

Isolation and Characterization of a Novel *p,p'*-DDT-degrading Bacterium: *Aeromonas* sp. Strain MY1 from Agricultural Soil

Abstract

Background: Despite the banned on the use of DDT and other Persistent Organic Pollutants (POPs) by the Stockholm Convention for their toxicity, emerging shreds of evidence have indicated that DDT is, however, still in use in developing countries. This might increase the global burden of DDT contamination and its hazardous effects.

Aim: This study focused on the isolation and characterization of *p,p'*-DDT-degrading bacterium from a tropical agricultural soil.

Methodology: Standard isolation procedure was used for the screening and isolation of the strain. The 16S rRNA and phylogenetic analyses were used to identify the isolate and established protocols were followed to characterize the strain.

Results: A new strain belonging to the genus *Aeromonas* was isolated from agricultural soil using minimal salt-*p,p'*-DDT enrichment medium. The 16S rRNA sequencing was used to identify the strain and the partial sequence was deposited in the NCBI GenBank as *Aeromonas* sp. Strain MY1. This mesophilic isolate was capable of utilizing up to 50 mgL⁻¹ of *p,p'*-DDT as the sole carbon source at an optimum pH of 7.5 and optimum temperature of 35 °C within 120 h under aerobic conditions. Fe²⁺ (0.2 mgL⁻¹) demonstrated a stimulatory effect on the *p,p'*-DDT degradation capacity by the strain MY1. However, Zn, Cu, Pb, Hg, Ag and Cr ions have demonstrated various patterns of inhibitory effect on the *p,p'*-DDT degradation capacity of the isolate at 0.2 mgL⁻¹. This isolate could be a promising candidate for the bioremediation of *p,p'*-DDT contaminant.

Conclusion: *Aeromonas* sp. strain MY1 was capable of utilizing *p,p'*-DDT as a sole carbon source under aerobic conditions. The utilization capacity of the strain was influenced by some heavy metals, where Fe enhanced the utilization capacity of the isolate at a lower concentration, while Zn, Cu, Pb, Hg, Ag and Cr showed an inhibitory effect.

Keywords: DDT biodegradation, *Aeromonas* sp., 16S rRNA, Environmental contaminant, Heavy metals

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1. INTRODUCTION

Dichlorodiphenyltrichloroethane commonly known as DDT is the most notorious organochlorine pesticide (OCPs) that is still captivating the interest of many researchers due to its degrading impact on ecology and human health. Its toxic impact is more serious to the environment when there is co-contamination with metals [1]. Despite the banned on the use of DDT and other Persistent Organic Pollutants (POPs) by the Stockholm Convention for their

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toxicity, DDT is, however, still in use in developing countries of Africa, Asia, and the South Pacific [2,3]. It was reported that in Nigeria over 130,000 tons of pesticides are used annually for agriculture and disease-borne control [4] and consequently DDT was recently detected in water samples [5]. The unique nature of persistence in the environment demonstrated by DDT made it traceable in ambient air, drinking water, vegetables, honey, fish, breast milk and other food varieties [6,7,8]. In many cases, DDT levels in food items were reported to exceed the upper limits of daily intake stated by the U.S. Environmental Protection Agency [9].

DDT is known to accumulate in the environment, potentially dually bio-accumulating in biological systems [6,10]. One of the major health implications of DDT and its metabolites is its link to endocrine disruption [11,12]. Recently, exposure to DDT and its metabolites DDE (dichlorodiphenyldichloroethylene) were reported to be implicated in long-term impairments in muscle health [13], the cognitive decline [14] and some alterations in maternal metabolome [15]. Many studies have also documented the probable involvement of DDT in carcinogenicity [16,17]. It was also demonstrated to pose some risks of inducing apoptosis of skin fibroblasts in some aquatic animals [18].

Owing to its inherent environmental recalcitrance, the intensity of ecological and human health effects, still exploring the microbial consortia for novel isolates for the efficient elimination of residual DDT contaminants could be a valuable option. Since microorganisms are known to influence the fate of organochlorine contaminants in the environments. [This study focused on the isolation and characterization of a *p,p'*-DDT-utilizing bacterium inhabiting tropical agricultural soil.

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2. MATERIALS AND METHODS

2.1 Materials: The *p,p'*-DDT and other relevant chemicals used in this research were purchased from the Sigma Aldrich (United Kingdom) unless otherwise specified.

2.1.1 Sampling site: The sampling site is part of Phase I, Kano River Project that is located at the Kadawa Irrigation Scheme, Kura-Garun Malam in Kano State, Nigeria. The site has geographical coordinates of 11° 41' 0" North, 8° 22' 0" East. It has a history of uninterrupted agricultural practice and massive use of agrochemicals including DDT for at least three decades [19].

2.1.2 Soil sample collection: Soil sample was collected from the stated location. The sample was collected at the surface of the soil to the depths of 15 cm. The sampling was focused on these horizons because a large portion of microbial activity occurs in these horizons. The soil sample was mixed evenly and 20 g was carefully put into a sterile container and kept at 4 °C for bacterial isolation.

2.1.3 Nutrient Medium: For the bacterial isolation purposes, Luria-Bertani (LB) medium was prepared as described by Pant et al. [20].

