Biodegradation of Chlorpyrifos and 3,5,6-trichloro-2-pyridinol (TCP) by *Ochrobactrum* sp. CPD-03: Insights from genome analysis on organophosphorus pesticides degradation, chemotaxis and PGPR activity

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Abstract

Organophosphorus pesticides (OPs) are xenobiotic chemicals, present in the environment at unnaturally high concentrations. Chlorpyrifos (CP) is a widespread OP pollutant that poses hazards to non-target organisms along with human beings. The present study reports a newly isolated strain, *Ochrobactrum* sp. CPD-03 form a paddy field in Bhubaneswar, Odisha, India which could degrade CP in 48 hours with a degradation efficiency of 85-88% in minimal salt medium. The CP degradation at various concentrations (100 mg L^{-1} to 1000 mg L^{-1}) by CPD-03 was curve fit into a pseudo-first order kinetics [Y= (Y_0 - Plateau)*$e^{(-K*X)}$ + Plateau)]. Whole genome analysis indicated the presence of arylesterase (EC 3.1.1.2) and showed the existence of several other genes accountable for the various other xenobiotic compounds degradation. CPD-03 showed chemotactic features towards CP at different concentrations along with other OPs including monocrotophos, coumaphos, diazinon, methyl parathion, and malathion, respectively. Moreover, few new metabolites during CP degradation by CPD-03 were found. This strain was also found to degrade a major metabolite, TCP (3,5,6-trichloro-2-pyridinol) during CP degradation. CPD-03 also displayed potential plant growth promoting activity in inoculated rice seedlings. Taken altogether, our results highlight the potentials of this new isolate *Ochrobactrum* sp. CPD-03 in bioremediation and application in OP-contaminated ecosystem.

Keywords: Biodegradation, Chlorpyrifos, *Ochrobactrum* sp. CPD-03, Organophosphorus hydrolase, Chemotaxis, Plant growth promotion

1. Introduction

Extensive use of organophosphorus pesticides (OPs) have triggered alarm over environmental pollution and food safety, apart from contact toxicity. OPs are the most toxic substances known and are widely used for insect pest control [1]. Corresponding to this, release of pesticides into the atmosphere leads microbes to either detoxify the residues of these toxic compounds and their metabolites or use them as new sources of carbon and energy sources. Mineralization of these OPs is significantly more challenging since microorganisms need to use specific enzymes to turn such pesticides into certain intermediates in a key metabolic pathway [2]. However, given adequate exposure time, microbial cells may develop pathways to mineralize pesticides that have been implemented [3].

Chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate) is moderately toxic [4] among OPs commonly used for protecting agricultural crops from insects, fungi, weeds and other pests, which affect the economic growth of crops. It enters inside the insects through ingestion, by contact or absorbed through cuticles, gut [5] and affects the nervous system by inhibiting the acetylcholinesterase activity, ultimately causing death of the target insect [6]. Chlorpyrifos (CP) hydrolysis leads to the formation of TCP (3,5,6-trichloro-2-pyridinol), a key metabolite having considerable water solubility [7, 8]. TCP has a half-life between 65-360 days in soil [9], water solubility owing to its neutral pH [10], creates comprehensive soil and aquatic habitat contamination, posing important environmental ecotoxicological hazards relative to CP [11]. The enhanced resistance to microbial degradation of TCP is due to the existence of three chloride in its chemical structure [12, 13] and upon release of these chlorine atoms inhibits the growth of the CP degrading microbes in the neighboring environment [14].

Living species being exposed to these CP residues present in soil and water, affects ecosystem balance [15]. CP toxicity has been shown to be liable for human health, including nervous system disorders, immune system defects, liver damage and endocrine disruption [16, 17]. Hence, remediation of the CP contaminated sites to mitigate the effects of these toxic residues are highly important. Certain causes of pollution of this pesticide include industrial effluents, product waste, leakage, accidental spills and remediation. Therefore, techniques to degrade CP and TCP from contaminated fields need to be established in order to reduce the adverse effects on human health, non-target species, and the ecosystem.

Bioremediation is one of the most promising methods due to its low cost, simple to use along with eco-friendly for decontaminating harmful pesticides [18]. Microbial pesticide degradation was identified as a primary mechanism for soil and water dissipation. Microbes containing the appropriate metabolic pathways play a key role in CP and TCP degradation [19, 20]. In the past few years several bacteria have been reported for degrading CP or TCP. These include *Enterobacter* sp. [21], *Pseudomonas* sp. [22, 23], *Bacillus* sp. [24, 25], *Klebsiella* sp. [26], *Sphingomonas* sp. [27], *Paracoccus* sp. [28], *Cupriavidus* sp. [29], *Brucella* sp., *Klebsiella* sp., and *Serratia marcescens* [30], *Stenotrophomonas* sp. [31] and *Stenotrophomonas* sp. [32]. There are some instances where the bacterial species could lose its degradation ability under high pressure of TCP, thus making difficult for CP degradation in contaminated areas [33]. Recently, *Ochrobactrum* sp. JAS2 was reported showing the ability of CP & TCP degradation in spiked soil within 24 h & 94 h respectively along with plant growth promoting ability [34].

Only a few bacterial genera have been investigated for degrading CP and TCP. The main objective of this study was therefore to screen the active microbial strain(s) from paddy field

soil for CP and TCP degradation followed by genome analysis and the formation of new metabolite signatures by GC-MS. This analysis would deepen our understanding and would provide valuable source of information to expand its potential as a biodegrader of OPs pesticides.

2. Materials & methods

2.1 Soil Samples, cultivation media and chemicals

Soil samples were collected from a paddy field near Kalinga Institute of Social Science (KISS), Bhubaneswar Odisha, India [20.3551° N, 85.8187° E]. Preliminary screening of CP tolerant bacteria was performed using minimal salts media (MSM) [Per litre contains; NH_4Cl, 0.5g/L; $MgSO_4.7H_2O$, 0.3g/L; $KH_2PO_4.H_2O$, 0.2g/L; $FeSO_4.7H_2O$, 0.01g/L; $Ca(NO_3)_2.4H_2O$, 0.01g/L;] supplemented with CP (100 mg L^{-1}). Analytical grade CP (PESTANAL® 99.99%,) and TCP (PESTANAL® 99.99%) were procured from Sigma-Aldrich, USA. Another commercial grade CP (TRICEL 20% EC and HILBAN 50% EC) were also purchased from local market for soil enrichment purpose. Chemicals and other reagents used in this study were of analytical grade (HiMedia, India and Merck, Germany).

2.2 Screening & isolation of CP degrading bacteria

CP degrading bacteria was isolated from rhizospheric soil by culture enrichment technique. Briefly 100 g of soil treated with CP (100 mg l^{-1}) was inoculated into a 200 ml of MSM media. These soil samples were incubated and enriched for bacterial culture at 30±2 °C in a shaker incubator at 120 rpm for a week. Post incubation, 10 ml of grown culture was transferred for another week of incubation to new MSM amended with CP (200 mg L^{-1}). Likewise, three consecutive transfers in sterile MSM supplemented with CP (100 mg L^{-1}) was performed upon serially dilution and plated on solid media (MSM with CP @100 mg L^{-1}).

Post 96 h incubation, the pure colonies were obtained by repeated streaking. Isolate CPD-03 was further selected based on its CP degradation potential. Degradation efficiency was calculated [(Residual amount in blank control − residual amount in sample)/ (Residual amount in blank control) x 100]

2.3 Morphological, biochemical and taxonomic identification of the bacterial strain CPD-03

Isolate CPD-03 was preliminarily characterized by morphological parameters by scanning electron microscope (Nova^{TM} NanoSEM 450) and Gram staining (HiMedia, India) followed by biochemical assays using BIOLOG® [35]. Genomic DNA was extracted followed by a 16S rRNA sequencing [36] was performed. The 16S rRNA gene was obtained by PCR amplification with universal bacterial primers 27F and 1492R. PCR reaction was set at 95°C denaturation for 5 min, followed by 30 cycles of 95°C for 45s, 55°C for 45s, and 72°C for 90s, and a final step of 72°C for 5 min. The PCR amplified product was purified and sequenced by SciGenome, Ahmedabad, India. The obtained 16s rRNA gene sequence was aligned with the 16S rRNA sequences of type strains with homology of 97% or higher with CPD-03 and were obtained from EzTaxon (eztaxon-e.ezbiocloud.net). The sequence alignment was performed using ClustalX [37]. Phylogenetic tree was constructed with MEGA version 7.0 [38].

2.4 Inoculum preparation (Resting cell assay)

Pure cultures of bacterial strain CPD-03 were cryopreserved in 25% glycerol at -80^{0}C. The bacterial strain was revived and inoculated in a 100 ml Erlenmeyer flask with 20 ml sterile MSM supplemented with CP (100 mg L^{-1}) before each set of experiments. Flask was incubated at 30 ± 2 °C with 120 rpm in a shaker incubator. The bacterial cells were harvested

after 44- 48h at 4500 rpm for 5 min followed by washing with sterile saline (0.9% NaCl). The washed bacterial cells were resuspended in sterile MSM to obtain a cell density of 1-1.5x10^8 cells ml^{-1}. This bacterial resting cell suspension was used for further biodegradation experiments, organophosphorus hydrolase (*opd*) gene localization, chemotaxis assays and plant growth promoting response (PGPR) activities.

2.5 Biodegradation of CP & TCP in aqueous medium

Biodegradation of CP (100 mg L^{-1}) & TCP (100 mg L^{-1}) was studied (in separate and in mixed) in 50 ml resting cell suspension (Supplementary Fig 1a&b). An additional carbon source molasses (0.1% v/v) [39] was added to enhance the growth. MSM with the same concentration of CP and 0.1% molasses was treated as control. All the experimental flasks were kept for incubation at 30±2°C and 120 rpm. Degradation under various concentration of CP (100 mg L^{-1} to 1000 mg L^{-1}) was studied in 50 ml resting cell as mentioned above were monitored till 12 h in an interval of 2 h and later till 48h in an interval of 6 h for determining the rate of CP degradation using high performance liquid chromatography (HPLC) (Agilent Technology 1260 Infinity, USA).

