Research Article

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ISOLATION AND BIOCHEMICAL CHARACTERISATION OF MALATHION DEGRADING BACTERIA

Radhika Sharma*, Aakriti Saraswat, Divya Pandey and Kriti Maheshwari
Department of Biotechnology, The IIS University, Gurukul Marg, Mansarovar, Jaipur- 302020, India

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Abstract: Agriculture is the major occupation of people of India, around 60-70% of its population is engaged in agriculture. Annually millions and tons of pesticides are used in fields to have healthy and quality field produce, which in turn accumulates in our ecosystem and food chain as well. The toxicity levels of these pesticides are reported in amphibians, aves, fishes and even human beings. Malathion (S-(1, 2 dicarethoxyethyl)-O, O-dimethyldithiophosphate) causes inhibition of human acetylcholinesterase leading to suffocation succeeded by death. Malathion could be degraded either chemically or biologically. Chemical degradation leads to production of malaxon which is 50 times more toxic then the parent compound. Our present study aims at biodegradation of malathion using enrichment technique. The presence of degradation was confirmed by Thin Layer Chromatography. Further, morphological and biochemical tests were performed on the isolated bacteria which indicated that they might belong to various genera.

Keywords: Bioremediation, xenobiotics, Malathion

INTRODUCTION

India is an agricultural based country; more than 70% residents are engaged in farming. Due to increasing population demand for food also increases, sometimes a single pest could destroy the entire healthy field produce due to which the produce of that field is no more useful and this not only creates food scarcity for growing population but also effects the economic health of a farmer and the country, to overcome such issues millions of tons of pesticides are applied annually in modern agriculture to increase the production through controlling harmful effects caused by the target organisms including insects, fungi, bacteria, viruses as well as grasses grown in between the economical crops(Liu and Xiong, 2001). Malathion[S- (1, 2 dicarethoxyethyl)-O, O-dimethyl dithiophosphate] toxicity is due to the presence of carboxyester group. The major environmental concern of used pesticides is their capacity to leach down to subsoil and contaminate the ground water (Kookana *et al*., 1998) or if immobile, they would persist on the top soil where it could accumulate to toxic level in the soil and become harmful to microorganisms, plants, animals and man (Amakiri,1982). Excessive and persistent use of pesticides results in deterioration of the environment. Malathion also inhibits an enzyme, acetylcholinesterase (AChE) in humans that breaks down acetylcholine, a chemical essential in transmitting nerve impulses across junctions between nerves. Without functioning AChE, acetylcholine accumulates, producing rapid twitching of voluntary muscles, incoordination, convulsions, paralysis, and ultimately death (Cremlyn, 1991).

Chemical degradation is cost exhausting method and sometimes leads to production of biproducts which are more toxic then parent compound, malaxon a biproduct produced by oxidation of malathion is much more toxic then malathion, so in order to overcome these issues we adopt bioremediation approach.

Bioremediation, the use of microorganisms, by virtue of their bio concentrating and metabolic properties, to degrade, sequester, or remove environmental contaminants. Bioremediation methodology to treat xenobiotics such as pesticides in soil have gained considerable attention owing to its ecofriendliness and have been employed successfully in many countries (Enrica, 1994; Ritmann *et al*., 1988)

The present study was conducted to isolate and characterize bacteria capable of using malathion as sole carbon source and thus helping in overcoming the persistence of malathion in environment.

MATERIALS AND METHODS

Pesticide: Malathion of commercial grade was purchased from local dealer

Media: Nutrient agar plates were prepared by dissolving a known amount of nutrient agar in sterile water according to manufacturer's instructions.

Inoculum: Soil from the field (Durgapura research center) routinely treated with pesticides was used as inoculum because this soil contains larger fraction of microorganisms capable of surviving pesticide treatment or using pesticide as energy source.

***Corresponding Author:**
Radhika Sharma*
Department of Biotechnology,
The IIS University, Gurukul Marg, Mansarovar,
Jaipur- 302020, India.

