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Research Article

BIODEGRADATION OF DDT BY *ACHROMOBACTER* SP. STRAIN Y12A IN BROTH MEDIUM

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Abstract- Ubiquitous presence of DDT owing to its excessive use and recalcitrance has been a matter of concern worldwide. The current study is an attempt to address this problem by isolating a bacterium possessing the capability of utilizing DDT as a sole Carbon source by selective enrichment technique. The isolated strain was identified to belong to bacteria *Achromobacter* on the basis of 16S rRNA gene similarity. The study revealed that strain Y12A was the most efficient degrader, degrading 81.25% DDT within 10 days of incubation. The degrading ability of the strain escalated at optimum conditions of 30ºC temperature, pH 7 and 50 mgL^{-1} of DDT concentration. Metabolites obtained during DDT degradation were DDE, DDD and DDMU. Hence, DDT degrading efficiency of the isolated strain Y12A could be utilized in integration with DDMU utilizing strains in consortia to achieve mineralization of DDT. To the best of our knowledge, this is the first instance when member of *Achromobacter* genus has been reported to possess DDT degrading ability. The study reveals the degradation potential of *Achromobacter* sp. strain Y12A for the bioremediation of DDT from polluted sites.

Keywords- *Achromobacter*, biodegradation, bioremediation, DDT, Yamuna.

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Introduction

Organochlorine pesticides including DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane) had been extensively employed for preventing agriculture pests as well asvector borne diseases affecting humans, which has resulted in grievous contamination of both terrestrial and aquatic ecosystems [1]. Several environmental regulatory agencies including US-EPA have categorized DDT as a priority POP (Persistent Organic Pollutant) because of various properties it exhibits like endocrine and neurological disruption, carcinogenic effect and teratogenic effect on non-target life forms including humans [1,2]. Additionally, its persistent and lipophilic nature ultimately results in bio-magnification along the food chain which can prove detrimental to ecosystems as well as human health which is affirmed by few instances viz the presence of DDT in adipose tissues and breast milk of humans [3,4]. Umpteen sites all over the world have been reported having DDT contamination, owing to its excessive use and recalcitrant nature [5]. Hence, its ubiquitous presence and pernicious effects on living beings has made it a matter of concern globally [6]. Recently, various studies have reported high level of pesticide contamination including DDT in agricultural crops, processed food and water based food like fishes as well [6-8]. Hence, remediation of DDT contaminated sites holds great significance.

DDT concentration can be reduced from the contaminated sites by various physico-chemical ways like photolysis, volatilization, runoff and biodegradation. However, DDT degradation using microbes is a propitious, minimally hazardous, profitable and environmental friendly strategy for the remediation of DDT contaminated sites [1]. It involves transformation of toxic xenobiotics into metabolites which are simpler and lesser toxic as compared to the parent compound [9]. However, extreme recalcitrant and toxic nature of DDT makes its degradation exceptionally difficult in the natural environment. This has led to the soaring vogue for the use of microbes for attaining enhanced degradation and complete mineralization [10]. So far, many strains having the capability of degrading DDT have been isolated [1,2,11-17]. For instance, Qu et al, 2015 reported around 80% degradation of 50 mg L^{-1} of DDT in 30 days by *Chryseobacterium* sp. PYR2 [11]. Similarly, *Serratia marcescens* NCIM 2919 degraded 42% of initial 50 mg L^{-1} of DDT in 10 days of incubation [18]. DDT degrading potential of members of *Achromobacter* genus has not explored yet. Degradation of chlorinated biphenyl which is a structural analogue of DDT has been reported by two species of *Achromobacter* [19]. So, it might be a potent degrader of DDT as well.

In the present study, a bacterial strain capable of utilizing DDT as a sole carbon and energy source was isolated from the soil of agriculture fields near Yamuna river, Delhi with history of DDT contamination. The isolated strain was identified as *Achromobacter* sp. Y12A. To the best of our knowledge a bacterium belonging to genus *Achromobacter* has been reported for the first time for DDT degradation. Various factors affecting degradation like substrate concentration, pH, and temperature were also examined for the isolate.