2.6 Sample extraction and HPLC analysis

The resting cell along with CP (100 mg L^{-1} along with molasses (0.1% v/v) from biodegradation assay, was harvested at 7500 rpm for 10 min for obtaining a cell-free media and extracted with organic solvents. The supernatant was pulled out for extraction with an equal volume of ethyl acetate and this step was repeated twice. The organic layer thus obtained was aspirated, pooled and dehydrated over a column of anhydrous sodium sulfate, evaporated at 45±2°C using rotavapor (Eyela, Japan), reconstituted in methanol (HPLC Grade) and filtered through 0.22 μm membrane filter. Residual CP and TCP was quantified

using a reverse-phase C18 column (ZORBAX Eclipse Plus, Agilent Technologies, USA) fitted with Agilent Technologies 1260 Infinity HPLC system equipped with a binary pump, UV/VIS detector, thermostat column compartment (TCC) column oven and auto sampler, with array detection based on peak area and retention time of the pure standard. The mobile phase used for the analysis contained a mixture of methanol and water in a ratio of 85:15 at a flow rate of 1.2 mL min^{-1}. Water (HPLC grade) was acidified to pH 3.2 using orthophosphoric acid. Injection volume was 20 µL. The recoveries of OPs added at concentrations of 1, 2, 5, 10, 50 mg/L, ranged from 97.0% to 100.0%. The linear range of the calibration curves was obtained from 1 to 50 mg/L.

2.7 Degradation kinetics

CP degradation rates has been fitted with a pseudo first order kinetic model $Y = (Y_0 - \text{Plateau}) * e^{(-K*X)} + \text{Plateau}$; Y_0 = the Y value when X (time) is zero and is expressed in the same units as Y, Plateau = the Y value at infinite times and expressed in the same units as Y. K = rate constant and expressed in reciprocal of the X axis time units. Half-life is in the time units of the X axis. It is computed as ln(2)/K using statistical software Prism8.

2.8 Identification of metabolites

The resting cell containing CP (100 mg L^{-1}) along with molasses (0.1% v/v) from biodegradation assay, was also assessed for the identification of CP degradation and simultaneous metabolites formation. The extraction was performed as mentioned above in section 2.6. Samples were analysed in gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies, USA) fitted with a HP-5MS capillary column (30.0 m x 250 µm x 0.25 µm) in mass spectrometer (Agilent 7890B ION TRAP MS) equipped with a manual injector containing split/split less capillary injection system along with an array detection of

total scan from 30-500 nm. The column temperature was kept for 5 min at 80^0C initially and increased for 5 min at 8^0C min-1 to 200^0C, then increased for 5 min at 15^0C min^{-1} to 260^0C. The ionization energy was kept at 70 eV, and the temperatures of the transfer line and the ion trap were kept 280^0C and 230^0C, respectively. The amount of sample injection was 1.0 μL at 250^0C with 1:50 split mode. Helium was used as a carrier gas at a flow rate of 1.5 ml min^{-1}. The CP and intermediates found by the GC-MS study matched authentic standard compounds from the National Standards and Technology Institute (NIST, USA) database (http://chemdata.nist.gov).

2.9 Molecular identification & localization of organophosphorus hydrolase (*opd*) gene

For identification of organophosphorus hydrolase (*opd*) gene, the resting cell (1-1.5x10^8 cells ml^{-1}) was used for genomic DNA isolation as mentioned section 2.3. The *opd* gene of strain CPD-03 was amplified by PCR with forward primer (OPDF, 5′-GCGGATCCATGCAAACGAGAAGGGTTGTGC-3′, and reverse primer OPDR, 5′-GCCTCGAGTCATGACGCCCGCAAGGTC-3′; *BamHI* and *XhoI* restriction sites, respectively, are underlined). The reagents used for PCR were obtained from Promega (Madison, WI) and a total volume of 25 μl reaction mixture for PCR amplification was carried out. Each reaction mixture consists of 2× green reaction mixture (10 mM $MgCl_2$, 10 μM each dNTPs, 1 U of Taq DNA polymerase along with 10x buffer), 10 pmol of each primer along with the template DNA and nuclease free water for volume makeup. The PCR amplification program was as follows: initial denaturation at 94°C for 5 min, 32 cycles of cyclic denaturation at 94°C for 60s followed by annealing at 55.1°C for 45s and extension at 72°C for 60s including a final extension at 72°C for 5 min.

To localize the presence of *opd* gene, CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}) were centrifuged and processed as per Deng et al., 2015 with a slight modification followed by

estimation with enzymatic assays [40]. Briefly, CPD-03 resting cells were harvested at 8500×g for 15 in 4^0C. Cell-free extract was obtained by passing the supernatant through a 0.22µm membrane filter and hence treated as extracellular (EC) and stored at 4^0C. The cell pellet was washed twice with normal saline in the Tris-Cl buffer solution (50 mmol / L, pH 8.0) and re-suspended to obtain cell suspension (CS). One half of the CS was stored at 4^0C, and the other half was re-suspended in Tris-Cl buffer and sonicated (4^0C, 400W) for 8min. The collected suspension was then centrifuged at 8000 ×g for 10 min in 4^0C. The resulting supernatant was filtered through a 0.22µm to obtain crude intracellular (IC) enzyme. *Opd* activity was calculated as the number of moles of *p*-nitrophenol produced in unit time.

2.10 Assessment of plant growth promoting activity

Evaluation of the effect of CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) on plant growth was done by quantitative measurement of root length in inoculated rice seedlings followed by indole acetic acid (IAA) production [41], ammonia production [42], and HCN production [43, 44]. The data were subjected to statistical analysis using Prism8.

2.11 Chemotaxis towards CP

The chemotactic property of CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) towards CP along with other OPs was examined qualitatively in swarm plate assays and quantitatively with capillary tube assay as per the procedure described earlier [45, 46]. Citrate (0.5%) was used as the positive control [47]. The chemotactic response was expressed as chemotaxis index (CI), and calculated as the ratio of the number of CFUs obtained from the capillary tube containing the test compound (CP) to CFUs obtained from a control capillary that contains only the chemotaxis buffer (100 mM potassium phosphate pH 7.0 and 20 µM EDTA; without any chemotactic compound).

2.12 *De novo* genome sequencing and assembly

Illumina HiSeq System 2000 (Illumina, Inc.) was used for whole-genome sequencing. Libraries were generated in a paired end manner. Data obtained from sequencing were *de novo* assembled followed by entire quality control performed using NGSQC Toolkit. Velvet (V 1.2.10) [48] was used for primary assembly that resulted a total size of the contig of 4,65,9698 (~4.6Mb). *De novo* Genome Validation and Quality Control was performed by Bowtie2 (v 2.2.2) [49]. Quality control of assembled genome based on genomic elements were performed by ARAGORN (v1.2.36) [50]. Genome Annotation and functional characterization were performed using RAST. All the bio-informatics work was performed in collaboration with Bionivid Technology Pvt. Ltd., Bangalore, India. Genome sequence including the necessary information for the CPD-03 strain was deposited in NCBI under the accession number RSEU00000000. Considering the ability of this strain for degrading CP, the whole genome of this strain was checked for the occurrence of the CP degrading core genes/proteins. The CPD-03 genome was also compared with other eight more *Ochrobactrum* strains obtained from the NCBI database.

3. Results & discussion

3.1 Isolation and identification of CP degrading bacterial strain CPD-03

Several bacterial strains were isolated from rhizosphere soil of paddy fields near KIIT University area which could utilize CP as carbon source for their growth. CPD-03 was distinguished from other isolates for its ideal growth response during isolation. Further CPD-03 ($1.2x10^8$ cfu/ml) showed a CP degradation efficiency of 82 ±2% in MSM supplemented with CP (100 mg L^{-1}) (Supplementary Fig.1a) and hence selected for further study. CPD-03

was able to degrade TCP (100 mg L^{-1}) separately with a degradation efficiency of 60 ±2% (Supplementary Fig.1b).

CPD-03 is Gram-negative and showed regular rod shaped cells which was observed in scanning electron microscopy (Fig. 1a). A detailed comparison of the physiological and biochemical properties of CPD-03 with its closest phylogenetic species of *Ochrobactrum* was performed (Supplementary Table 1a-c). A phylogenetic tree including strain CPD-03 and related type strains within the clade of *Ochrobactrum* (obtained from EzBioCloud) was also compared (Fig. 1b). 16S rRNA gene sequence analysis illustrated that, CPD-03 shared maximum homology of 99.8% with *Ochrobactrum intermedium* LMG 3301^T followed by 99.2% with *Ochrobactrum ciceri* Ca-34^T of the genus *Ochrobactrum* [51], hence designated as *Ochrobactrum* sp. CPD-03. The 1396 bp 16S rRNA nucleotide sequence of CPD-03 strain was submitted in GenBank under Accession No. KT366923.

