Kinetics of dimethoate biodegradation in bacterial system

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Abstract

The present study is an investigation on the kinetics of dimethoate biodegradation and an estimation of residual dimethoate in bacterial culture by spectrophotometry. The methylene chloride extract of the culture medium was used for determination of dimethoate through its reaction with 1 chloro-2, 4 dinitrobenzene to produce methylamine whose absorbance at 505 nm gave an estimation of dimethoate content. The dimethoate standard curve follows Beer’s law at 505 nm with a slope of 0.0129 absorbance units per µg/mL. The regression equation relating concentration of dimethoate (x) with the absorbance is (y): y = 0.037 + 0.0129x. The amount of residual dimethoate after 7 days were 0, 4, 17, 28 and 29 µg/mL; the rate constants were 0.775, 0.305, 0.225, 0.167 and 0.127 each per day, and the efficiency of dimethoate degradation were 100%, 96%, 83%, 72% and 71%, for Bacillus licheniformis, Pseudomonas aeruginosa, Aeromonas hydrophila, Proteus mirabilis, and Bacillus pumilus respectively. Dimethoate remediation could be attained through bacterial metabolism of the pesticide and colorimetric analysis might be useful in the estimation of dimethoate within a detection limit of 5-100 µg/mL.

Materials and Methods

Bacterial strain

Five bacterial strains, namely, Bacillus licheniformis, (B. licheniformis) F102, Pseudomonas aeruginosa (Ps. aeruginosa) W171, Aeromonas hydrophila (A. hydrophila) O102, Bacillus pumilus (B. pumilus) KS23 and Proteus mirabilis (P. mirabilis) C114 isolated, respectively from the intestine of Labeo rohita, Ganges river water, intestine of Clarius batracus, soil sample and urinary tract infection, were taken for the present study. All the strains are capable of tolerating high amount of dimethoate in nutrient agar medium.

Culture condition

The bacterial strains were grown in 50 mL Mineral salts (MS) solution containing 100 µg/mL of dimethoate as the sole source of carbon at 28°C for 24 h. From this culture, bacterial inoculum amounting to approximately 5x 10^5 CFU/mL was prepared as described earlier for all the strains. In separate conical flasks containing 100 mL MS solution supplemented with 100 µg/mL of dimethoate, was inoculated 0.1 mL of the bacterial inoculum of 5x10^6 CFU/mL and incubated at 28°C for up to seven days.

Methylene chloride extract of dimethoate

An aliquot of 1 mL of the bacterial culture fluid cells were taken out aseptically from 100 mL stock culture after every 24 h for up to 7 days and treated as described for the preparation of acetonitrile extract. The cells were removed from the culture by centrifugation at 4000 x g for 20 min. The supernatant was extracted twice with equal volumes of methylene chloride, shaken vigorously and centrifuged at 4000 x g and the organic fraction was transferred to a separate tube. The methylene chloride extract was used for the estimation of unknown amount of dimethoate.

Preparation of 99% dimethoate

The dimethoate (95% purity), obtained from Rallis India Limited, Bangalore, India, was purified to 99%, as described earlier by DebMandal et al.

Preparation of standard curve

The standard curve for dimethoate was prepared, as has been described earlier. A range of dimethoate solutions containing 0 to 100 µg/mL of 99% dimethoate was prepared in methylene chloride in a series of glass-stoppered test tubes. One drop of 0.5% lanolin solution was added to each tube to prevent loss during the evaporation. The solvent was evaporated in a 70°C water bath. The dimethoate present in each test tube was hydrolyzed with 1 mL of 0.5 N methanolic sodium hydroxide solution followed by heating for 10 min in a water bath pre-adjusted to 60°C. The resulting reaction mixture was immediately cooled in a cold-water bath. To each of the tubes, 0.1 mL of the 1-chloro 2, 4 dinitrobenzene reagent was added and shaken vigorously. The absorbance at 505 nm gave an estimation of dimethoate concentration.

Introduction

Organophosphorus compounds such as dimethoate [O, O-dimethyl S-methyl-carbamoyl-methyl phosphorodithioate], has both plant and animal systemic insecticidal properties and constitutes the largest class of insecticide used worldwide.