Materials and Methods

Enrichment and isolation

Soil sampling was carried out from the agriculture fields along the bank of river Yamuna (28º34' 05.8"N,077º17'44.3"E) in Delhi with history of DDT contamination. The top 15 cm soil was collected randomly, mixed well, air dried, passed through 2 mm sieve and maintained at 4ºC until any further analysis. Enrichment of DDT degrading bacteria was firstly carried out in soil with DDT followed by enrichment in Carbon Free Media (CFM): 1 g K_2HPO_4, 1 g KH_2PO_4, 1 g NH_4NO_3, 0.01 g $Fe(SO_4)_3$, 0.02 g $CaCl_2$, in 1000 ml distilled H_2O (pH 7.0). The autoclaved media was supplemented with 50 ppm DDT as sole carbon source. At weekly intervals, the culture was regularly transferred to fresh medium with increasing DDT concentration for five times. The increase in the optical density of culture medium was taken as a validation of bacterial consumption of DDT. Initially pure strains

were screened on the basis of their discrete colony morphology, like shape, size, color, surface, margin etc. The isolates were further purified by repeatedly streaking on fresh CFM agar plates supplemented with 50 ppm DDT. Analytical grade reagents and chemicals have been used in this study.

Degradation study and identification of metabolites

The capability of the bacterial isolates to consume DDT as an exclusive source of carbon was assessed. The isolates were cultivated in Erlenmeyer flasks each containing 100 mL of CFM supplemented with 50 mg L^{-1} of DDT. The inoculation of experimental flasks was done with 2 mL of overnight grown culture in Luria Bertini (LB) washed with PBS (phosphate buffer saline) (pH 7, 0.05 M). Flasks without bacterial inoculums having DDT were taken as control. All the flasks were incubated in a rotary shaker at 30ºC at 150 rpm. To check the degradation of DDT and formation of metabolites, culture was sampled out and extracted using ethyl acetate after specified intervals. The extracted samples were subjected to analysis in Gas Liquid Chromatography (Agilent 7890A) equipped with a 63 Ni Electron Capture Detector (ECD) and HP-05 column (30 m ×320 mm × 0.25 mm). 250 ºC temperature was maintained at the injection port. The initial oven temperature was set at 90 ºC (1 min hold), Ramp 1: 250 ºC at 10 ºC min^{-1} (4 min hold) Ramp 2: 280 ºC at 30 ºC min^{-1} (3 min hold). Detector temperature was maintained at 300ºC temperature and 30 mL min^{-1} nitrogen was used as makeup gas. The $OD_{\lambda 595nm}$ of the broth was also monitored. Among the isolated strains, strain Y12B exhibited highest degradation of DDT as well as growth in CFM. Hence, it has been selected for further studies.

Morphological and biochemical characterization of the strain

Initially, isolated strain Y12A was examined for colony morphology, shape and pigmentation. For biochemical characterization, gram staining of the strain was performed. After that, KB003:Hi25 Kit, (Himedia, India) was utilized to determine the phenotypic characters of the isolated strain.

Identification of strain Y12A

16S rRNA gene sequencing was utilized for the identification of bacterial strain Y12A. The amplification of the isolated genomic DNA was performed in a thermal cycler (C1000, Bio-Rad, India) using 16S rDNA universal primers 1492R (50-TACGGCTACCTTGTTACGACTT-30) and 8F (50-AGAGTTTGATCCTGGCTCAG-30) [20]. PCR reaction mix of 25 µl final volume comprised of 6 µl template, 2.5 µl reaction buffer, 2 µl dNTP's, 2.5 µl Primer 1492R, 2.5 µl Primer 8F, 0.5 µl taq polymerase and nuclease free water for making up the volume. Amplified PCR product was examined on Agarose gel (1%) and 1.5 kb band size was excised and eluted using gel extraction kit (Wizard SV Gel and PCR cleanup system, Promega, USA). Eluted DNA products were sent for commercial sequencing to SciGenom Labs Pvt. Ltd., Kerala. The nucleotide sequence of strain Y12B obtained from SciGenom Labs was then subjected to BLAST using NCBI/BLAST program.