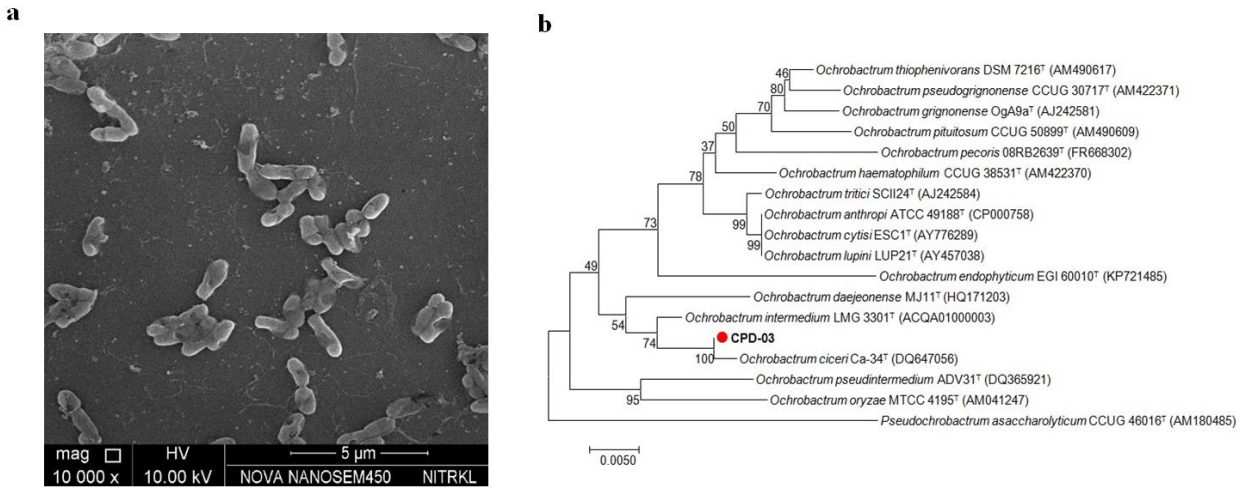

Fig.1. a. Scanning electron microscopy of CPD-03; b. phylogenetic tree of CPD-03 based on comparison of 16S rRNA sequence similarity of closely related *Ochrobactrum* sp. conducted in MEGA version 7.0

3.2 Biodegradation of CP and TCP

Degradation of CP (100 mg L^{-1}) and TCP (100 mg L^{-1}) when added together were assessed using CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in MSM along with molasses (0.1% v/v). This resting cell approach showed CP and TCP degradation efficiency of 88±2% and 62±2%, respectively in 48 h (Fig. 2a). TCP degradation was monitored separately using CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in MSM along with molasses (0.1% v/v) with a degradation efficiency of 62.2±2% (Fig. 2b). Earlier reports have shown TCP can be degraded by *Alcaligenes* sp. [52], *Burkholderia* sp. [53], *Pseudomonas* sp. [54], *Pseudomonas* sp., *Bacillus* sp. and *Agrobacterium* sp. [55], *Trichosporon* sp. [56], and *Pseudomonas* sp. [57] when used as sole carbon source. Previous report has shown *Ochrobactrum* sp. JAS2 had degraded not only CP and TCP in aqueous medium within 96 h when used together. The present study showed, CPD-03 could degrade CP at various concentrations (100 mg L^{-1} to 1000 mg L^{-1}) and it was clearly evident that higher concentration reduced the CP degradation efficiency (Fig. 2c). This correlates with a previous report where higher concentration of pesticides has inhibited the bacterial growth hence decreasing the degradation rate.

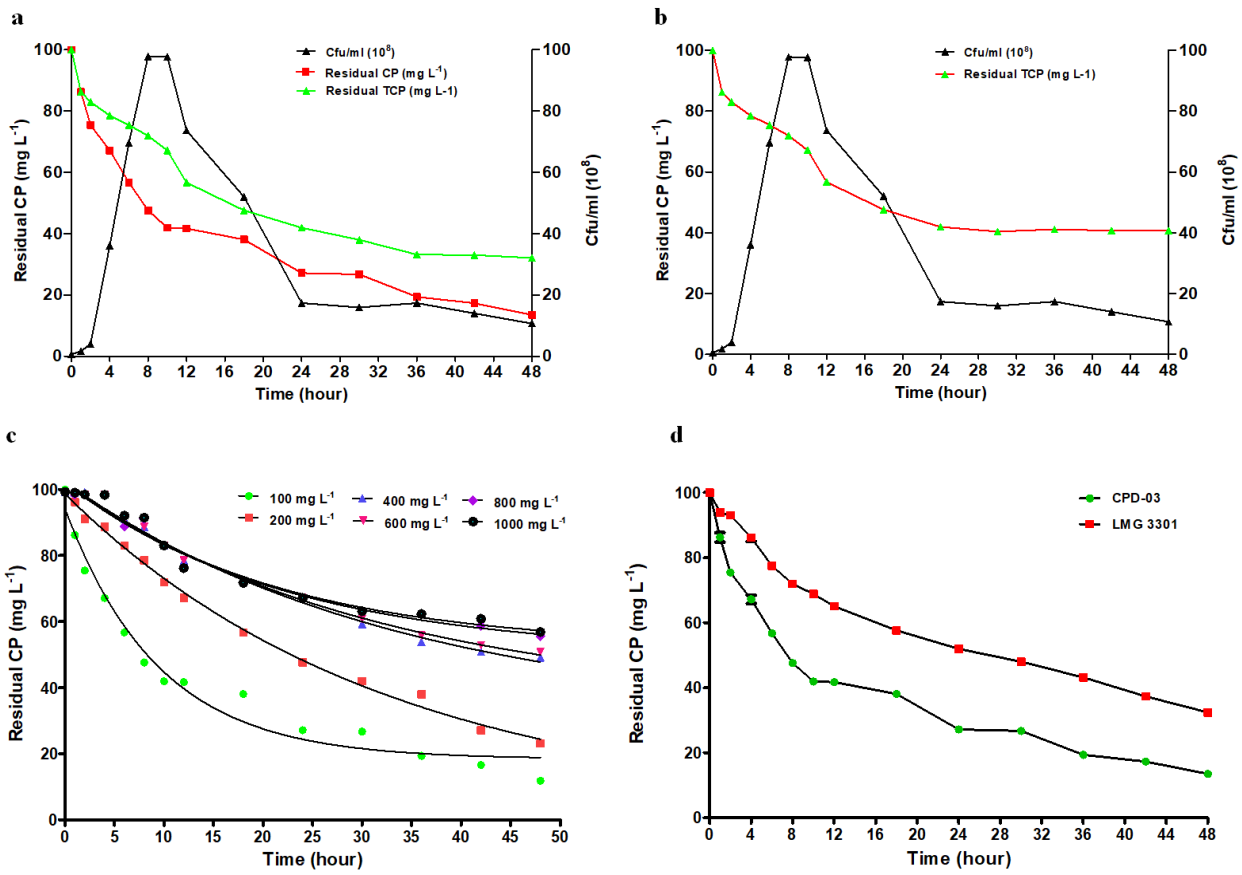

Fig.2. a. Degradation of CP (100 mg L^{-1}) and TCP (100 mg L^{-1}) by CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in a MSM supplemented molasses (0.1% v/v) showing formation of its intermediate TCP and its further degradation; b. degradation of TCP (100 mg L^{-1}) by CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in a MSM supplemented molasses (0.1% v/v); c. degradation of CP at various concentrations (100 mg L^{-1}, 200 mg L^{-1},400 mg L^{-1},600 mg L^{-1},800 mg L^{-1}, and 1000 mg L^{-1}) by CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in MSM supplemented molasses (0.1% v/v); d. comparision of CP (100 mg L^{-1}) degradation by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}) and LMG 3301^T (1.2-1.4x10^8 cells ml^{-1}). Data were represented in the mean of three replicates and analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

CP degradation using CPD-03 resting cell in MSM supplemented with molasses (0.1%) revealed very good compliance with pseudo first order kinetics. Various pesticide

degradation studies were previously reported following a pseudo first order kinetics [58, 59]. A recent study had reported the CP degradation by *Ochrobactrum* sp. JAS2 also followed a pseudo first order kinetics [34]. CPD-03 degraded 100 mg L^{-1} CP with a half-life of 6.559 h and TCP 100 mg L^{-1} with a half-life of 12.08 h. CPD-03 was compared with its phylogenetically closest neighbor *Ochrobactrum intermedium* LMG 3301^T for its ability to degrade CP (100 mg L^{-1}) and was found to show higher degradation efficiency of 88.34% as compared to LMG 3301^T with a degradation efficiency of 60.34% (Fig. 2d).

3.3 Identification of metabolites

Degradation of CP by *Ochrobactrum* sp. CPD-03 was monitored in GC-MS, has resulted in some new metabolites (Fig. 3). Previous studies have shown the formation of TCP upon CP mineralization and its immediate degradation upon subsequent hydrolysis steps through ring cleavage [15]. However, in our study, few new downstream metabolites like (a) cis-Vaccenic acid, (b) Octadecanoic acid, (c) Benzoic acid, 4-hydroxyl 3-methoxy, (d) n-Hexadecanoic acid, (e) 9-Hexadecanoic acid were obtained after CP mineralization which could add new insights into the metabolic pathways. cis-Vaccenic acid is a byproduct from the activity of 3-oxoacyl-[acyl-carrier protein] reductase which was present in CPD-03 genome. The presence of this enzyme has previously been shown in case of poly aromatic hydrocarbon (PAH) degradation [60]. Similarly, Octadecanoic acid was found to be a metabolite during biodegradation of endocrine-disrupting chemicals in earlier reports [61], Benzoic acid, 4-hydroxyl 3-methoxy was reported in beta-cypermethrin degradation [62], n-Hexadecanoic acid production and degradation due to microbial activity has been studied [63] and 9-Hexadecanoic acid was reported to produce during bioremediation of *n*-alkanes [64]. This could possibly draw new details into the metabolic pathway in terms of pesticide degradation.

