International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 07, July 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

ISOLATION AND CHARACTERIZATION OF LINDANE DEGRADING BACTERIA FROM ENRICHMENT ON MULTIPLE PESTICIDES

Satish Kumar[1], Rajkumar Bidlan[2], Jai Gopal Sharma[3]

Department of Biotechnology, Delhi Technological University, Delhi- 110042, (India)

ABSTRACT

Lindane had been widely usedin agriculture, public health projects to control pests and in cosmetics industry. Mixed commercial formulated pesticides like organophosphates and pyrethroids aregainingacceptancein theglobal market. The extreme use of pesticides has generated numerous environmental problems leading to disturbance in biogeochemical cycles and ecosystem. It becomes imperative to develop strategies that clear off the environment residues theresidues of organochlorines, organophosphates and pyrethroidspesticides. Soil microflora is the basic and major eco-friendly agent for minimization of these pesticides. Weenricheda consortiumon mixed commercial formulations of organophosphates and pyrethroids, followed by acclimatization by lindane (isomer of organochlorine pesticide hexachlorocyclohexane). During screening, only four bacterial strains survived in nutrient broth induced with 10 ppm lindane. However, 18 bacterial strains able to degraded a thin film of lindane (0.6%) on nutrient agar plate. The four bacterial strains viz. SRJ1, SRJ2, SRJ3 and SRJ4 were used for morphological, biochemical testing and antibiotic susceptibility for identification. These four strains may be constituted into a defined microbial consortium and applied to the pesticide-contaminated sites for efficient treatment.

Keywords: Pesticides, Lindane, Bacterial consortium, Biochemical test, Antibiotic sensitivity.

I. INTRODUCTION

Gamma-hexachlorocyclohexane (γ-HCH) commonly known as lindane has been broadly used in agriculture in the last decades due to insecticidal properties. It is also used for public health and medical purposes in the past and in present [1]. Few isomers of HCH don't have insecticidal properties, consequently dumped as waste and its accumulation causes many environmental problems [2]. AccordingtoWeber *et al* [3] an estimate6 million tons of various isomers of HCH havebeen dumped all over world. Due to the extensive worldwide use, this isomer of HCH has been detected in environmental samples. HCH andDDT were used in the last 30years of their ban and residues of these pesticides existed in the environment of China [4]. Tech-HCH has been shown totoxic affect the seeds germination, growth and reduce the length of seedling[5]. Lindane is a neurotoxin that affects the nervous system and other organs [6]. Due to the toxicity and persistence in nature of γ-HCH, contaminated sites with this compound has been targeted for remediation. A lot of information is available on the biodegradation of γ-HCH and other isomers of HCH using pure and mixed bacterial cultures [7-9]. This study summarizes the isolation ofγ-HCH degrading bacterial strains which were enriched by organophosphates and pyrethroids mixed commercial formulations pesticides. In our laboratory, we tried to grow the best bacterial

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

strains capable of degrading more than one type of pesticide. Main objective of this study is to isolate potential strain from enriched on multiple pesticides and morphological, biochemical testing and antibiotic sensitivity for identification. The outcome of this work may be applied to contaminated sites with a similar load of pesticides, because mostly commercial formulations of pesticides are directly used on site by farmers and common people. Such strategies can be helpful in cleaning our environment, leading to better health of plants and animals in the ecosystem.

II. MATERIALS ANDMETHODS

2.1 Chemicals and media

Lindane(97% purity)was purchased from Sigma-Aldrich Company (USA).Commercial formulations of organophosphates and pyrethroids (Chloropyriphos, Profenofos, Cypermethrin, Deltamethrin, Triazophos, Lambda- Cyhalothrin, Dichlorvos, Cypermethrin) pesticides were obtained from Hindustan Pulverising Mills (HPM) Chemicals & Fertilizers Limited, insecticide India Ltd and Bharat Insecticides Ltd, India. Nutrient agar, antibiotic disks and nutrient broth were from HiMedia Lab Pvt Ltd and Titan Biotech Ltd respectively. All other chemicals used for the biochemical tests were ofanalyticalgrade and from standard company. Thenutrient broth/agar media and mediafor the biochemical tests were prepared accordingtoBidlan [10].