The bacteria capable of degrading malathion was isolated by using enrichment technique (khan *et al.*, 2004). The soil sample was mixed thoroughly and sieved to remove pebbles and any impurity present in it. Then to 50grams of soil 1ml of pesticide was added and slurry was prepared using distilled water and this was incubated for 48 hours at 37°C in an incubator. 1 gm of this incubated soil was diluted in 10 ml of distilled water. This soil was streaked on nutrient agar plates in laminar air flow. The plates were incubated for 24-48 hours in an incubator at 37°C. Subsequently nutrient agar plates were prepared in which yeast and beef extract was supplemented step by step with malathion and colonies from precursor plate was used as inoculums (fig 1) (table 1)

Table.1: Enrichment of media with Malathion

Plates number	Beef extract concentration	Yeast extract concentration	Malathion concentration	Inoculum used
Plate 1	0.75	0.75	0.00	Incubated soil
Plate 2	0.70	0.70	0.05	Colonies from plate 1
Plate 3	0.65	0.65	0.10	Colonies from plate 2
Plate 4	0.55	0.55	0.20	Colonies from plate 3
Plate 5	0.45	0.45	0.30	Colonies from plate 4
Plate 6	0.25	0.25	0.50	Colonies from plate 5
Plate 7	0.05	0.05	0.70	Colonies from plate 6
Plate 8	0.00	0.00	0.75	Colonies from plate 7

The colonies obtained on plate 8 use malathion as sole carbon source and are capable of degrading malathion.

QUALITATIVE ANALYSIS OF DRGRADATION USING THIN LAYER CHROMATOGRAPHY:

The colonies so obtained in 100% malathion containing plate (lacking carbon source) were inoculated in nutrient broth containg malathion as sole carbon source and incubated for 24-48 hours. After incubation TLC (thin layer chromatography) was performed on a TLC plate.3 spots were marked on TLC plates according to following table.

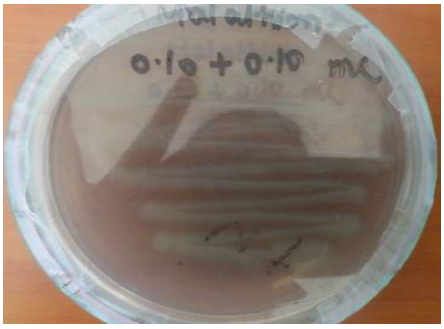
Spot number	Spot type
Spot 1	Pure pesticide
Spot 2	Nutrient broth containing pesticide as sole carbon source + inoculums from plate 8
Spot 3	Nutrient broth

The solvent system of TLC containing petroleum ether, acetone, and butanol was allowed to run over TLC plates. After that, plates were dried and examined under UV light at 265 nm. (fig2**).** After performing TLC morphological (gram's staining) and biochemical characterization (gelatin hydrolysis, carbohydrate fermentation, catalase test, citrate utilization test, nitrate reduction, indole test) of microorganism was done.