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2.1.4 Preparation of minimal-salt-*p,p'*-DDT enrichment medium: This medium was prepared following the medium compositions and description by Pant et al. [20] with slight changes: in a litre of distilled water, 0.1 g CaCl₂·2H₂O, 0.08 g Ca(NO₃)₂·4H₂O, 0.5 g MgCl₂·6H₂O, 1.0 g Na₂SO₄, and 1.0 g KH₂PO₄ were dissolved to form the minimal salt medium. Then before inoculation, 0.05 mg mL⁻¹ of *p,p'*-DDT was added to the minimal salt

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medium to form the minimal-salt-*p,p'*-DDT enrichment medium. Thus, inoculation into the final medium ensures that the sole carbon and energy source for the isolate was from the *p,p'*-DDT only, and thus, significant formation of biomass in this medium would only occur if the isolate can utilize the *p,p'*-DDT. Before inoculation, the media was usually autoclaved.

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2.2 Isolation of *p,p'*-DDT-degrading *Aeromonas* sp. strain MY1 from soil samples:

Isolation of *p,p'*-DDT-degrading *Aeromonas* sp. strain MY1 was carried out using a modified isolation procedure described by Pant et al. [20]. A 25 ml suspension of LB medium containing 0.5 g of air-dried soil was prepared. The suspension was incubated for 48 h at 30 °C on a shaker at 100 rpm. After the incubation, the LB medium was allowed to settle down for 2 h. Then, a 100 µl from the cleared LB supernatant was used to inoculate 4 ml of minimal-salt-*p,p'*-DDT enrichment medium. The enrichment culture was then incubated for 168 h at 30°C on a rotary shaker at 100 rpm. After the incubation, 100 µl of the enrichment culture was transferred into another 4 ml of fresh minimal-salt-*p,p'*-DDT enrichment medium, and the incubation step was repeated. After six sequential sub-culturing, the isolate was inoculated on to minimal-salt-*p,p'*-DDT enrichment agar plates containing 0.05 mg mL⁻¹ and incubated for 24 h at 30 °C and the isolate formed was preserved. This ensures adequate exposure of the isolate to the *p,p'*-DDT.

2.3 DNA extraction: The bacterial genome extraction was done using the DNA Biospin Bacteria Genomic Extraction Kit, Bioflux Co. (Gapan) following manufacturer's instruction.

2.4 The 16S rRNA nucleotide sequencing

For amplification of the target gene from the extracted genomic DNA, 16S rRNA gene primers [16SRNA BAC27F: 5'-AGA GTT TGA TCC TGG CTC AAG-3' and 16SRNA BAC1492R: 5'-GGT TAC CTT GTT ACG ACT T-3'] were used. Polymerase chain reaction (PCR) conditions were set as described by Sangwan et al. [21]. After the final elongation cycle, the size of the DNA fragment was compared with the Hyper Ladder-1K marker Bioline (Lot No: H4-111B). The 3 µl PCR product was mixed with 5XDNA loading buffer blue (1.5 µl) Bioline (Lot No: hLBB-415704) and introduced onto 1% agarose gel electrophoresis that has been subjected to ethidium bromide staining. The electrophoresis was run for 35 minutes under 120V and 300mA current. The product was then visualized with the Syngene Gel Documentation System of Ingenius, England (IG31459). The presence of a product of the expected size was considered to be a positive result. The PCR product obtained was gel purified and subjected to 16S rDNA sequencing using the protocols described by Sanger et al. [22]. Then, DNA sequence alignment was done using ClustalW 2.0.12 and the sequence was subjected to the BLAST search program at the NCBI website (<http://www.ncbi.nlm.nih.gov/>) to identify the isolate.

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2.5 Phylogenetic analysis of *Aeromonas* sp. Strain MY1

Phylogeny and evolutionary history of the *Aeromonas* sp. strain MY1 was constructed using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method [23]. The phylogenetic tree was constructed using MEGA version 6 software program.

2.6 Characterization of *Aeromonas* sp. strain MY1 in *p,p'*-DDT enrichment medium by using One Factor at a Time (OFAT)

In the characterization of *Aeromonas* sp. strain MY1 in the *p,p'*-DDT enrichment medium, we have determined the optimum *p,p'*-DDT concentration (as a sole carbon source), pH, temperature, incubation time, and effect of some heavy metals on the growth of the isolate. The turbidity of the medium is regarded as the index of the biomass and growth of *Aeromonas* sp. strain MY1 in the *p,p'*-DDT enrichment medium, which was determined spectrophotometrically as optical density (OD) at 600_{nm}.

Before inoculation, *Aeromonas* sp. Strain MY1 was pre-cultured in LB broth for 48 h and the turbid broth was centrifuged. The supernatant was removed while the cell pellets were introduced into a fresh MSM medium and used as an inoculum source. The *p,p'*-DDT degrading capacity of *Aeromonas* sp. strain MY1 was determined *in vitro* by adjusting the cell density (OD_{600nm}) of the inoculum source to 0.5, the cells (100 µL) were then inoculated into MSM media containing varying concentrations *p,p'*-DDT (10, 20, 30, 40, 50, 60 and 70 mgL⁻¹) at varying pH ranges (5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) and incubated at different temperatures (20, 25, 30, 35, 40 and 45 °C) and different incubation period (24 h, 48 h, 72 h, 96 h, 120 h, 144 h and 168) under shaking condition (150 rpm). The experiments were conducted one factor at a time in triplicate.