2.2 Enrichment, isolation and screening of bacterial strains

The enrichment technique was developed according to Kumar *et al* [11] with slight improvements that is first enriched with mixed commercial formulations of organophosphates and pyrethroids followed by pure isomer of organochlorine i.e., γ-HCH. These cultures were gradually acclimated to increasing concentrations of above commercial formulations pesticides. The pure cultures isolated from the enriched consortium by repeated streaking were further streaked for dense growth onto nutrient agar plates and allowed to grow for 24 h. These culture plates were then sprayed with 0.6% lindane (in acetone) under aseptic conditions. The acetone was allowed to evaporate leaving a thin lindane film behind. The plates were incubated for another 48 hatroom temperature(RT, 25-30 ^{o}C). and observed for the zone of clearance in the lindane film due to degradation by the respective cultures. The 18 positive cultures were further screened in nutrient broth as well as on nutrient agar plate supplemented with 10 ppm lindane (Fig. 2).

2.3 Culture and inoculum

Four pure bacterial isolates SRJ_1, SRJ_2, SRJ_3 and SRJ_4 were used in this study. These pure cultures wereinoculated tonutrient broth and incubated for 24 h at room temperature. Fresh24 h old cultures were used asinoculum for morphological and biochemical tests. Rest of the inoculum was maintained at 4 ^{o}C till further use. Each culture was checked for purity before carrying out the morphological and biochemical tests by plating on the nutrient agar plates. Only the pure cultures were, later, mixed together to get the defined consortium.

2.4 Morphological and biochemical behavior of bacterial strains

Morphological study including cell shape, cell arrangement and gram staining was done with the help of light microscope. For biochemical behavior of cultures, many tests such as catalase, nitrate reduction, indole

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

test, H_2S production, citrate utilization test, starch hydrolysis and gelatin hydrolysis were performed using the standard protocols [10].

2.5 Antibiotic sensitivity

Seven antibiotics were used Kanamycin (5 □g/disc; K-5), Norfloxacin (5 □g/disc; Nx-5), Streptomycin (10 □g/disc; S-10), Rifampicin (5 □g/disc; Rif-5), Penicillin-G (10 □g/disc; P-10), Cefixime (5 □g/disc; Cfm-5) and Nalidixic acid (30 □g/disc; Na-30). These antibiotic disks were placed on the surface ofnutrient agar which has already been inoculated overnight with pure bacterial strain (Fig. 3).

III. RESULTS AND DISCUSSION

3.1 Enrichment, screening of the individual isolates

The mixed population that got enriched with a gradual increase in concentrations of non-organochlorine pesticide mixture consisted of many morphologically distinct colonies on nutrient agar plates (Fig. 1).The agar plate screening reveals that some of the individual isolates of the consortium enriched on non- organochlorine pesticide could degrade lindane provided as a thin film.These bacterial strains were incubated for 48h and lindane solution was sprayed on the three pure strains grown in one culture plate(Fig. 2). This was an indication that 18 cultures showed clearance of lindane film, they have potency for degrading lindane. Further these 18 bacterial strains were grown in nutrient agar plate in presence of 10 ppm lindane as well as in nutrient broth using 10 ppm lindane as a carbon source. Only four bacterial strains survived in presence of 10 ppm lindne innutrient broth. These four distinctbacterial strains were maintained on minimal agar media augmented with 1/10 NB and 10 ppm lindane for future studies.

Acclimation of the consortium for DDT degradation by Bidlan and Manonmani [12] also resulted in the ultimate survival of four strains comprising *Serratia marcescens* DT- 1P and other three *Pseudomonas* strains. Pannu and Kumar [9] isolated 78 strains out of which only 9 strains could clear the lindane film after 7 days of incubation and only 3 strains RP-1, RP-3and RP-9 were able to withstand 100 ppm of this insecticide. The other strains were able to tolerate lindane concentrations from 20-60 ppm. Similar observations with limiting cadmium were described by Kumar *et al.* [13].