Effect of different DDT concentrations, pH and temperature on its biodegradation

The effect of varying temperature range was explored by growing the strain at varying temperatures (10ºC-40ºC) with 50ppm DDT incubated at 150rpm in a rotary shaker, under optimal pH. At constant temperature, the effect of pH (4, 5, 6, 7 and 8) was studied, similarly. To affirm the optimum DDT concentration, bacterial strain was cultivated in differing DDT concentrations (0, 25, 50, 75 and 100 ppm). Control flasks were devoid of bacterial strain. Samples were taken out at regular intervals for degradation and growth studies.

Results and Discussion

Enrichment, isolation and degradation study of isolated bacterium

Through selective enrichment technique, morphologically distinct bacterial strains were isolated namely Y10A, Y12A, Y14B, Y15C [Table-1]. During the incubation period, these strains varied notably in their growth and degradation ability in CFM. Among the isolated strains, strain Y12A had shown highest DDT degradation i.e., 81.6% of 50mgL^{-1} DDT getting degraded within 10 days of incubation. An increase in the degradation rate was observed with the passage of incubation time. It can be inferred that constant subjection of bacteria to rising DDT concentrations enhanced its capability to degrade DDT [14, 21]. In similar studies, 62.12% of 10 ppm DDT was reportedly degraded during 10 days of incubation by *Alcaligenes* sp. [14] and 31.20% of 25 mg L^{-1} DDT was found to be degraded by *Bacillus* strain GSS in 120 h [22]. DDT is known to possess antimicrobial properties at high concentrations (>20mgL^{-1}) and restricted number of strains hold the ability to degrade higher DDT concentrations [14]. 5.43% of DDT concentration was found to be diminished in the control flask. This decrease in DDT concentration in the uninoculated flask is accredited to abiotic loss.

Table-1 *Percent degradation rate of the isolated strains*

Strains	Percent Degradation Rate
Y12A	81.6
Y10A	55.22
Y14B	48.16
Y15C	47.25

An increase in $OD_{\lambda 595nm}$ of culture media by 0.690 also accompanied DDT degradation. Since no increase was observed in optical density of control flasks, the increased OD in the inoculated flasks indicates utilization of DDT by strain Y12A as a source of its growth. Previous studies have also demonstrated an increase in the $OD_{\lambda 595nm}$ by bacterial culture in presence of DDT [14,23].

In the present study, degradation of DDT by strain Y12A consisted of initial dehydrochlorination and dechlorination into DDE and DDD respectively and further dechlorination into DDMU. [Fig-1] represents the probable pathway of DDT biodegradation by the strain Y12A. Various previous studies have reported formation of similar metabolites during the course of DDT degradation utilizing *Chryseobacterium* sp. PYR2, *Serratia marcescens* NCIM 2919 and *Pseudomonas putida* [11, 18, 24]. Pan *et al*., have also reported mineralization of DDT via DDMU. Present study has shown the metabolite formation till DDMU [25]. Similarly, Bajaj *et al*., has reported DDT metabolism into DDE, DDD and DDMU in culture broth by Rhococcus sp. strain IITR03[27]. To achieve complete mineralization of DDT we have isolated three more isolates. Thus, further study of another isolate's capability to degrade DDMU is required.