It is nerve toxin, carcinogen, teratogen, has high oral and dermal acute toxicity, is an eye irritant, and is readily absorbed through lungs. In view of its toxicity, it is important to remove dimethoate from the environment. Naturally occurring microorganisms have a physiological versatility and catabolic potential to degrade a large number of organic or synthetic compounds. Several bacterial species can utilize an insecticide as a sole source of carbon and energy for growth, which is manifested by consumption of the parent compound, and which can be detected and estimated by various methods. Several methods for the determination of dimethoate compound have been reviewed based on column, paper, and thin layer chromatography, paper electrophoresis, colorimetry, gas chromatography, and radiometry, tandem mass spectrometry. The toxicity of dimethoate in freshwater airbreathing catfish Heteropneustes fossilis was carried out by bioassay method. The investigations, reported herein, were undertaken for the quantitative estimation of dimethoate when it is introduced directly as a sole source of carbon and energy in bacterial system; another aspect of this investigation was to study the dynamics of dimethoate biodegradation using bacterial isolates from different sources.
added, stoppered and shaken for 10 min. Next, 2 mL of absolute ethyl alcohol was added and swirled. The absorbance of the resulting red colored solution was measured in spectrophotometer at 505 nm. Absolute ethyl alcohol was taken as the blank solution and the standard curve was prepared by plotting the absorbance reading against micrograms of dimethoate.

Colorimetric estimation of residual dimethoate from bacterial cultures

Each of the bacterial culture taken at regular interval of day 1, 2, 3, 4, 5, 6, and 7 was extracted as described above with methylene chloride and dimethoate in the culture was treated with methanolic sodium hydroxide and 1 chloro-2, 4 dinitrobenzene to form the red colored compound, whose absorbance was measured at 505 nm. The unknown amount of residual dimethoate present due to biodegradation was determined from the standard dimethoate curve.12

Biodegradation dynamics

The dynamics of dimethoate biodegradation in liquid culture, of bacterial strains, *B. licheniformis* F102, *Ps. aeruginosa* W171, *A. hydrophila* O102, *B. pumilus* KS23, and *Pr. mirabilis* C114 was examined at an initial concentration of 100 µg/mL dimethoate, after a period of seven days.12 The efficiency of dimethoate degradation was evaluated with respect to their percent biodegradation, rate constants, and half-lives for dimethoate degradation.

Results

The dimethoate standard curve follows Beer’s law at 505 nm, in the range of 5-100 µg/mL of dimethoate, and it has a slope of 0.0129 absorbance units per µg/mL. The blank solution (absolute ethyl alcohol) gives an average absorbance of 0.034. The regression equation relating concentration of dimethoate (x) with the absorbance is (y): y = 0.037 + 0.0129x. Herein it was found that 1 µg/mL dimethoate was equivalent to an absorbance of 0.0129 absorbance units per µg/mL. The unknown amount of dimethoate in liquid culture, of bacterial strains, *F102, W171, O102, C114 and KS23* strains were estimated to be 0.775, 0.305, 0.225, 0.167 and 0.127 per day each, respectively (Figure 4). The half-lives (T½) for dimethoate were estimated to be 0.894, 2.268, 3.072, 4.135 and 5.456 days for *F102, W171, O102, C114 and KS23* strains, respectively (Figure 4); no abiotic decrease in dimethoate concentration in control flasks over a seven-day incubation period was observed.

Discussion

Biological remediation strategies are environmentally desirable, and considered a cost-effective option. In the present study, we have demonstrated the removal of dimethoate (at 100 µg/mL) in enrichment cultures using five different bacterial strains. Several authors have reported on the quantitative determination of dimethoate compound. Pandey et al.9 used bioassay method to determine the toxicity of dimethoate in catfish *Heteropeuceus fossilis* and found out the LC50 values for dimethoate to be 2.98-3.38 mg/L for upto 4 days. Pagliuca et al.1 reported that 37 among 135 raw milk samples were positive for traces, and 10 samples showed organophosphorus pesticide contamination in a range 5-18 µg/kg. Xiang et al.3 monitored dimethoate utilization by *Ps. aeruginosa* using SPQC (series piezoelectric quartz crystal) and HPLC (high performance liquid chromatography) with determination limit of 1.08 ng in HPLC. Hadjidemetriou et al.13 studied the dissipation of dimethoate in citrus foliage by gas-liquid chromatography using 5 % OV-101 coated on a Carbowax 20M surface-modified support (Ultra-Bond 20M). Gamón et al.14 quantified pesticides in fruit and vegetables using gas chromatography/tandem mass spectrometry by spectral confirmation of the matrices after...
extracting the residues from samples with ace-tonic solution followed by a mixture of dichloromethane-petroleum ether. Szymczyn
and Malczewska13 presented a GC method for the determination of six organophosphorous
pesticide residues including dimethoate in cabbage, in which the chopped cabbage was
blended with acetone, extracted with n-hexa-ne:methylene chloride (1:1) mixture. The extract was then purified on a GC column and
eluted with the mixture of methylene chloro-
ne:cyclohexane (1:1) at the flow rate of 0.5
mL/min. Dimethoate was extracted from hexa-
e system with HBr followed by hydrolysis and determination of the evolved hydrogen sul-
phide from dimethyl sulphate, as methylene blue. Herein, the parent compound dimethoate
was extracted with methylene chloride, fol-
lowed by alkaline hydrolysis, and colorimetric determination of the resultant methylamine by
reaction with 1-chloro-2, 4-dinitrobenzene. Both dimethoate and omethoate react to form
the color by the dinitrochlorobenzene colori-
metric procedure, but omethoate cannot be
determined in the same sample, because gradu-
ally it tends to get lost in the clean up proce-
dure.