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2.7 Effects of heavy metals on *p,p'*-DDT utilization capacity of *Aeromonas* sp. strain MY1

The effects of heavy metals (Fe, Zn, Cu, Pb, Hg, Ag and Cr) on *p,p'*-DDT degrading capacity of *Aeromonas* sp. strain MY1 was determined *in vitro* by inoculating into MSM-*p,p'*-DDT enrichment media containing varying metal concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mgL⁻¹) and incubated on a rotary shaker (140 rpm) at 30 °C and pH 7.5 for 168 h. The OD was measured spectrophotometrically at 600_{nm} after blanking with freshly prepared MSM medium containing *p,p'*-DDT for the determination of cell growth as an index of *p,p'*-DDT degradation capacity of *Aeromonas* sp. strain MY1 [1]. The experiments were set up under aerobic conditions.

3. RESULTS AND DISCUSSION

3.1 Identification of the bacterial strain

The preliminary morphological and biochemical characteristics of *Aeromonas* sp. strain MY1 on LB agar showed a creamy, shiny colony. Microscopic observation revealed a rod-shaped Gram's negative, motile, non-spore and non-capsule forming bacteria that is positive to catalase, cytochrome oxidase, acetate, and citrate but negative to urease tests (data not presented here).

3.2 16S rRNA and phylogenetic analyses: For identification of bacteria, although there are many options available, DNA-DNA relatedness using the 16S rRNA sequencing is, however, the most accurate and reliable means of identification [24,25] relative to conventional phenotypic methods that show a low level of accuracy and reliability [26]. The 16S rRNA PCR amplification product of *Aeromonas* sp. strain MY1 revealed about 1200 bp upon running on 1% agarose electrophoretic gel (Figure 1). Soltani et al. [27] reported a similar

base-pair range for some *Aeromonads* isolated from Southern and Northern provinces of Iran. The partial sequence of the 16S rRNA from *Aeromonas* sp. strain MY1 has been determined and deposited in the NCBI Biodata Bank under the accession number MN530936.

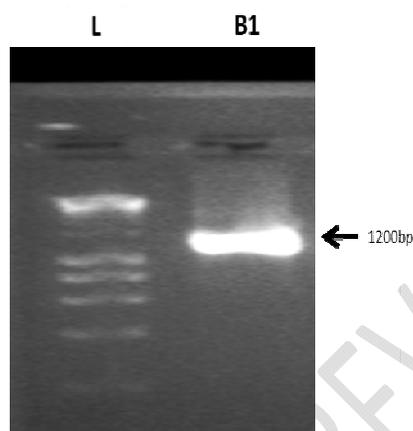


Figure 1: Agarose gel electrophoretic image of the 16SrRNA amplicon of *Aeromonas* sp. Strain MY1 isolate coded as B1. L represents Hyper Ladder-1K marker Bioline (Lot No: H4-q 111B)

The partial sequence of 16S rRNA of the *Aeromonas* sp. strain MY1 contains 678 nucleotides, which upon blasting in the NCBI Biodata Bank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) showed that the organism belongs to the genus *Aeromonads*. More than 60% of the first hundred sequence homology output (blast) of the *Aeromonas* sp. strain MY1 showed more than 94% sequence similarity with various *Aeromonas* species, with *Aeromonas sanarellii* strain A2-67 and *Aeromonas enteropelogenes* strain CECT having the highest sequence similarity of 98.22% each, whereas *Aeromonas cavernicola* strain MDC 2508 showed the least similarity of 94.09% (data not presented here). Several kinds of research documented that the genus *Aeromonas* has a closely related interspecies sequence homology, showing high levels of similarity that is close to 100% [28,29,30].

The phylogenetic tree for the 16S rRNA gene of *Aeromonas* sp. strain MY1 was derived from the genetic matrices of the first seventeen representatives from *Aeromonas* species as obtained from the blast analysis and other three DDT degrading species as outliers. The evolutionary tree was constructed using the MEGA 6 program by using the Neighbor-Joining method as stated in the methodology section. Apart from the outliers used in this study, the phylogeny showed minimum divergence (branch lengths) between all the *Aeromonas* species used as indicated by higher bootstrap values of 99-100%. However, the tree sandwiches *Aeromonas* sp. strain MY1 in between *Aeromonas enteropelogenes* strain CECT 4487 and *Aeromonas enteropelogenes* strain DMS 6394 in a single cluster (Figure 2) suggesting stronger closeness with *Aeromonas enteropelogenes* species. This finding conforms to Martínez-Murcia et al. [28] that the depth of phylogeny of the 16S rRNA gene for the genus *Aeromonas* is narrow,

revealing interspecies identity close to 100%. Also, Thornton et al. [31] observed that some *Aeromonads* strains showed up to 99.6% identity.