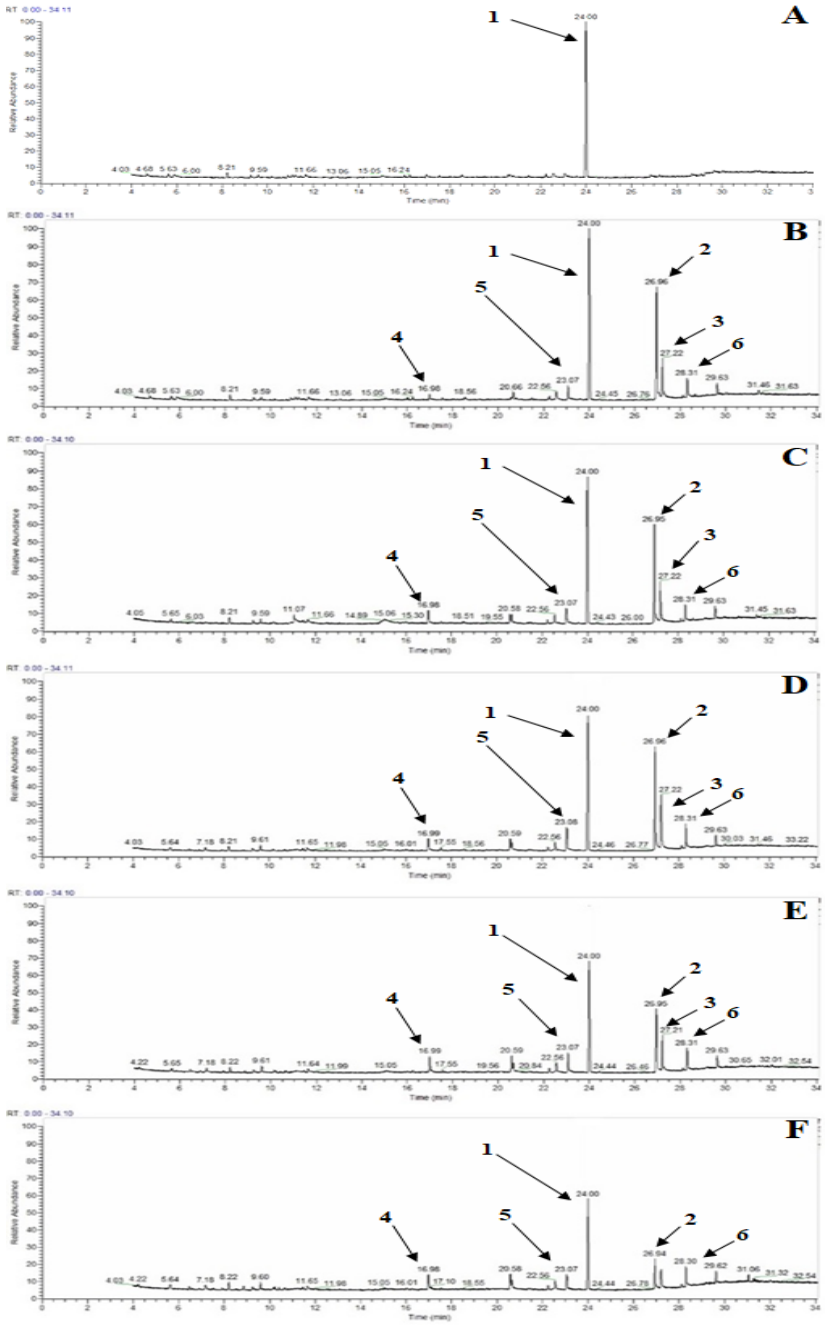

Fig.3 GC-MS chromatograms showing the metabolites formed during CP (100 mg L^{-1}) degradation by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}). A: 0 h, B: 2 h, C: 4 h, D: 6 h, E: 8 h, and F: 10 h respectively. 1: chlorpyrifos, 2: cis-Vaccenic acid, 3: Octadecanoic acid, 4: Benzoic acid, 4-hydroxyl 3-methoxy, 5: n-Hexadecanoic acid, 6: 9-Hexadecanoic acid, respectively.

3.4 Identification and localization of *opd* gene in CPD-03

CPD-03 was found to contain the gene encoding organophosphorus hydrolase (*opd*, ~1098 bp), which was successfully amplified using the primers OPDF and OPDR (Fig. 4a). This genes could possibly transform the complex OPs to generate *p*-nitrophenol have been previously reported [65]. Since CPD-03 is a Gram-negative bacteria, organophosphorus hydrolases potentially are secreted into the extracellular space from periplasmic region, hence can be extracted in more quantities [66]. A maximum amount of *p*-nitrophenol (2±0.2 mmol/L) produced by treatment of the CPD-03 resting cells (extracellular cell free content, EC) with CP for 30 min (Fig. 4B) as compared to the intracellular and cell suspension. This result is consistent with the earlier report of *Flavobacterium* sp. MTCC 2495 [67].

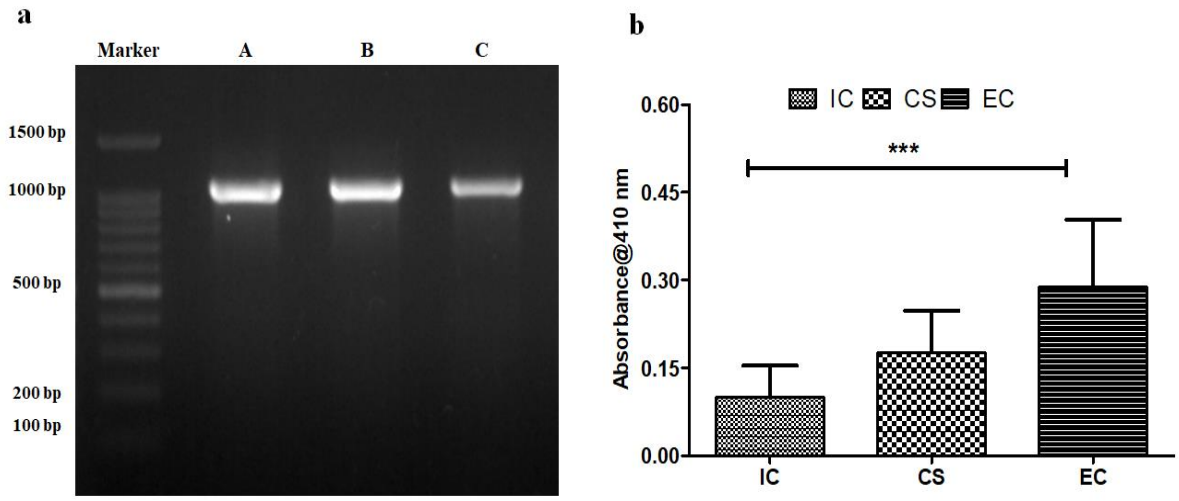

Fig.4. a. Amplification of ~1098 bp *opd* gene; b. enzyme activity of CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}); where IC: intracellular, CS: cell suspension and EC: Extracellular. Data analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

3.5 CPD-03 genome analysis and presence of arylesterases gene

The draft genome sequence of *Ochrobactrum* CPD-03 has been submitted in GenBank under the accession RSEU00000000. The version described in this paper is version RSEU01000000 and comprises 4,660,061bp (~4.6 Mb), 31 contigs and 25 scaffolds (N50: 403.18 kb) (Supplementary Table 2). A comparative genomics approached was analyzed using KAAS (KEGG Automatic Annotation Server) [68] for functional annotation of proteins and metabolic pathways present in CPD-03. The minimal sets of metabolic pathways were further annotated and reconstructed against the predicted protein families using a web-based tool MinPath [69] and were clustered using ClustVis [70]. In addition, comparative genome analysis of other *Ochrobactrum* genomes were assessed for the capability to degrade the aromatic and relative compounds (Supplementary Fig. 2). This feature could also demonstrate the potential genes of CPD-03 strain for possible application in case of aromatic compound degradation.

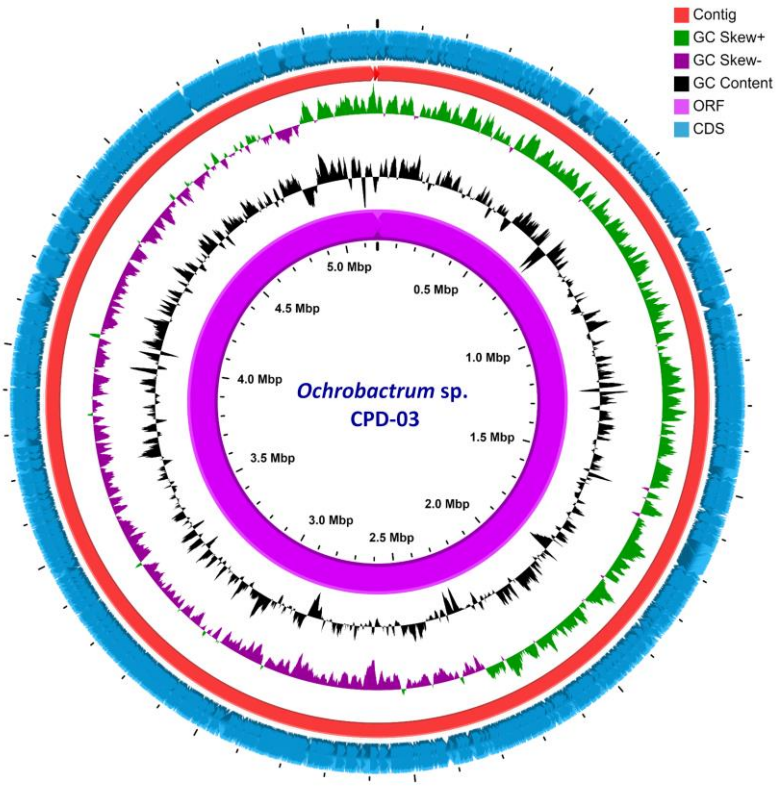

Fig. 5 Genome map of *Ochrobactrum* sp. CPD-03

Although microorganisms have been known to possess numerous enzymes with capability to degrade several xenobiotic compounds, only ten organophosphorus hydrolase genes were reported to degrade CP. Organophosphorus hydrolase (OPH; EC 3.1.8.1) is an ortholog of arylesterase (EC 3.1.1.2), are known to catalyze the hydrolysis of a wide range of OPs and are produced by few microbes. OPH uses OPs as substrates rather than inhibitors or antigens. Due to its bioremediation potential, OPH has been used in industrial, health care and military purposes [71]. As of now, many arylesterases were isolated from mammals, known as serum paraoxonases responsible for hydrolyzing paraoxon along with other organophosphorous esters [72]. Previous studies have reported arylesterases from insect pests displaying resistance against oxo-analogs of organophosphorous insecticides such as parathion and chlorpyrifos [73].