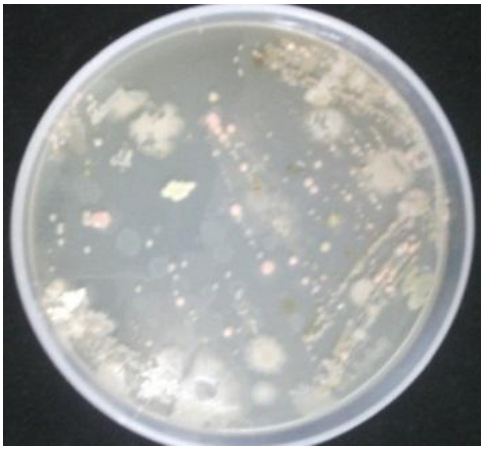

Fig. 1:Mixed populations from culture flask after enriched by multiple pesticides.

International Journal of Advance Research in Science and Engineering
Vol. No.6, Issue No. 07, July 2017
www.ijarse.com
IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

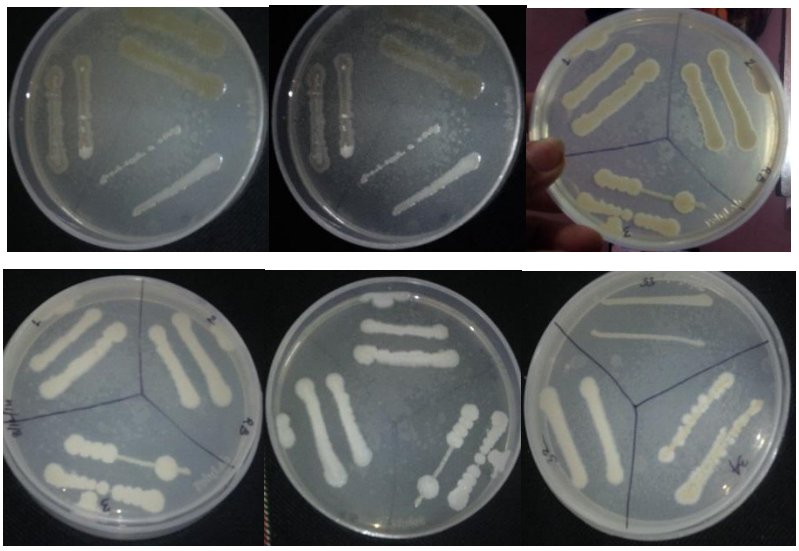

Fig. 2:Bacteria capable of lindane degradation from mixed populationenriched by multiple pesticides.

3.2 Morphological and biochemical behavior of bacterial strains

Morphology including cell shape, cell arrangement and Gram staining were done with the help of a light microscope. For biochemical behavior, many tests such as catalase, nitrate reduction, indole test, H_2S production, citrate utilization test, starch hydrolysis and gelatin hydrolysis were carried out. Response of cultures can be observed in Table 1.

Table 1:Biochemical testing (triplicate) of four isolates SRJ_1, SRJ_2, SRJ_3 andSRJ_4

S.No.	Test	Bacterial strains											
		SRJ1			SRJ2			SRJ3			SRJ4		
1	Shape	Rods			Cocci			Short rods			Cocci		
2	Motile (Aerobic)	Non- Motile			Non- Motile			Motile			Non- Motile		
3	Motile (Anabolic)	Non- Motile			Non- Motile			Motile			Non- Motile		
4	Gram staining	+	+	+	+	+	+	+	+	+	-	-	-
5	Catalase	-	-	-	+	+	+	+	+	+	+	+	+
6	Nitrate Reduction	+	+	+	+	+	+	+	+	+	+	+	+
7	Indole Test	-	-	-	-	-	-	-	-	-	-	-	-
8	H_2S Production	-	-	-	-	-	-	-	-	-	+	+	+
9	Citrate utilization Test	-	-	-	-	-	-	-	-	-	-	-	-
10	Starch hydrolysis	-	-	-	-	-	-	+	+	+	-	-	-
11	Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