The B. licheniformis F102 strain isolated from fish intestine degraded 100% of
dimethoate in liquid culture at day four. The rate constant of dimethoate degradation was
0.775 per day for the F102 strain, and half-life of
dimethoate was calculated as 0.894 day when the strain was incubated in liquid media.
Another bacillus, B. pumilus KS23 strain, a soil bacterium, exerted 71% degradation of
dimethoate at day 7 with a rate constant 0.127 per day, and for this strain half-life of
dimethoate was 5.456 days. The water bacteria
Ps. aeruginosa W171 strain demonstrated an efficiency of degrading 96% dimethoate at day
7 with rate constant 0.305 per day, and a 2.268
days half-life of dimethoate as was calculated for this strain. For other two strains, P.
mirabilis C114 and A. hydrophila O102, that showed the efficiency of degrading respective-
ly 72% and 83% of dimethoate, the respective rate constants was calculated as 0.167 and
0.225 per day, with half-life of dimethoate as 4.135 days and 3.072 days, respectively.

Deshpande et al.14 found that Ps. aeruginosa
and B. megaterium demonstrated an efficiency of more than 95% for dimethoate de-
gradation after 8 days of incubation, and for the other strains used in their study, the efficiency of dimethoate degradation ranged between 64% and
90%. Liang et al.4 showed that a Raoultella
sp. was able to remove up to 75% of dimethoate via co-metabolism through optimization of car-
bon and energy source using response surface
methodology. Okeke et al.15 using Pandoraea
sp., reported that after 8 weeks of incubation, 89.9% and 93.3% degradation were achieved with γ and α hexachlorohexane (150 µg/mL
for each) isomers, respectively. The rate con-
stants for γ and α hexachlorohexane, were
0.28 and 0.32, respectively, and the half-lives were 2.51 weeks and 2 weeks, respectively.
Kanrar et al.16 determined the dissipation pat-
tern, residue level and half live values of imi-
dacloprid in soil, water, plant, grain husk and
strand samples in rice ecosystem. Schmalko et
al.17 studied degradation kinetics of dimethoate in yeba mate plants by capillary gas chromato-graphy technique. Half-life times in plants ranked between 9.8 and 11.8 days and the
dimethoate concentration decreased to a 22.7% of its initial value (in dry basis); while
during seasoning step (at 45°C), half-life time
was 17.3 days. Bo and Xin-Huai18 found out
that the half life periods of dimethoate and other organophosphates were in a range of 4.0
to 10.3 h, at 63°C-100°C; and that lactic bacte-
ria in Rhodia and Danisco starter cultures increased the degradation of dimethoate in bovine milk to 11.7% and 20.4% respectively.
Hui-mei et al.19 determined the half-lives of
dimethoate in cabbage and soil to be 2 and 1.5
days respectively, through GC detection, with
90.25-110.39% recovery and a variation coeffi-
cient of 1.51-5.83%. Hadjidjemetriou et al.13 showed that dimethoate residues were best characterised by two first-order kinetic
processes; the half-life values of dimethoate were 2.2 days for the 1-10 days portion, and 7.0
days for the 10-49 days portion.

In this communication, it has been estab-
lished for the first time that the colorimetric estima-
tion is useful in detecting dimethoate
(within a limit of 5-100 µg/mL) in bacterial
system, in order to study the dynamics of the pesticide (dimethoate) biodegradation.

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