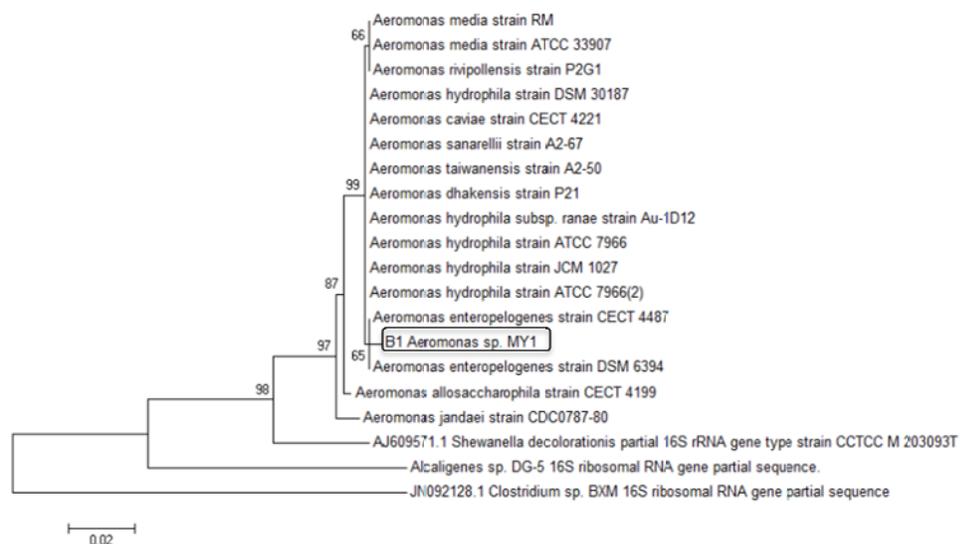


Figure 2: Evolutionary relationships of taxa of *Aeromonas* sp. Strain MY1 isolate. The strain was shown in a box coded as B1 *Aeromonas* sp. MY1

3.3 Growth response of *Aeromonas* sp. strain MY1 in *p,p'*-DDT enrichment medium

Many researchers have reported the isolation of bacterial species capable of tolerating $< 20 \text{ mgL}^{-1}$ of *p,p'*-DDT as a sole carbon source [20]. Though some isolates were documented to tolerate up to 50 mgL^{-1} when other carbon sources were supplemented [32,33,34]. However, *Aeromonas* sp. strain MY1 demonstrated higher tolerance and utilization capacity of up to 50 mgL^{-1} of *p,p'*-DDT as the sole carbon source under aerobic setup. An increase in the concentration greater above 50 mgL^{-1} however, lowers the organism's efficiency to utilize the pesticide contaminant (Figure 3). A similar scenario was documented by Mwangi et al. [35] that DDT concentration above 50 mgL^{-1} lowers the growth of the DDT degrading isolates. The ability of a microorganism to depend on DDT as sole carbon and energy source for significant growth depends on the organism's capacity to mineralize the DDT. Microbial mineralization of DDT involves multiple biological reactions [34]. The *Aeromonas* sp. strain MY1 demonstrated a minimal lag phase of $< 24 \text{ h}$ and precipitated its highest biomass of 0.152 ($\text{OD}_{600\text{nm}}$), in 120 h (Figure 3), efficiently mineralizing the DDT in the medium. This was perhaps, achieved because the strain MY1 possesses effective machinery for the efficient utilization of *p,p'*-DDT as a sole carbon source. Although the majority of literature on *Aeromonads* has focused on their clinical implications, information on their ability to biotransform DDT is scarcely available. However, *Aeromonas hydrophila* was reported to biotransform DDT [36,37].

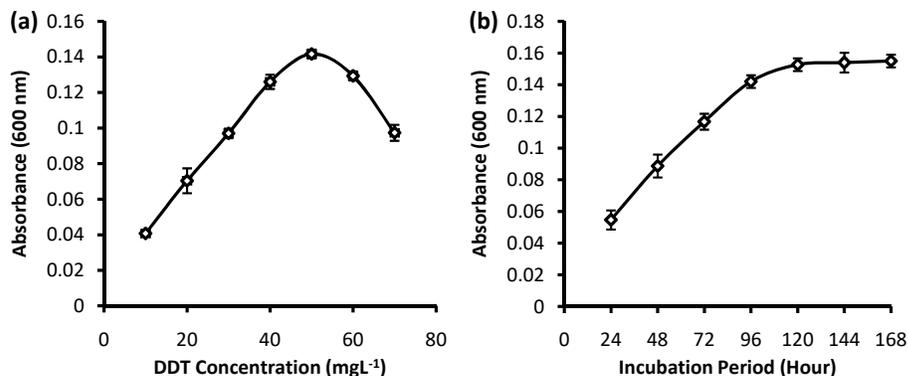


Figure 3: Effects of *p,p'*-DDT concentration (a) and incubation time (b) on the growth of *Aeromonas* sp. Strain MY1 in *p,p'*-DDT enrichment medium

3.4 Effects of pH, temperature and inoculum size on the growth of *Aeromonas* sp. strain MY1 in *p,p'*-DDT enrichment medium

As a complex process, bacterial DDT mineralization is largely influenced by some environmental determinants such as pH, temperature and concentration of the DDT [38,39]. Concerning this, the strain MY1 demonstrated some mesophilic characteristics by exhibiting maximum growth at pH 7.5 and 35 °C, whereas fluctuations in the above parameters lower the biomass and decrease the OD of the isolate (Figures 4). This is not surprising by considering the environmental conditions of the tropical region where the organism was isolated. Data from a pilot study on the physicochemical characteristics of the soil from the sampling site fall within a similar range of pH and temperature values upon which the organism had been inherently adapted to (data not presented here). Pant et al. [20], Fang et al. [34] and Mwangi et al. [35] also reported isolates that degrade DDT close to the above pH and temperature ranges.