3.3Antibiotic sensitivity of bacterial strains

The diameters of the zone of inhibition are shown in Fig 3. During the incubation period, the antibiotics/chemicals diffuse outward from the disks into the agar. This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic disks indicates that the respected organism is susceptible to that antibiotic and the presence of growth around the antibiotic disk indicates the organism is resistant to that particular antibiotic. This area of no growth around the disk is known as a zone of inhibition, which is uniformly circular with a confluent lawn of growth in the media. The diameters of the zone of inhibition are measured including disk using a scale (Table 2).

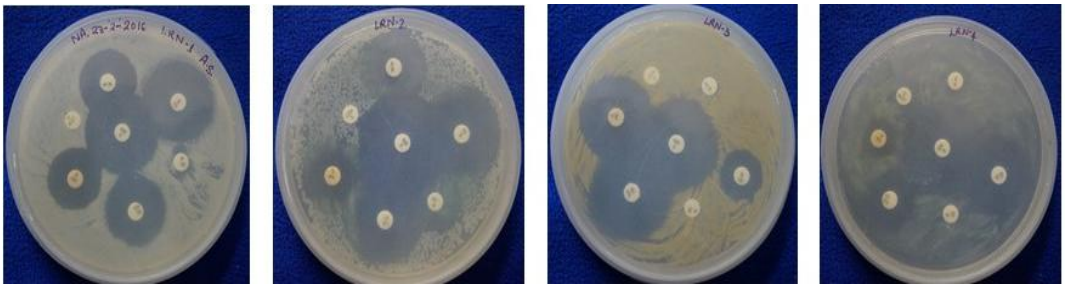

Fig. 3: Antibiotic sensitivity. The test was conducted using antibiotic-impregnated discs placed over the culture spread on nutrient agar and incubating for 18 h at R.T.

Table 2:Antibiotic sensitivity of four isolates SRJ1, SRJ2, SRJ3 and SRJ4

	SRJ1		SRJ2		SRJ3		SRJ4	
	Sensitivity	Radius(mm)	Sensitivity	Radius(mm)	Sensitivity	Radius(mm)	Sensitivity	Radius(mm)
K-5	+	11	+	6	-	-	+	5
Nx-5	+	14	+	17	+	16	+	16
S-10	+	15	+	13	+	8	+	13
Rif-5	+	10	+	9	+	14	+	6
P-10	-	-	-	-	-	-	-	-
Cfm-5	-	-	+	10	-	-	-	-
Na-30	+	11	+	10	+	15	+	9

Enrichment process was succeededwith thesame compound [5, 9, 10, 14, 15, 16, 17]orthe correspondents of the compound [18, 19] that needs to be acclimatization and degraded bythemicrobes. Many microbes were present in environment that naturally degrade toxic substance, such as *Eschericiacoli*was obtained from animalsfaeces which degraded 10%of the added 0.04%lindane,whenenrichment of soil and sewage could notyield anypromisingstrain [20].

In our study, it was observed that there was utilization of organophosphatesand pyrethroids as a carbon source during enrichment process. Similar study was done by Siddique *et al* [21] where 3 bacterial strains exhibited

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

almost equal capability to utilize endosulfan as a sole carbon source. Morphological and biochemical tests are helpful for identification of bacterial strains. From health point of view antibiotic resistance/ susceptibility is a very important parameter. Here we used 7 antibiotics with different concentrations like 5 □g/disc, 10 □g/disc and 30 □g/disc, while Naphade*et al* [22] used 15 different antibiotics and Kumari*et al* [23] applied two different antibiotics with different levels, like 10 □g/ml to 50 □g/ml.