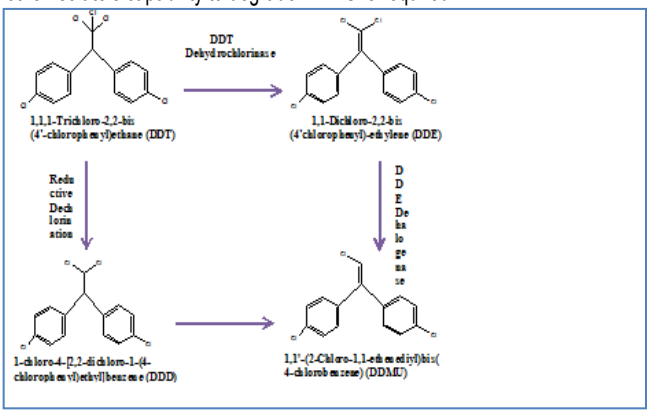

Fig-1 Proposed pathway for the degradation of DDT by *Achromobacter* sp. Y12A

Morphological and biochemical characterization of the strain

The identification of the bacterial strain was done based on the colony and cell morphology, gram staining, physiological growth conditions and biochemical tests [28]. The strain formed translucent grey-white colored round, mucoid colonies when grown on LB. The isolated strain was gram negative and tested positive for oxidase activity and for consumption of D-glucose, D-xylose, adipate, caprate, Phenylacetate and L-Malate.

16S rRNA partial gene sequencing and phylogenetic analysis

On the basis of 16S rRNA partial gene sequencing, BLAST results showed 99% similarity to A*chromobacter* sp., Taxonomy: Bacteria; Proteobacteria; Betaproteobacteria; Burkholderiales; Alcaligenaceae; *Achromobacter*). Based on the aforementioned identifications, the strain was named as *Achromobacter* sp. Y12A. The phylogenetic tree for *Achromobacter* sp. Y12A is illustrated in [Fig-2]. The accession number provided for the 16S rRNA sequence of strain Y12A submitted to the GenBank is MF138131.

Effect of different DDT concentrations, pH and temperature on its biodegradation

Variations in environmental factors may also affect the growth and degradation potential of microorganisms. The optimum temperature for the growth of strain Y12A was obtained at 30ºC and growth rate was discouraged as the temperature was increased or decreased beyond this point [Fig-3]. Degradation rates have shown similar pattern as shown by the growth [Fig-4]. After attaining the highest degradation by strain Y12A at 30ºC, a dip in the degradation rates were observed at 40ºC. In all control flasks without the isolated strain Y12A, the degradation percentages of DDT were less than 9.5%. Similar results have been reported in various studies, *Alcaligenes* sp. Kk [14] and *Pseudoxanthomonas* sp. wax [1] have also shown highest growth rate and degradation of DDT at 30ºC and least at extreme temperatures.

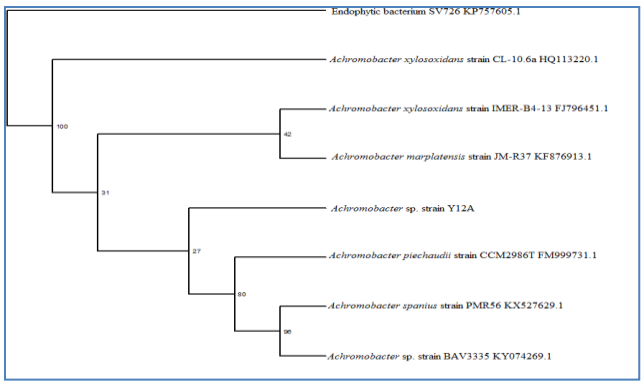

Fig-2 Phylogenetic tree of *Achromobacter* sp. Y12A constructed using Neighbour joining method.

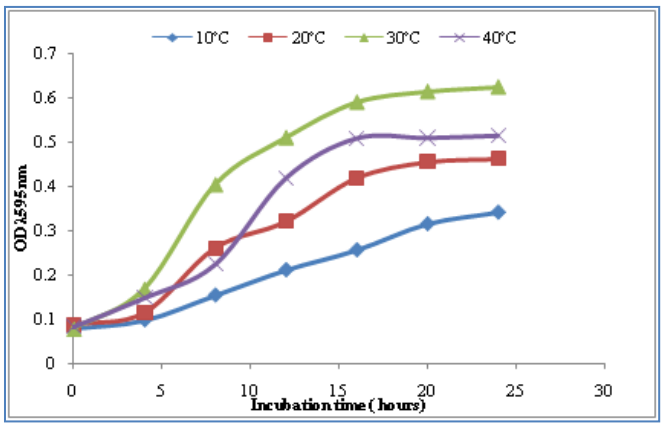

Fig-3 Growth kinetics of *Achromobacter* sp. Y12A in CFM (50mgL^{-1} DDT) at different temperature