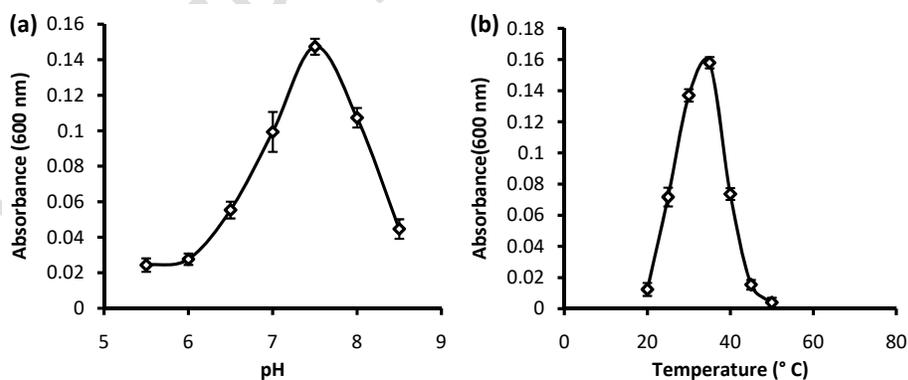


Figure 4: Effect of pH (a) and temperature (b) on the growth of *Aeromonas* sp. Strain MY1 in *p,p'*-DDT enrichment medium

3.5 Effects of heavy metals on *p,p'*-DDT utilization capacity of *Aeromonas* sp. strain MY1

Virtually all agricultural sites are co-contaminated with organic and metal contaminants. The presence of these pollutants must have some effects on the microbial consortia and the way these microbes biotransform the accumulated contaminants. Thus, assessing the effects of Fe, Zn, Cu, Pb, Hg, Ag and Cr on the *p,p'*-DDT utilization capacity of strain MY1 is important. The growth of *A. sp.* strain MY1 in *p,p'*-DDT medium was enhanced by a presence of 0.2 mgL^{-1} of Fe^{2+} relative to the absence of this divalent ion. However, a further increase in the concentration to 0.4 mgL^{-1} decreased the growth of the organism (Figure 5). Depending on the concentration, Fe^{2+} showed both stimulatory and inhibitory effects. The stimulatory effect of Fe^{2+} could be the result of an increase in biogenic Fe^{2+} formation as reported by Glass, [40] and Cao et al. [37] with a possible increase in the reductive biotransformation and utilization of *p,p'*-DDT observed in the strain MY1. Indeed, iron is required for various metabolic processes in bacteria. Thus, it is not surprising when iron enhanced the growth of strain MY1 in *p,p'*-DDT medium. The enhancement of the reductive transformation of DDT by *Aeromonas hydrophila* HS01 in the presence of zerovalent iron was also reported by Cao et al. [36].

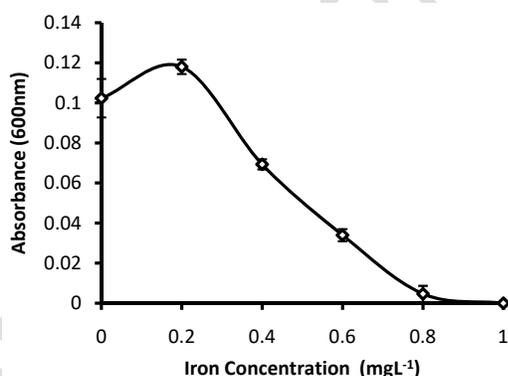


Figure 5: Effect of iron (II) ions on the growth of *Aeromonas* sp. Strain MY1 in *p,p'*-DDT enrichment medium

The other metals tested (Zn, Cu, Pb, Hg, Ag, and Cr) showed an inhibitory effect on the *p,p'*-DDT utilization capacity of strain MY1 with Pb and Cr (Figures 6) exhibiting a maximum inhibitory effect at the minimum concentration of 0.2 mgL^{-1} used in this study.

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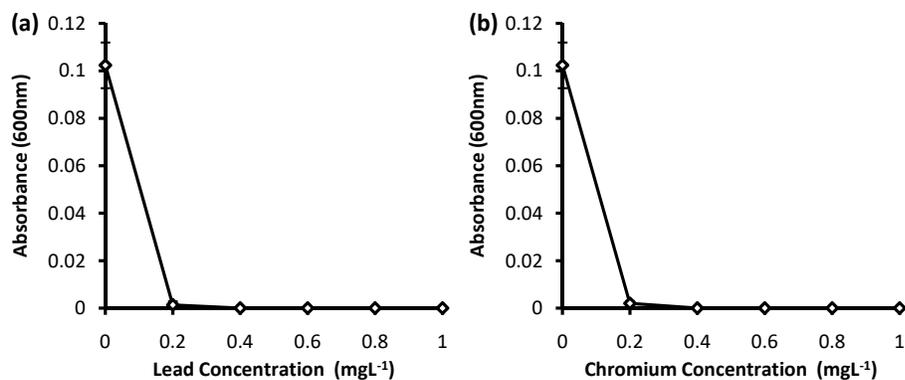


Figure 6: Effects of lead (a) and chromium (b) ions on the growth of *Aeromonas* sp. Strain MY1 in *p,p'*-DDT enrichment medium