IV. CONCLUSION

Thefact that our four bacterial strains may be constituted into a defined microbial consortium that could degrade different commercial formulations of pesticides.The consortium is best suited for application in the contaminated water bodies. The microbial components of the consortium, namely SRJ1, SRJ2, SRJ3 and SRJ4 can also be exploited for various bioremediation processes where their individual capabilities can be explored before application. The avenues are still open for a better combinatorial bioremediation strategy to clean our environmentforthe upcominggenerations.

V. ACKNOWLEDGEMENTS

The first two authors thank the University Grants Commission, India for providing financial assistance as JRF/SRF and PDF respectively. The authors would like to thank the Hon'ble Vice Chancellor, Registrar, Dean-PG and DR-PG, all from Delhi Technological University for their constant support and encouragement during this work. We also thank to Prof. D. Kumar (HoD), Mr. C. Bihari and Mr. J. Singh of Biotechnology Department, DTU for their timely support.

REFERENCES

[1] S. Mohapatra, and M. Pandey, Biodegradation of Hexachlorocyclohexane (HCH) Isomers by White Rot Fungus, Pleurotusflorida. *Journal of Bioremediation and Biodegradation, 6, (2015), 280. doi:10.4172/2155-6199.1000280*

[2]E.Villanneau, N.P.Saby, D.Arrouays, C.C.Jolivet, L.Boulonne, G.Caria, A.Bispo, and O. Briand, Spatial distribution of lindane in top soil of Northern France. Chemosphere 77(9), 2015, 1249-55. doi: 10.1016/j.chemosphere.2009.08.060.

[3] R.Weber, C.Gaus, M.Tysklind, P. Johnston, M.Forter, H.Hollert, H.Heinisch, I.Holubek, M.Lloyd- Smith, S.Masunaga, P.Moccarelli, D.Santillo, N.Seike, R.Symons, J.P.M.Torres, M.Verta, G.Barbelow, J.Vijgen, A.Watson, P.Costner, J.Woelz, P.Wycisk, andM. Zennegg, Dioxin- and POP- contaminated sites-contemporary and future relevance and challenges. *Environmental Science and Pollution Research., 15, (2008), 363-393.* DOI: http://dx.doi.org/10.1007/s11356.008.0024-1.

[4] M.Grung, Y. Lin, H. Zhang, A. O. Steen, J. Huang, G. Zhang, andT. Larssen, Pesticide levels and environmental risk in aquatic environments in China — A review. *Environment International, 81, (2015), 87–97. DOI: http://dx.doi.org/10.1016/j.envint.2015.04.013.*

[5] R. Bidlan, M. Afsar, andH.K. Manonmani, Bioremediation of HCH- contaminated soil: elimination of inhibitory effects of the insecticide on radish and green gram seed germination. *Chemosphere*, 56, (2004)*803- 811. doi: http://dx.doi.org/10.1016/j.chemosphere.2004.01.015.*

[6] N. Agrawal, andS.K. Shahi, An environmental clean-up strategy- Microbial transformation of xenobiotic compounds. *International Journal of Current Microbiology and Applied Sciences*, 4(4), (2015), 429-461.

[7] Y. Chen, Organophosphate- induced brain damage: mechanisms, neuropsychiatric and neurological consequences and potential therapeutic strategies. *Neurotoxicology, 33, (2012) 391- 400. DOI: http://dx.doi.org/10.1016/j.neuro.2012.03.011*.

[8] H. Kumari, S.K. Gupta, S. Jindal, P. Katoch, and R. Lal, *Sphingo biumlactosu tens* sp. nov., isolated from a hexachlorocyclohexane dump site and *Sphingo biumabikonense* sp. nov., isolated fromoil-contaminated soil. *International Journal of Systematic and Evolutionary. Microbiology, 59 (9), (2009), 2291 http://dx.doi.org/10.1099/ijs.0.004739-0*

[9]R. Pannu, andD. Kumar, Biodegradation study of γ- hexachlorocyclohexane using selected bacteria from agricultural soil.*African Journal of Microbiology Research, 8(36), (2014), 3335-3346. DOI: http://dx.doi.org/10.5897/AJMR2014.6842.*