Comparative genomics of CPD-03 genome with other *Ochrobactrum* genomes showing presence of arylesterase was performed. Top 25 hits for Arylesterase sequences were obtained from UniProt (https://www.uniprot.org/) and analyzed against the same present in CPD-03 strain. A phylogenetic tree based on arylesterase sequences was constructed to validate the closeness of this enzyme among top hits from the database. The highest sequence homology was with *Ochrobactrum intermedium* LMG 3301^{T} which was also phylogenetically closest (Supplementary Fig. 3). Comparison of *opd* sequence obtained from whole genome with the amplified *opd* gene sequence revealed that these two shared similarity in genetic and protein level (Supplementary Fig. 4). The position of arylesterase was annotated on genome of CPD-03 using a rapid prokaryotic genome annotation tool, Prokka [74] (Supplementary Fig. 5). CPD-03 shared numerous putative pathways for aerobic degradation of aromatic compounds (Supplementary Table 3). As of now, only two reports are available on TCP biodegradation genes [75]. Based on the previous reports along with the

CPD-03 genome, a metabolic pathway has been proposed (Supplementary Fig. 6) which indicated the presence of gene clusters responsible for CP degradation by CPD-03. The characteristic feature of the strain CPD-03 as revealed by in silico analysis, showed its ability to degrade benzoate, aminobenzoate, chloroalkane and chloroalkene, chlorocyclohexane and chlorobenzene, suggesting that it might have acquired genes and pathways for mineralization of these xenobiotic compounds from the soil ecosystem.

3.6 Chemotactic response of strain CPD-03 towards various OPs

Bacterial chemotactic movement under a chemoattractant helps bacterium find metabolizable substrates for growth and survival. The chemoattractant is often a compound that also acts as a source of carbon and energy [76]. Chemotaxis has recently become a widespread phenomenon of bacterial metabolism against various xenobiotic compounds, and the use of these bacteria in bioremediation may therefore be beneficial [77].

Qualitative and quantitative assays for chemotaxis were performed with resting cells of CPD-03 with CP along with various other OPs like monocrotophos, methyl parathion, diazinon, malathion, and coumaphos separately. The strongest chemotactic response was observed for CP as compared to other OPs (Fig. 6a). Moreover, various concentration of CP also had the similar effect of chemotaxis (Fig. 6b, Supplementary Fig. 7). Additionally, putative gene clusters were also found in the genome of *Ochrobactrum* CPD-03, validating the chemotactic effects towards chemoattractant (Supplementary Table 3). To the best of our knowledge, our results suggested the first report of the genus *Ochrobactrum* for chemotaxis towards CP along with other OPs.

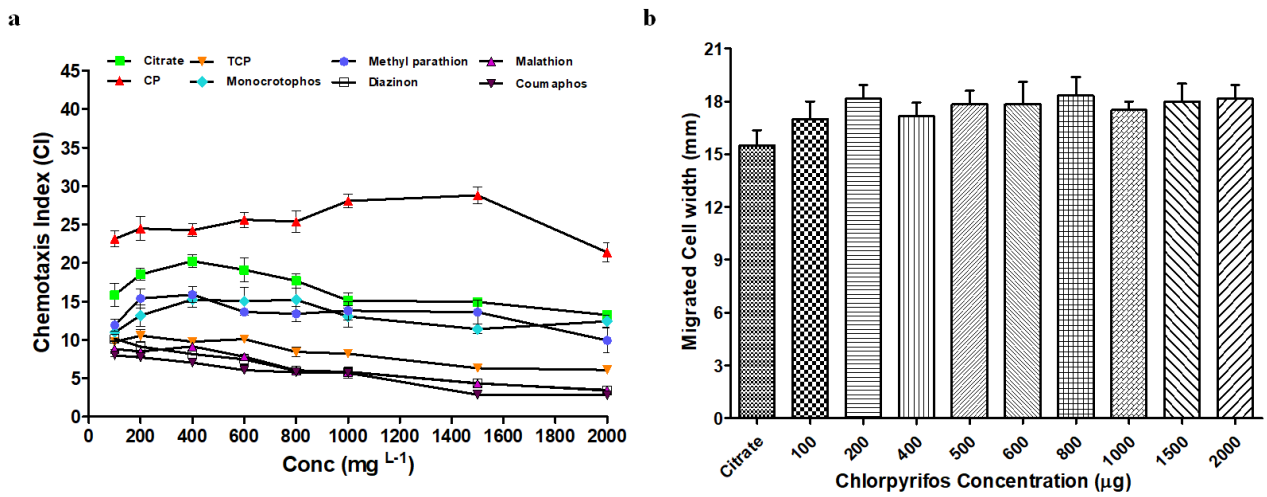

Fig.6 a. Chemotaxis index of CPD-03 resting cells CPD-03 (1-1.5x10^8 cells ml^{-1}) towards CP, TCP, monocrotophos, methyl parathion, coumaphos, diazione and malathione as competitive attractant at various concentrations (from 200 mg L^{-1} to 2000 mg L^{-1}). Values are presented as arithmetic means and error bars indicate standard deviations based on three independent replicates. b. migrated cell width of chemotactic movement by CPD-03 towards various concentration of CP (100 mg L^{-1}, 200 mg L^{-1}, 400 mg L^{-1}, 600 mg L^{-1}, 800 mg L^{-1}, 1000 mg L^{-1}, 1500 mg L^{-1} and 2000 mg L^{-1}) along with citrate (0.5%) as positive control. Data analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

3.7 Assessment of Plant growth promoting ability of CPD-03

Rhizospheric bacteria live on and around the paddy fields have been contributing towards degradation of toxic xenobiotic compounds in contaminated ecosystem and shown to have potential for accelerating degradation process [78]. Paddy field environment comprised of phosphorus in soils (in both organic and inorganic forms) [79]. As a result, microbes associated with phosphate solubilizing ability would give an insight into the available forms of phosphate to the plants. In our present study, CPD-03 was found to solubilize the tricalcium phosphate post incubation, which was a qualitative analysis i.e. the agar media of

the pikovskaya containing bromophenol turned from blue to yellow due to a decrease in the pH of the medium confirming a positive feature of phosphate solubilization (Fig. 7a). In plants, production of ammonia is accountable for the indirect plant growth promotion via pathogens control and d HCN is a key compound during this metabolism [80]. CPD-03 showed a substantial increase in ammonia production as compared to the control (*E.coli*) (Fig. 7b). CPD-03 had shown to produce HCN (Fig. 7c). It is reported that, microbes isolated from various agricultural sites have the ability to produce phytohormone auxin indole-3-acetic acid (IAA) as secondary metabolites [81]. Generally, IAA secreted by microbes stimulate seed germination followed by increase the rate of xylem and root development, controls vegetative growth process, regulates photosynthesis, formation of pigments, production of several metabolites, and resistance to stress [82]. In our study, CPD-03 produced 40.33 mg L^{-1} IAA as compared to the control (*E.coli*) which had produced 22.36 mg L^{-1} of IAA, respectively (Fig. 7d). CPD-03 inoculation was also positively affected the increased root length (Fig. 7e). This result is consistent with the previous report on *Ochrobactrum* sp. JAS2. Genome analysis had shown putative gene clusters present in *Ochrobactrum* CPD-03, validating the potential effects of PGPR activity. However, the gene clusters for HCN production, phosphate solublization, ACC deaminase activity, and ammonia production were not found. The genome shows the presence of several hypothetical proteins which might be contributing for these above mechanisms or presence of ortholog sequences (Supplementary Table 4) which has still not been studied but may be of interest in future.

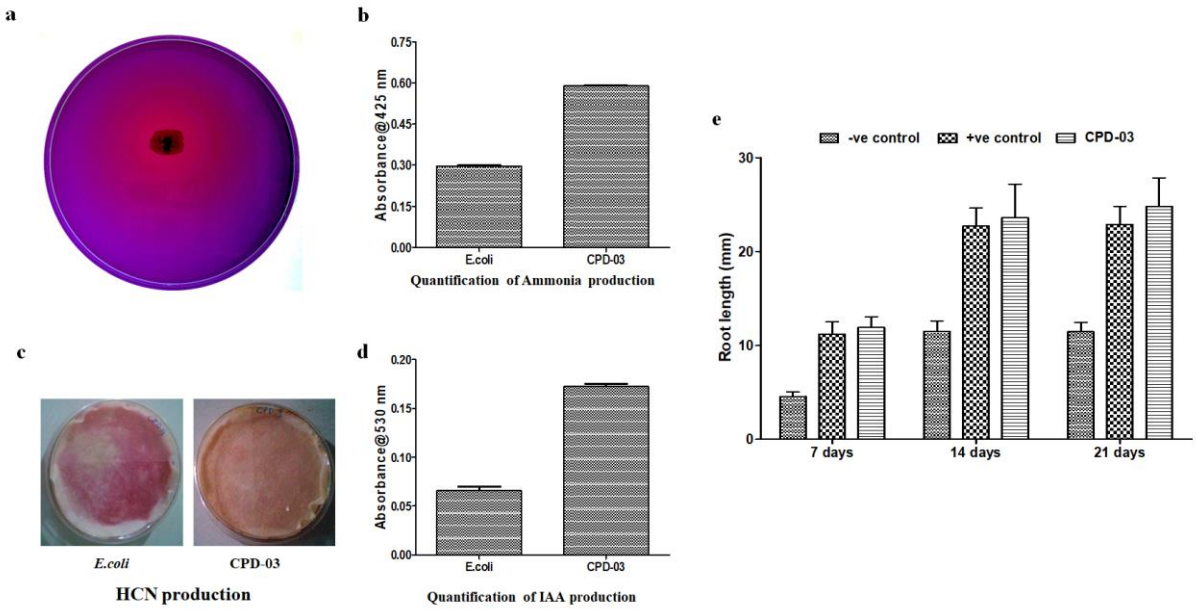

Fig.7(a-e). a. Phosphate solubilization on Pikovskaya's agar supplemented with tricalciumphosphate by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}); b. quantification of ammonia production; c. HCN production and d. quantification of IAA production by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}); e. Effect of root length upon CPD-03 inoculation on rice seed; -ve control: culture free, +ve control: *Bacillus* sp., lab stock for positive PGPR activity. *E.coli* was used as a negative control. Data analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

