S. Kavitha, Department of Chemistry, National College, Tiruchirappalli, Tamil Nadu for GCMS analysis of the sample.

REFERENCES

[1] C Cervantes; G Ji; JL Ramirez; S Silver; *FEMS Microbiology Reviews*.,**1994**, 15, 355-367.
[2] AM Baya; PR Brayton; VL Brown; DJ Grimes; E Russek-Cohen; RR Colwell; **1986**, *Applied and Environmental Microbiology*., 51, 1285-1292.
[3] NF Burton; MJ Day; AT Bull; **1982**, *Applied and Environmental Microbiology*., 44, 1026-1029.
[4] GS Sayler; SW Hooper; AC Layton; JM Henry King; **1990**, *Microb. Ecol*., 19, 1-20.
[5] S Chapalamadugu S; GR Chaudhry; **1992**, *Crit. Rev. Biotechnol*., 12, 357-389.
[6] CM Serdar; DT Gibson; DM Munnecke; JH Lancaster; **1982**, *Appl. Environ. Microbiol*., 44, 246-249.
[7] WW Mulbry; JS Karns; PC Kearney; JO Nelson; CS McDaniel; JR Wild; **1986**, *Appl. Environ. Microbiol*., 51, 926-930.
[8]WW Mulbry; JS Karns; **1989**, *J. Bacteriology*.,171: 6740-6746.
[9] PHA Sneath; SN Mair; M Elisabeth Sharpe; JG Holt; In Bergeys manual of systematic Bacteriology, Williams and ailkins, Baltimore, USA, **1994.**
[10] T Maniatis; EF Fritsch; J Sambrook; a Laboratory Manual, Cold springer Harbor Laboratory, Cold spring Harbor, **1982.**
[11] FE Hahn; Chiak J;**1976**, *Antimicrobial. Agents and Chemotherapy*., 9, 77-80.
[12]S Malghani; N Chatterjee; Hu Xue Yu; Zejiao Luo; **2009**, *Braz. J. Microbiol*. 40(4) 893-900.
[13] AE Bahig; EA Aly; AA Khaled; KA Amel; **2008**, *Malaysian Journal of Microbiology*., 4(2): 42-50.
[14] H Masahito; H Motoko; T Shinichi; **2000**, *Applied and Environmental Microbiology*., 66(4) 1737-1740.
[15] MS Mohamed; **2009,** *Electronic Journal of Biotechnology*., 12(4)
[16] JS Karns; **1990**, *ACS Symposium Series.*, 426, 141-152.
[17] D Siddavattam; S Khajamohiddin; B Manavathi; BP Suresh; M Merrick; **2003**, *Applied and Environmental Microbiology*., 69(5): 2533-2539.
[18] PR Fisher; J Appleton; JM Pemberton; **1978**, *Journal of Bacteriology*., 135(3): 798-804.
[19] D Siddavattam; **1995**, *Biochemistry and Molecular Biology International*., 36(3): 627-631.
[20] AG Kulkarni; BB Kaliwal; **2015**, *Journal of Bioremediation and Biodegradation*., 6(2): 281-287.
[21] DM MManisha; M Shyamapada; P Nishith Kumar; **2005**, *Journal of Biomedicine and Biotechnology*., 3, 280-286.
[22] NM Deshpande; PK Dhakephalkar; PP Kanekar; **2001**, *Letters in Applied Microbiology*., 33, 275-279.
[23] H Masahito; H Motoko; N Tadahiro; **1999**, *Applied and Environmental Microbiology*., 65(3):1015-1019.
[24] S Umamaheswari; M Murali; **2010**, *Journal of Environmental Biology*., 31(6) 957-964.
[25] S Umamaheswari; P Anitha; S Gokilavani; **2013**, *Journal of Microbiology and Biotechnology Research*., 3(6): 15-20.
[26] CM Serdar; Gibson DT; Munnecke DM; JH Lancaster; **1982**, *Appl. Environ. Microbiol*., 44, 246-249.
[27] GR Chaudhary; Huang GH; **1988,** *Journal of Bacteriology*., 170, 3897.
[28] S Somara; D Siddavattam; **1995**, *Biochem. Biol. Int.* 36, 627-631.
[29] P Segers; M Vancanneyt; B Pot; U Torck; B Hoste; D Dewettinck; E Falsen; K Kersters; P De Vos; **1994**, *Int J. Sys. Bacteriol*. 44, 499-510.