[10] R. Bidlan, Studies on DDT degradation by Bacterial strains. In: Isolation, purification and identification of microbes capable of DDT- degradation. *Ph.D. thesis, University of Mysore, India., 2003, pp 90-142.*

[11] S. Kumar, R. Bidlan, and J. G. Sharma, Degradation of lindane by sludge enriched on mixed commercial formulations of organophosphate and pyrethroid pesticides. *International Journal of Current Microbiology and Applied Sciences, 5(5), (2016), 138- 152 (DOI: http://dx.doi.org/10.20546/ijcmas.2016.505.015*

[12] R. Bidlan, and H. K. Manonmani, Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by *Serratia marcescens* DT-1P. *Process Biochemistry, 38 2002), 49-56. DOI: http://dx.doi.org/10.1016/S0032-9592(02)00066-3*.

[13] A. Kumar, S.S.Cameotra, and S. Gupta, Screening and characterization of potential cadmium biosorbentAlcaligenes strain from industrial effluent. *Journalof Basic Microbiology., 51, (2010) 1-7.*

[14] H.K. Manonmani, D.H.Chandrashekaraiah, S.N. Reddy, C.D.Elcey, and A.A.M.Kunhi, Isolation and acclimation of a microbial consortium for improved aerobic degradation of □- hexachlorocyclohexane. *Journal Agriculture and Food Chemistry*, 48, (2000) 4341–4351. DOI**:** *http://dx.doi.org/10.1021/jf990712c*.

[15] V. Nagpal, andK.M.Paknikar, Integrated approach for the enhanced degradation of lindane. *Indian Journal Biotechnology*, 5(Suppl), (2006), 400-405.

[16] H.M.R. Murthy, andH.K. Manonmani, Aerobic degradation of technical hexachlorocyclohexane by a defined microbial consortium. *Journal of Hazardous Materials, 149(1), (2007), 18-25. DOI: http://dx.doi.org/10.1016/j.jhazmat.2007.03.053*.

[17] N. Deepthi, N.K. Rastogi, andH.K. Manonmani, Degradation of DDT by a defined microbial consortium: optimization of degradation conditions by response surface methodology. *Research Journal Microbiology, 2, (2007): 315- 326. DOI: http://dx.doi.org/10.3923/jm.2007.315.326*.

[18] D.D. Focht, andM. Alexander, DDT metabolites and analogs: ring fission by *Hydrogenomonas,Science, 170(3953), (1970), 91-92. DOI: http://dx.doi.org/10.1126/science.170.3953.91*.

[19] R. Bartha, Isolation of microorganisms that metabolize xenobiotic compounds. In: Labeda D. P., editor. Isolation of Biotechnological Organisms from Nature. New York: Mc Graw Hill. (1990), 283- 305.

IJARSE
ISSN (O) 2319 - 8354
ISSN (P) 2319 - 8346

[20] A.J. Francis, R.J. Spanggord, and G.I.Ouchi, Degradation of lindane by *Escherichiacoli*. *Applied Microbiology, 29(4), (1975), 567- 568.*

[21] T. Siddique, B.C. Okeke, M**.** Arshad, andW.T. Frankenberger, Enrichment and isolation of endosulfan-degrading microorganisms. *Journal of Environment Qual. 2003 Jan-Feb;32(1):47-54.*

[22] S. R. Naphade, A.A.Durve, M. Bhot,J. Varghese,and N. Chandra,Isolation, characterization and identification of pesticide tolerating bacteria from garden soil. *European Journal of ExperimentalBiololgy, 2 (5), 2012, 1943-1951*.

[23] N. Kumari, A. Vashishtha, P. Saini, andE. Menghani, Isolation, identification and characterization of oil degrading bacteria isolated from the contaminated sites of Barmer, Rajasthan. *International Journal of Biotechnology Bioengineering Research, 4(5), (2013), 429-436.*