4. Conclusion

A new CP degrading bacterium of *Ochrobactrum* genus was isolated and characterized as *Ochrobactrum* sp. CPD-03 for its ability to degrade CP & its major metabolite TCP with chemotactic ability towards other OPs and additional plant growth promoting abilities. Our study highlights as the second report on a bacterial strain from the genus *Ochrobactrum* possessing the ability to degradation of both CP and TCP. Whole genome assessment revealed the presence of gene clusters responsible for CP biodegradation by CPD-03. In addition to this, several other genes were found responsible for degradation of aromatic and

relevant compounds suggested a versatile role for CPD-03 in the degradation of xenobiotic compounds. Based on phylogenetic and genomic analyses, CPD-03 was identified as belonging to the genus *Ochrobatrum*. Thus, the genome sequence of CPD-03 is a valuable source of information to understand the potential of this paddy field isolate to study its importance in hydrolyzing the OP compounds. This study provides the genetic information for its application for the degradation of OP pollutants in natural environment for further development of a biostimulation process. CP degrading ability makes this strain CPD-03 useful for decontamination of the pesticide polluted sites. Moreover, the plant growth promoting abilities could be of great potential for improved and persistent crop production during CP induced stressed agricultural ecosystem.

Authors' contributions

TN & VR designed the research and outline. TN performed the experiments and generated the result. BD performed microscopic analysis. TN & ANP performed genome analysis. TN & ANP performed comparative genomics. TN wrote the manuscript. TKA & VR critically reviewed the manuscript with appropriate technical inputs. Authors would like to thank Dr. Rasu Jaybalan, Department of Life Science, National Institute of Technology Rourkela for his assistance in microscopic work. All authors have read and approved the final manuscript.

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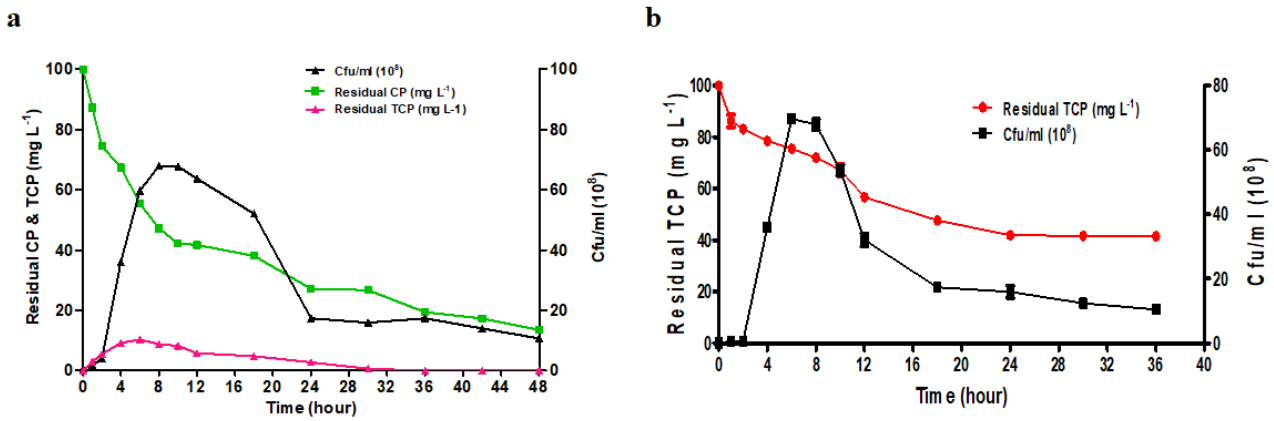
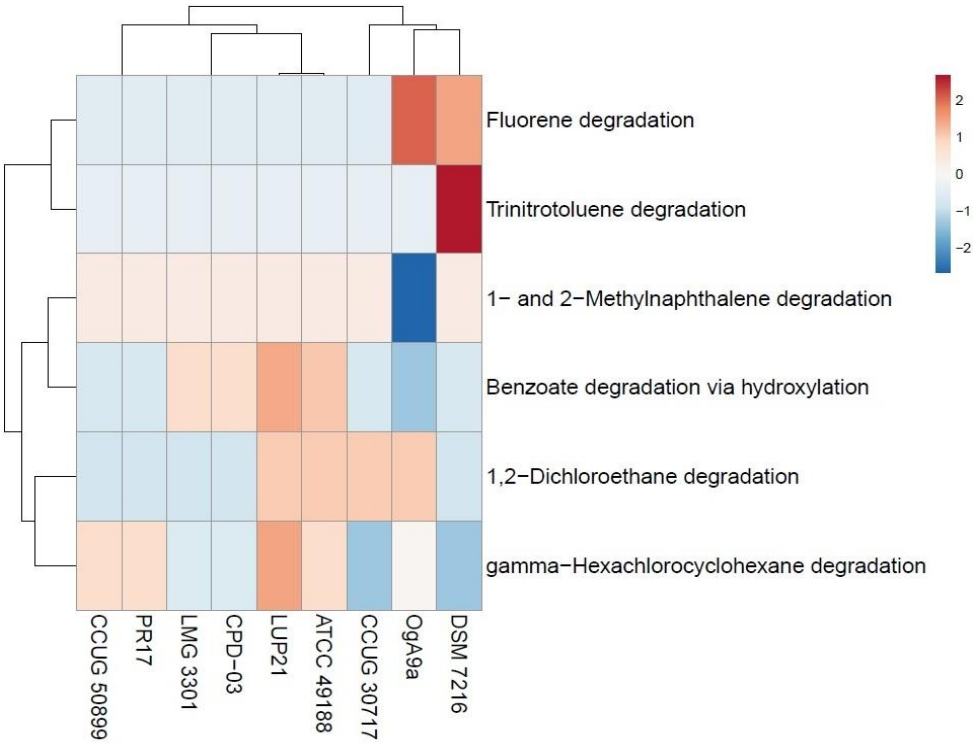
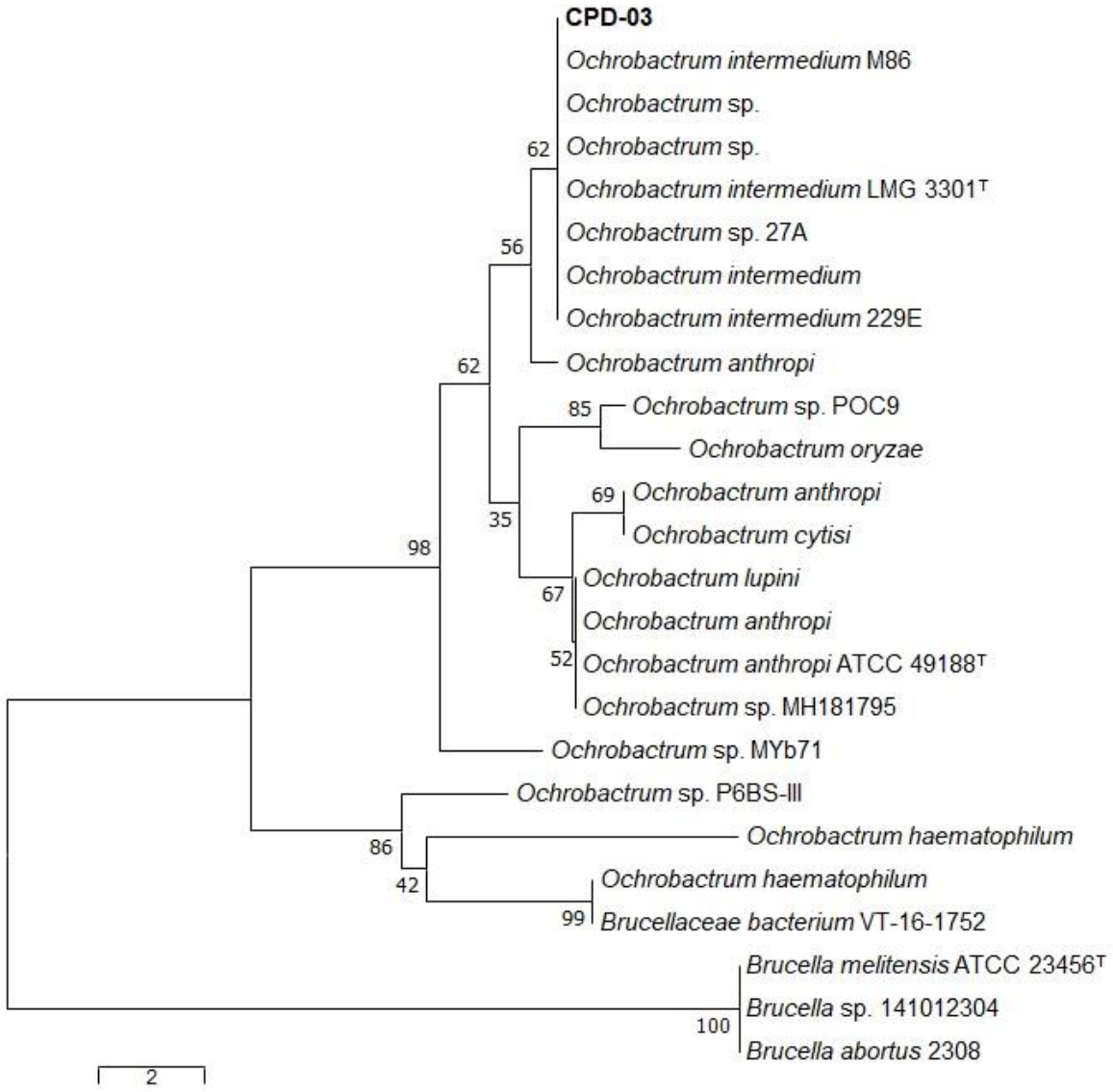