However, the pattern of inhibition demonstrated by Zn and Cu (Figure 7a and b) was more favourable to the growth of strain MY1 relative to that of Hg and Ag (Figure 7c and d). This could be linked to the essentiality of Zn and Cu for some metabolic processes in bacteria. However, it has been observed that the presence of both organic and metal pollutants resulted in metal toxicity in bacteria, mostly by interacting and inhibiting the bacterial enzymes and thus, inhibiting organic pollutant biodegradation [1,41]. Also, metal oxyanions, such as chromate, mimic the structure of essential non-metal oxyanions, such as sulfate [1]. Furthermore, mercuric and silver cations form harmful complexes that may disrupt some physiological functions, in addition to their inhibitory binding to the SH group of the variety of bacterial proteins [42].

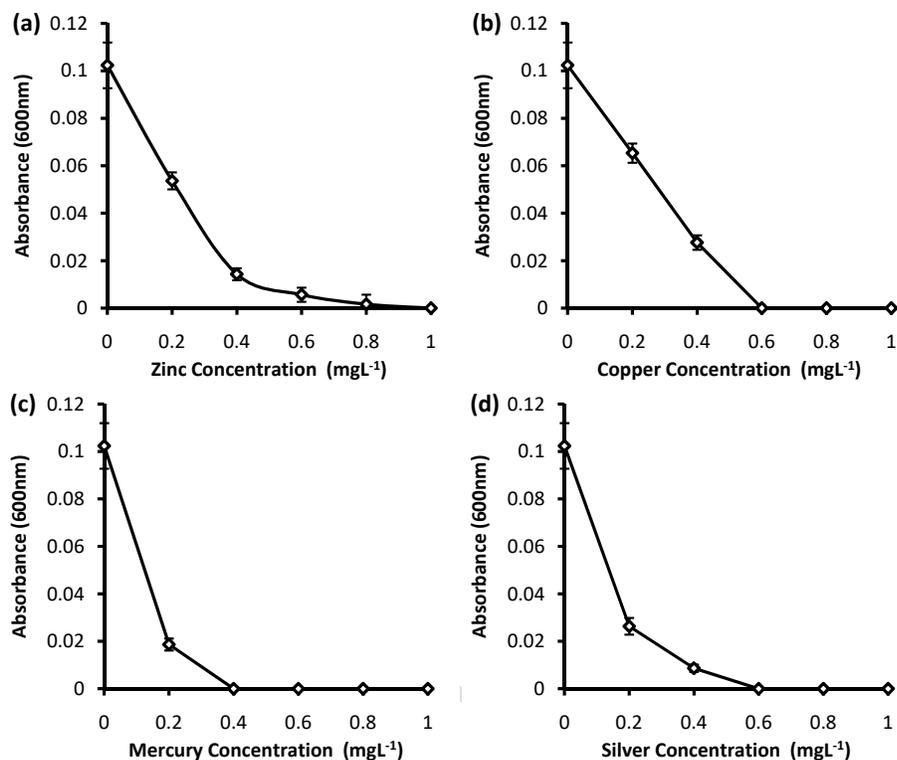


Figure 7: Effects of zinc (a), copper (b), mercury (c) and silver (d) ions on the growth of *Aeromonas* sp. Strain MY1 in *p,p'*-DDT enrichment medium

4. CONCLUSION

A new strain of the genus *Aeromonas* has been isolated from agricultural soil. Genomic 16S rRNA sequencing was used to identify the strain and the partial sequence was deposited in the NCBI Biodata Bank as *Aeromonas* sp. MY1 with accession number MN530936. The isolate was capable of utilizing up to 50 mg L⁻¹ of *p,p'*-DDT as only carbon and energy source at an optimum pH of 7.5 and 35 °C within 120 h. Fe²⁺ (0.2 mgL⁻¹) demonstrated a stimulatory effect on the *p,p'*-DDT degradation by strain MY1. However, Zn, Cu, Pb, Hg, Ag, and Cr ions showed inhibitory effects on the *p,p'*-DDT degradation capacity of the isolate at 0.2 mgL⁻¹. This isolate could serve a potential role of a bio-cleaner for the removal of *p,p'*-DDT contaminant.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because