RESULTS

0.1 ml Malathion

0.20 ml Malathion



0.40 ml Malathion

1.5 ml Malathion

Fig.1: plates containing different concentrations of malathion

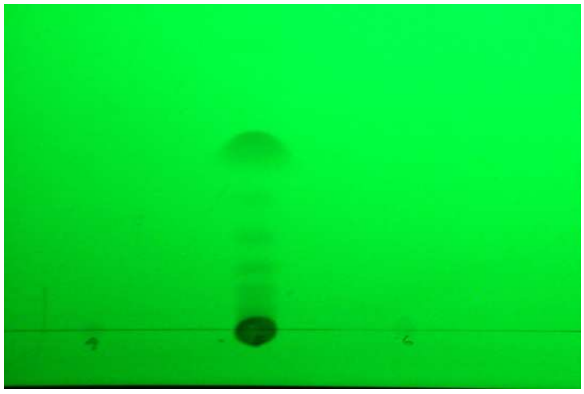


Fig.2: TLC plate observed at 240 nm

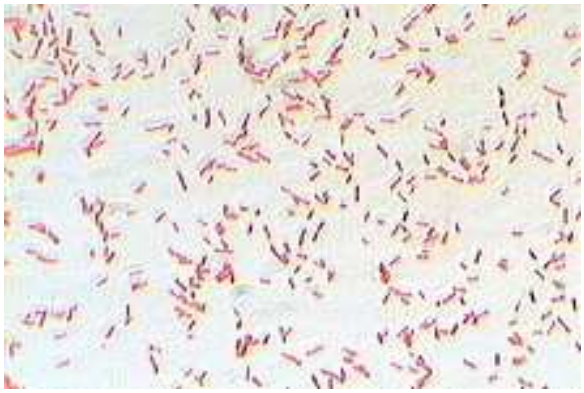
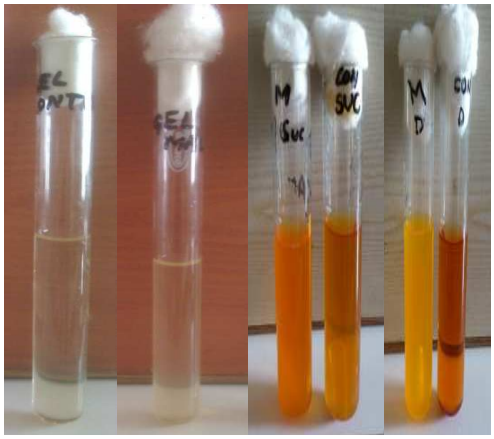
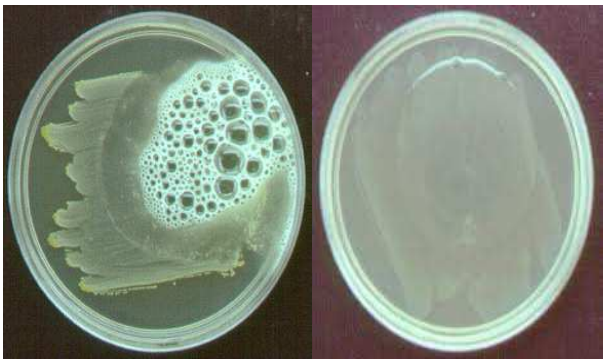


Fig.3: Gram staining (gram negative, rod shaped)



4A 4B 5A, 5B

Fig.4: Gelatin hydrolysis
Fig.5: Dextrose fermentation



6A 6B

Fig. 6: Catalase test

7A 7B 8A 8B 9A 9B

Fig.7: Citrate utilization
Fig.8: Nitrate reduction
Fig.9: Indole test

Fig.5:

NAME OF TEST	RESULT CONFERED
GRAM STANING (fig 3)	GRAM NEGATIVE, ROD SHAPED
GELATIN HYDROLYSIS (fig 4)	POSITIVE
SUCROSE FERMENTATION (fig 5)	POSITIVE
DEXTROSE FERMENTATION (fig 5)	NEGATIVE
CATALASE TEST (fig 6)	POSITIVE
CITRATE UTILISATION (fig 7)	POSITIVE
NITRATE REDUCTION (fig 8)	POSITIVE
INDOLE TEST (fig 9)	NEGATIVE

DISCUSSIONS

After performing TLC it was confirmed that bacteria is capable of degrading malathion because the movement was observed only in case of spot 2 (nutrient broth containing pesticide as sole carbon source + inoculums from plate 8), as pure pesticide components are quite large and cannot move through interstices of plate so movement can only be observed if pesticide components are broken in shorten fragment (characteristic of degradation).

CONCLUSION

The isolated bacteria were gram negative, rod shaped bacteria capable of hydrolyzing gelatin, utilizing sucrose, citrate, reducing nitrate and producing enzyme catalase.

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Conflict of interest: None Declared