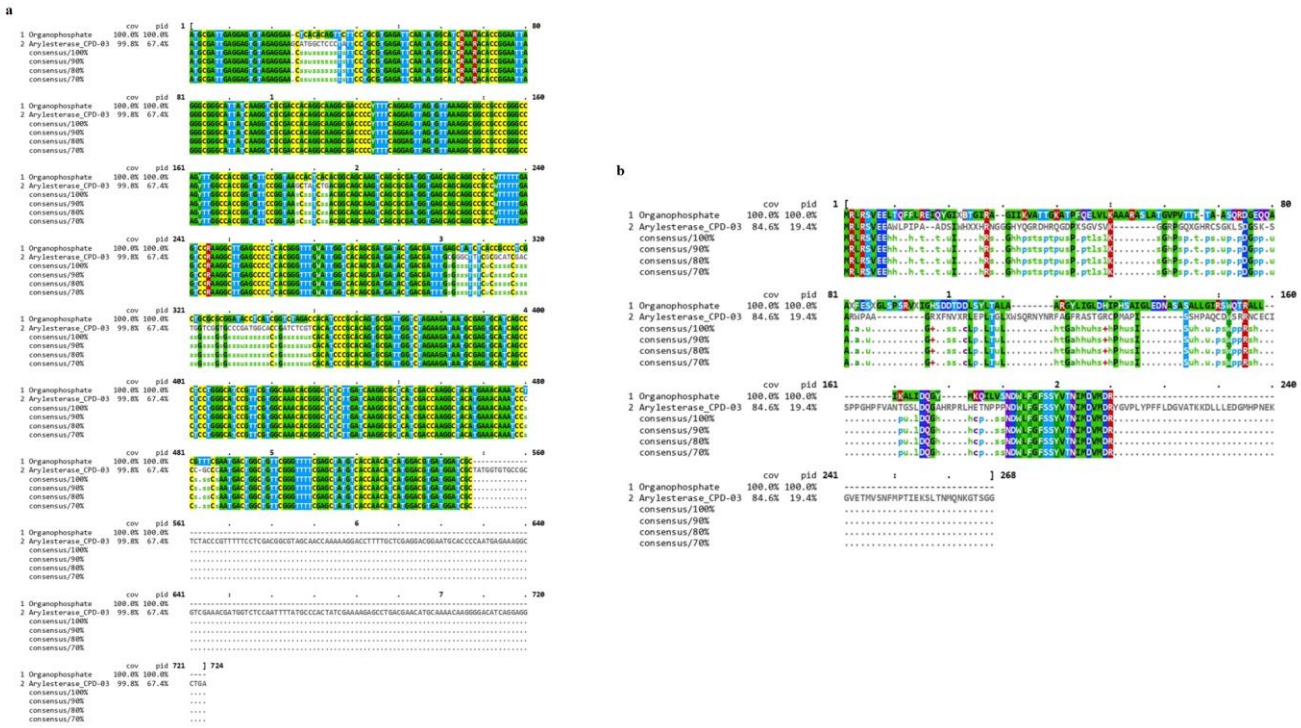
Supplementary Fig.1 a. Degradation of CP (100 mg L^{-1}) by CPD-03 ($1.2x10^8$ cfu/ml) leads to formation of TCP followed by its further degradation; b. degradation of TCP (100 mg L^{-1}) by CPD-03 ($1.2x10^8$ cfu/ml). Data were represented in the mean of three replicates and analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.0001$*** significance level



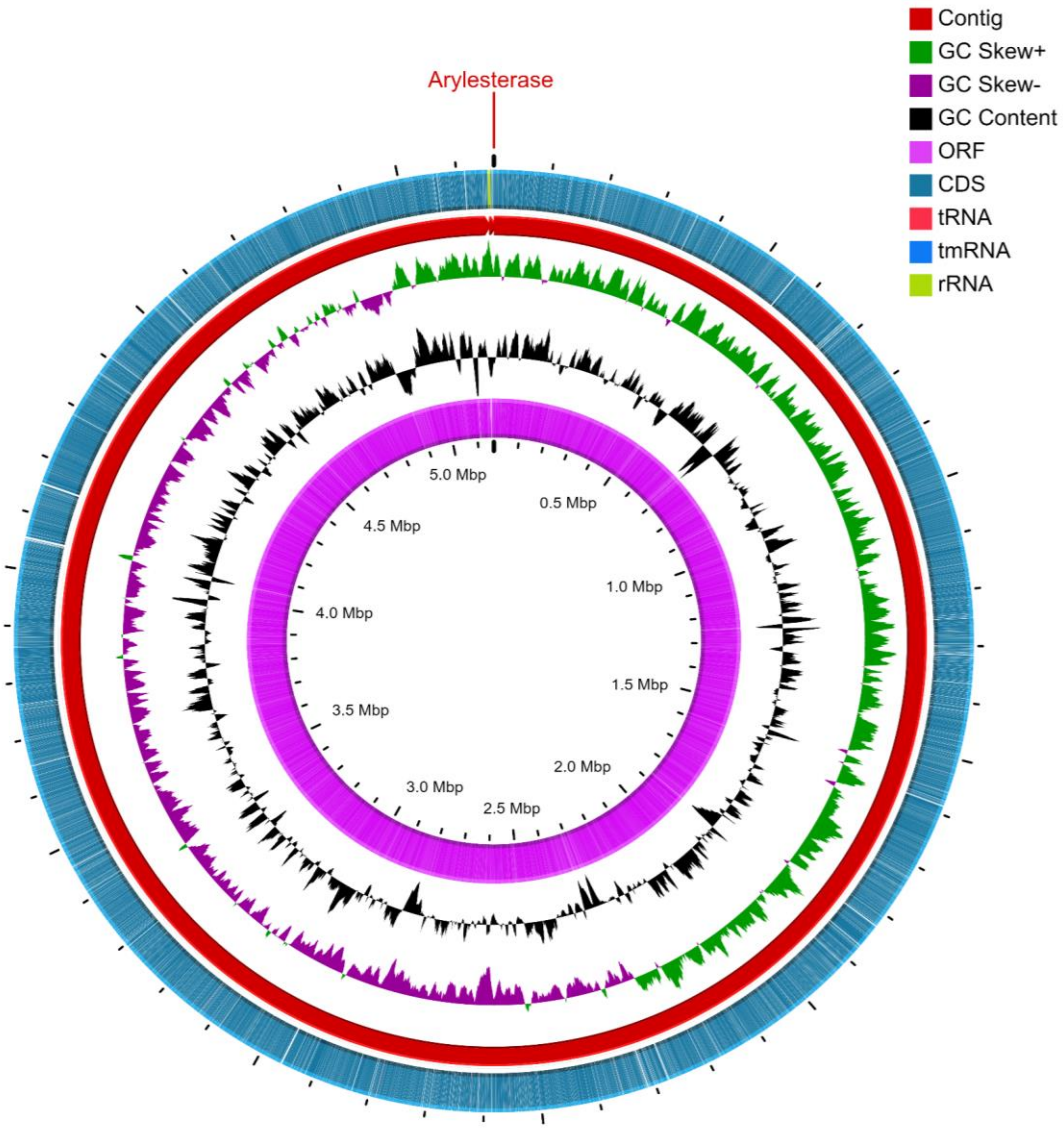
Supplementary Fig. 2. Heatmap and dendrogram of potential abilities of degradation of Aromatic and relative compounds predicted by genomic features of CPD-03 along with other *Ochrobactrum* strains (*Ochrobactrum thiophenivorans* DSM 7216^{T}, *Ochrobactrum pseudogrignonense* CCUG 30717^{T}, *Ochrobactrum pituitosum* CCUG 50899^{T}, *Ochrobactrum grignonense* OgA9a^{T}, *Ochrobactrum rhizosphaerae* PR17^{T}, *Ochrobactrum anthropi* ATCC 49188^{T}, *Ochrobactrum lupini* LUP21^{T}, *Ochrobactrum intermedium* LMG 3301^{T}). Information retrieved from MinPath Annotation Tool.