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we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Sandrin TR, Maier RM. Impact of Metals on the Biodegradation of Organic Pollutants, *Environmental Health Perspectives*. 2003;111(8):1093-1101.
2. UNEP. DDT expert group and its report on the assessment of scientific, technical, environmental and economic information on the production and use of DDT and its alternatives for disease vector control. *Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants Ninth meeting*, Geneva, 2019. 29 April-10 May 2019.
3. Abdul Kader M. (2019). Domination of Pollutant Residues Among Food Products of South-East Asian Countries, *Acta Scientific Pharmaceutical Sciences*. 2019;3(9): 75-79.
4. Asogwa EU Dongo LN. Problems Associated with Pesticide Usage and Application in Nigerian Cocoa Production: A Review, *African Journal of Agricultural Research*. 2009;4:675-683.
5. Ogbeide O, Tongo I, Ezemonye L. Risk Assessment of Agricultural Pesticides in Water, Sediment, and Fish from Owan River, Edo State, Nigeria, *Environmental Monitoring and Assessment*. 2015;187:654-666.
6. Bussolaro D, Filipak Neto F, Glinksi A, Roche H, Guiloski I.C, Mela M. et al. Bioaccumulation and Related Effects of PCBs and Organochlorinated Pesticides In Freshwater Fish *Hypostomus Commersoni*, *Journal of Environmental Monitoring*. 2012;14(8):2154-2163 doi:10.1039/c2em.10863a.
7. Mendes RA, Lima MO, de Deus RJA, Medeiros AC, Faial KCF, Jesus IM. et al. Assessment of DDT and Mercury Levels in Fish and Sediments in the Iriri River, Brazil: Distribution and ecological risk, *Journal of Environmental Science and Health B*. 2019;9:1-10. doi:10.1080/03601234.2019.1647060.
8. Thompson LA, Ikenaka Y, Darwish WS, Nakayama SM, Mizukawa H. Effects of the Organochlorine *p,p'*-DDT On MCF-7 Cells: Investigating Metabolic And Immunomodulatory Transcriptomic Changes *Environmental Toxicology and Pharmacology*. 2019;72:103249.
9. Sheldon M, Pinion JC, Klyza J, Zimeri A. Pesticide Contamination in Central Kentucky Urban Honey: A Pilot Study. *Journal of Environmental Health*. 2019;82(1): 8-13.
10. Devi NL. Persistent Organic Pollutants (POPs): Environmental Risks, Toxicological Effects, and Bioremediation for Environmental Safety and Challenges for Future Research. In: Saxena G., Bharagava R. (eds) *Bioremediation of Industrial Waste for Environmental Safety*. 2020;53-76 pp. Springer, Singapore
11. Mnif W, Hassine AH, Bouaziz A, Bargeti A, Thomas O, Roig B. Effect of Endocrine Disruptor Pesticides: A Review, *International Journal of research and Public Health*. 2011;8:2236-2303 doi: 10.3390/ijerph8062265.

12. Piazza MJ, Urbanetz AA. Environmental Toxins and the Impact of other Endocrine Disrupting Chemicals in Women's Reproductive Health JBRA Assisted Reproduction. 2019;23(2):154-164 doi: 10.5935/1518-0557.20190016
13. Truong KM, Cherednichenko G, Pessah IN. Interactions of Dichlorodiphenyltrichloroethane (DDT) and Dichlorodiphenyldichloroethylene (DDE) With Skeletal Muscle Ryanodine Receptor Type 1, Toxicological Sciences. 2019;170(2): 509–524 <https://doi.org/10.1093/toxsci/kfz120>
14. Medehouenou TCM, Ayotte P, Carmichael PH, Kröger E, Verreault R, Lindsay J et al. Exposure to Polychlorinated Biphenyls and Organochlorine Pesticides and Risk of Dementia, Alzheimer's Disease and Cognitive Decline in an Older Population: a Prospective Analysis from the Canadian Study of Health and Aging. Environmental Health. 2019;18(1):57 <https://doi.org/10.1186/s12940-019-0494-2>
15. Hu X, Li S, Cirillo P, Krigbaum N, Tran V, Ishikawa T. Metabolome Wide Association Study of Serum DDT and DDE in Pregnancy and Early Postpartum. Reproductive Toxicology. pii: 2019;S0890-6238(18):30588-4.
16. Hadara T, Takeda M, Kojima S, Tomiyama N. Toxicity and Carcinogenicity of Dichlorodiphenyltrichloroethane (DDT), Toxicological Research. 2016;32(1):21-33. <http://dx.doi.org/10.55487/TR.2016.32.1.021>
17. Cohn BA, Cirillo PM, Terry MB. DDT and Breast Cancer: Prospective Study of Induction Time and Susceptibility Windows, Journal of the National Cancer Institute. 2019; doi: 10.1093/jnci/djy198
18. Yu X, Yu RQ, Zheng X, Zhan F, Sun X, Wu Y. DDT Exposure Induces Cell Cycle Arrest and Apoptosis of Skin Fibroblasts from Indo-Pacific Humpback Dolphin Via Mitochondria Dysfunction, Aquatic Toxicology. 2019;213:105229. <https://doi.org/10.1016/j.aquatox.2019.105229> [Get rights and content](#)
19. Sangari DU. An Evaluation of Water and Land Uses in the Kano River Project, Phase I, Kano State. Journal of Applied Science and Environmental Management. 2006;11 (2): 105-111
20. Pant G, Mistry SK, Sibi G. Isolation, Identification and Characterization of p, p-DDT Degrading Bacteria from Soil, Journal of Environmental Science and Technology. 2013;6(8): 180-187.
21. Sangwan P, Kovac S, Kathryn ERD, Sait M, Peter HJ. Detection and cultivation of soil Verrucomicrobia. Applied and Environmental Microbiology. 2005:8402-8410
22. Sanger F, Nicklen S, Coulson AR. DNA Sequencing with Chain-Terminating Inhibitors. Biochemistry. 1977;4(12): 5463-5467
23. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution. 2013;30 2725-2729.
24. Roy RP, Bahadur M, Barat S. Isolation, Identification and Antibiotic Resistance of *Aeromonas* spp. and *Salmonella* spp. from the Fresh Water Loach, *Lepidocephalichthys guntea* and water of Terai River Lotchka, West Bengal, India. Zool Pol. 2013;58: 5-17.
25. Hong S, Farrence CE. Is It Essential to Sequence the Entire 16S rRNA Gene for Bacterial Identification? American Pharmaceutical Review. 2015;18(7):13 www.americanpharmaceuticalreview.com
26. Clarridge JE. 3rd. Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases, Clinical Microbiology Review. 2004;17(4):840-62 doi:[10.1128/CMR.17.4.840-862.2004](https://doi.org/10.1128/CMR.17.4.840-862.2004)