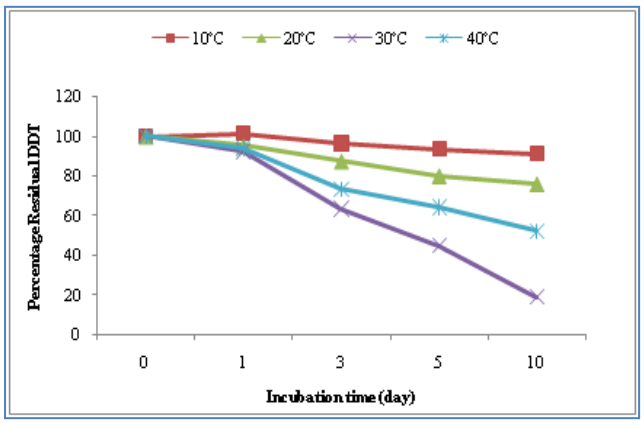

Fig-4 Degradation of DDT by *Achromobacter* sp. Y12A in CFM (50mgL^{-1}) at different temperature

Strain Y12A exhibited maximal growth at pH 7 followed by pH 8, 6, 5, 4 [Fig-5]. Degradation showed similar pattern as the bacterial growth [Fig-6]. Maximum degradation of DDT was obtained at pH 7,81.77% degraded within 10 days of incubation. Minimal growth as well as degradation was obtained at pH 4. Results show that pH influences the growth as well as degradation of DDT and bacterium requires near- neutral pH to degrade DDT. Pan et al. [26], Bidlan and Manonmani [21], Pant et al. [22] also promulgated the suitability of near-neutral pH range for DDT degradation by *Strentrophomonas* sp., *S. marcescens* DT-1P and *Bacillus* sp. GSS respectively. However, a different observation has been promulgated by Xie *et al.* [14] who showed weak acidic conditions (pH 6.0) as most conducive pH for the highest DDT degradation by *Alcaligenes* sp. KK.

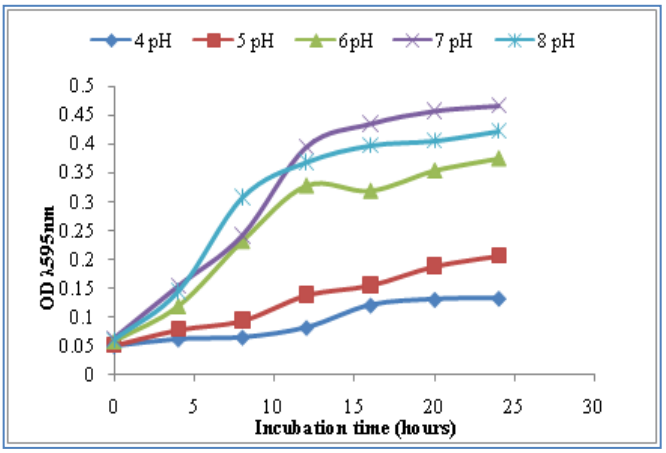

Fig-5 Growth kinetics of Achromobacter sp. Y12A in CFM (50mgL-1 DDT) at different pH

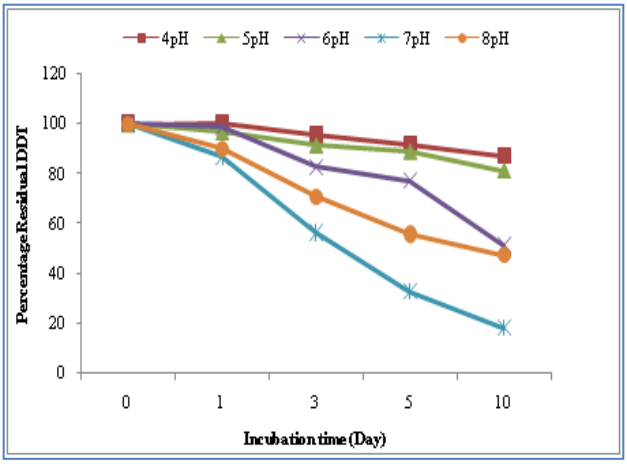

Fig-6 Degradation of DDT by *Achromobacter* sp. Y12A in CFM (50mgL^{-1}) at different pH range

Strain Y12A has shown increase in the growth with increasing concentration of DDT till 50ppm [Fig-7]. DDT degradation peaked at 50ppm degrading 82.04% DDT, further high concentrations showed inhibitory effect on DDT degradation while growth was more or less same [Fig-8]. It may be inferred that high DDT concentrations have proven toxic for the isolated strain Y12A. In accordance with our results, Li et al. [29] had shown removal of 69.0% and 30.7% of DDT at 40 and 60ppm level concentration of DDT respectively, by *Stenotrophomonas* sp. D-1 in broth, Chakraborty et al. [30] also reported a decrease in degradation with rising DDT concentrations from 50 to 500 ppm by mixed bacterial consortium (*Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., and *Flavobacterium* sp.) from activated sludge. These results indicated that DDT degradation evidently declines with rising concentrations as high concentrations of DDT inhibit bacterial growth as DDT possess antimicrobial properties at high concentration [14].

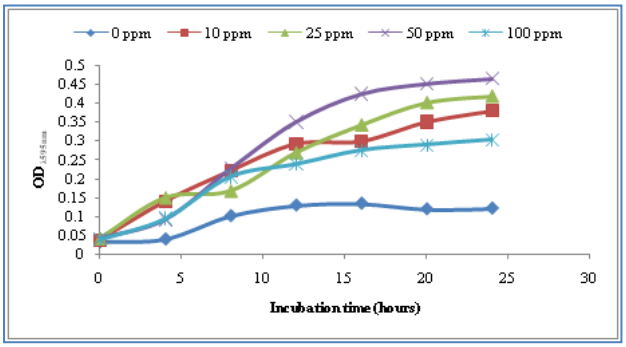

Fig-7 Growth kinetics of *Achromobacter* sp. Y12A in CFM at different DDT concentrations

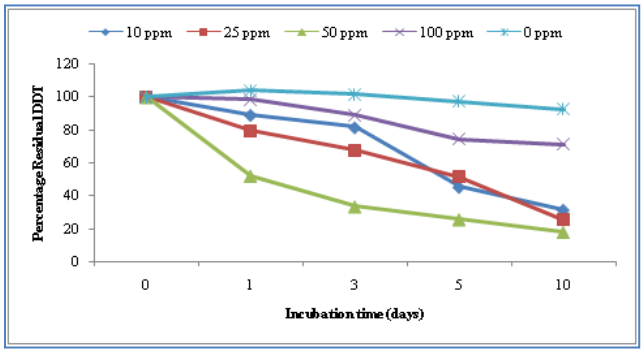

Fig-8 Degradation of DDT by *Achromobacter* sp. Y12A in CFM at different DDT concentrations

Conclusion

DDT degrading bacterium, strain Y12A was isolated from DDT contaminated soil. On the basis of 16SrRNA gene sequence analysis, strain Y12A was identified as *Achromobacter* sp. which can swiftly degrade DDT. Under optimum pH, temperature and concentration of DDT which is pH 7, 30ºC and 50ppm respectively, degradation rate escalates. The results indicate that *Achromobacter* sp. strain Y12A exhibits the capability of degrading DDT as a sole source of carbon. However, the strain belonging to *Achromobacter* genus has been reported for the first time for DDT degradation. It shows the potential to be used for bioremediation of DDT.

Abbreviations

16S rRNA: 16S ribosomal Ribonucleic acid
DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane
POP: Persistent Organic Pollutants

Ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors.

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Author Contributions: All author equally contributed

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