27. Soltani M, Moghimi SM, Ebrahimzade Mousavi H, Abdi K, Soltani E. Isolation, Phenotypic and Molecular Characterization of Motile Aeromonas Species, The Cause of Bacterial Hemorrhagic Septicemia in Affected Farmed Carp in Iran. *Iranian Journal of Veterinary Medicine IJVM* 2016;10 (3):209 -216
28. Martinez-Murcia AJ, Benlloch S, Collins MD. Phylogenetic Interrelationships of Members of the Genera Aeromonas and Plesiomonas as Determined by 16S Ribosomal DNA Sequencing: Lack of Congruence with Results of DNA-DNA Hybridizations. *International Journal of Systematic Bacteriology*. 1992;42(3):412-21.
29. Kupfer M, Kuhnert P, Korczak BM, Peduzzi R, Demarta, A. Genetic Relationships of Aeromonas Strains Inferred from 16S rRNA, gyrB and rpoB Gene Sequences. *International Journal of Systematic and Evolutionary Microbiology*. 2006;56:2743-2751 doi:10.1099/ijs.0.63650-0
30. Martin-Carnahan A. & Joseph S.W. (2015). *Aeromonas: Bergey's Manual of Systematics of Archaea and Bacteria*, pp 2-44 John Wiley and Sons, Inc. USA. <https://doi.org/10.1002/9781118960608.gbm01081>
31. Thornton JM, Austin DA, Austin B, Powell R. Small subunit rRNA gene sequences of *Aeromonas salmonicida* subsp. *smithia* and *Haemophilus piscium* Reveal Pronounced Similarities with *A. salmonicida* subsp. *salmonicida*. *Diseases of Aquatic Organism* 1999;35:155–158.
32. Kantachote D, Singleton I, McClure N, Naidu R, Megharaj M, Harch BD. DDT Resistance and Transformation by Different Microbial Strains Isolated from DDT-Contaminated Soils and Compost Materials, *Compost Science and Utilization* 2003;11:300-310
33. Barragan-Huerta BE, Costa-Pe'rez C, Peralta-Cruza J, Barrera-Cortes J, Esparza-García F, Rodríguez-Vázquez R. Biodegradation of Organochlorine Pesticides by Bacteria Grown In Microniches of the Porous Structure of Green Bean Coffee, *International Biodeterioration and Biodegradation*. 2007;59:239–244.
34. Fang H, Dong B, Yan H, Tang F, Yu Y. Characterization of a Bacterial Strain Capable Of Degrading DDT Congeners and Its Use in Bioremediation of Contaminated Soil, *Journal of Hazardous Materials*. 2010;184:281-289.
35. Mwangi K, Boga HI, Muigai AW, Kiiyukia C, Tsanuo MK. (2010). Degradation of Dichlorodiphenyltrichloroethane (DDT) by Bacterial Isolates from Cultivated and Uncultivated Soil. *African Journal of Microbiology Research*. 2010;4 (3):185-196
36. Cao F, Li FB, Liu TX, Huang DY, Wu CY, Feng CH. et al. Effect of Aeromonas hydrophila on Reductive Dechlorination of DDTs by Zero-Valent Iron, *Journal of Agriculture and Food Chemistry* 2010;58: 12366-12372 doi:10.1021/jf102902f
37. Cao F, Liu TX, Wu CY, Li FB, Li XM, Yu HY. et al. Enhanced Biotransformation of DDTs by an Iron- and Humic-Reducing Bacteria Aeromonas hydrophila HS01 upon Addition of Goethite and Anthraquinone-2,6-Disulphonic Disodium Salt (AQDS), *Journal of Agriculture and Food Chemistry* 2012;60: 11238-11244 dx.doi.org/10.1021/jf303610w.
38. Bidlam R, Manonmani HK. Aerobic Degradation of Dichlorodiphenyltrichloroethane (DDT) by Serratia marcescens DT-1P. *Process Biochemistry* 2002;38: 49-56.
39. Aislabie JM, Richards NK, Boul HL. Microbial Degradation of DDT and Its Residues—a Review. *New Zealand Journal of Agricultural Research*. 1997;48: 269-282.

40. Glass BL. Relation between the Degradation of DDT and the Iron Redox System in Soils. *Journal of Agriculture and Food Chemistry* 1972;20:324-327.
41. Murata T, Kanao-Koshikawa M, Takamatsu T. Effects of Pb, Cu, Sb, In and Ag Contamination on the Proliferation of Soil Bacterial Colonies, Soil Dehydrogenase Activity, and Phospholipid Fatty Acid Profiles of Soil Microbial Communities, Water, Air and Soil pollution 2005;164:103-118
42. Nies DH. Microbial Heavy-Metal Resistance. *Applied Microbiology and Biotechnology* 1999;51:730-750.

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