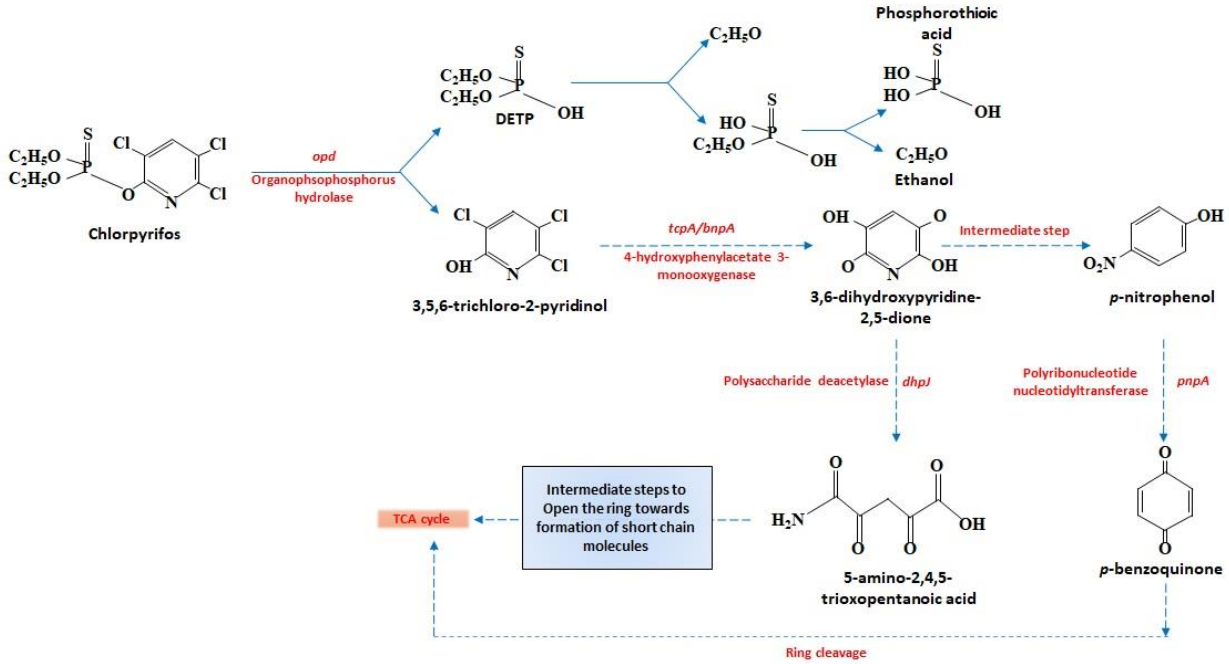
Supplementary Fig. 3: Phylogenetic tree with respect to the Arylesterase enzyme present in *Ochrobactrum* strains. These sequences were obtained from UniProt followed by the Top 25 hits were analyzed in MEGA 7.



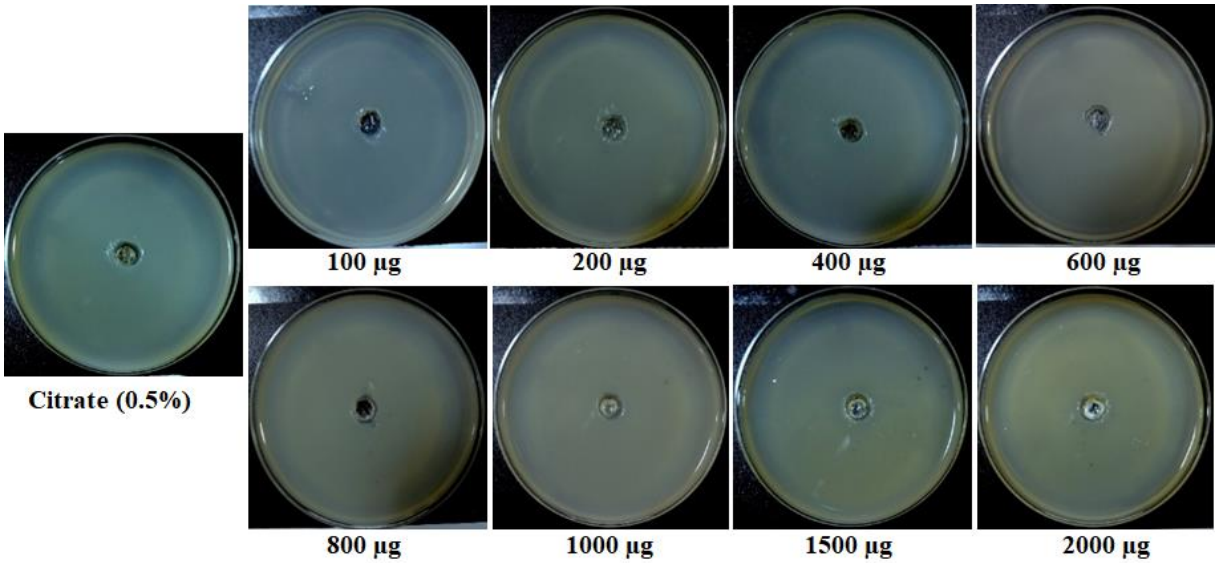
Supplementary Fig. 4: a. Nucleotide sequence similarity of organophosphate hydrolase and Arylesterase (EC 3.1.1.2) present in CPD-03 strain; b. Protein sequence similarity of organophosphate hydrolase and Arylesterase (EC 3.1.1.2) present in CPD-03 strain. The nucleotide sequence of organophosphate hydrolase gene was obtained after successful cloning followed by Sangers sequencing and the Arylesterase sequence was obtained from the whole genome. These sequences similarity comparison was performed in ClustalOmega.



Supplementary Fig. 5: Position of Arylesterase mapped on the genome of *Ochrobactrum* sp. CPD-03. This was located using rapid prokaryotic genome annotation (Prokka).



Supplementary Fig. 6. Proposed pathway for metabolic degradation of CP by *Ochrobactrum* sp. CPD-03 based on whole genome analysis



Supplementary Fig. 7. Chemotaxis of *Ochrobactrum* sp. CPD-03 resting cells of CPD-03 (1-1.5x10^8 cells ml^{-1}) towards various concentration of CP along with citrate (0.5%) as positive control citrate. Migrated cell width of CPD-03 showing the response towards CP at various concentration

Supplementary Table. 1a.

Test	**Results**
Catalase, Oxidase	+
NaCl tolerance	Up to 4%
Growth temperature	25^0C - 37^0C (30±2 ^{0}C optimum)
pH	5-8 (7 optimum)
Utilization of Dextrin, maltose, Cellobiose, Sucrose, N-Acetyl-D-Glucosamine, Citrate, Galactose, Glucose, Arabitol, myo-Inositol, Mannose, Fructose, Xylose	+

Notes.: (+, positive reaction). Data taken from GEN III plate of Biolog® System

Phenotypic characteristics of the strain *Ochrobactrum* sp. CPD-03

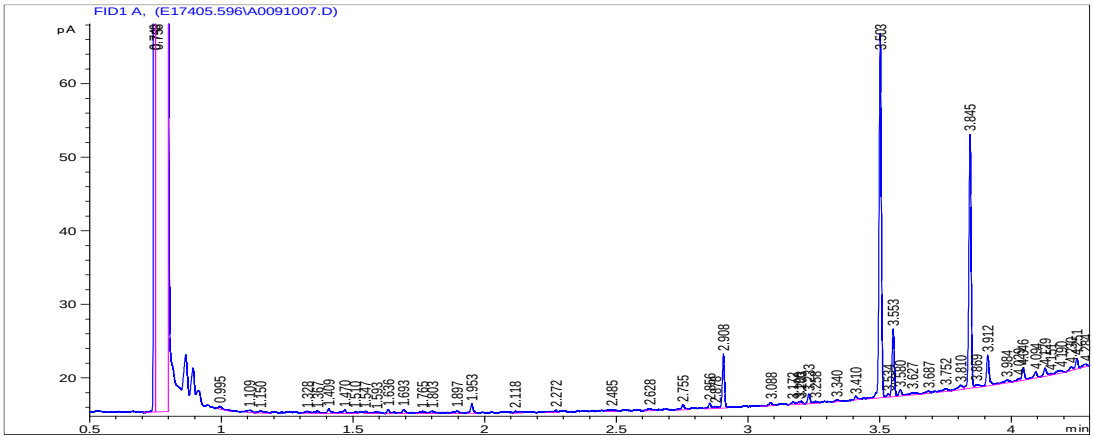
Supplementary Table. 1b.

Assimilation of	A	B	C
Tween 80	-	+	-
N-Acetyl-D-galactosamine	+	+	+
Adonitol	+	+	+
D-Arabitol	+	+	+
Cellobiose	+	+	+
Gentiobiose	+	+	+
L-Fucose	+	+	+
myo-Inositol	+	+	+
D-Mannitol	+	+	+
L-Rhamnose	+	+	-
D-Sorbitol	+	+	+
Raffinose	-	-	+
Sucrose	+	+	+
Trehalose	-	-	+
Citric acid	+	+	+
Glycerol	-	-	-

Differential biochemical properties of strain CPD-03 (A) and closet reference *Ochrobactrum* type strains Strains: (B) Ca-34^{T}; (C) LMG 3301^{T}. The table is arranged according to phylogenetic clustering. All data for CPD-03 are from this study and others obtained from Imran et al., 2010.

Supplementary Table 1c.

RT	Response	Ar/Ht	RFact	ECL	Peak Name	Percent
1.4094	1143	0.011	1.180	10.6984	11:0 anteiso	1.01
1.4701	1393	0.016	1.153	11.0051	11:0	1.20
1.5098	459	0.009	1.137	11.1740	10:0 2OH	0.39
1.6360	833	0.009	1.094	11.7107	12:0 anteiso	0.68
1.8971	849	0.014	1.027	12.7126	13:0 anteiso	0.65
2.1184	628	0.014	0.987	13.4833	12:0 3OH	0.46
2.2721	782	0.015	0.965	14.0005	14:0	0.56
2.8562	1163	0.010	0.912	15.8393	Sum In Feature 3	0.79
2.9082	8304	0.008	0.910	16.0001	16:0	5.66
3.0882	1047	0.013	0.902	16.5564	17:1 anteiso w9c	0.71
3.1724	725	0.012	0.899	16.8162	17:1 w8c	0.49
3.1956	379	0.006	0.899	16.8879	17:1 w6c	0.25
3.2028	545	0.008	0.898	16.9102	17:0 cyclo	0.37
3.2327	1643	0.009	0.898	17.0024	17:0	1.10
3.4100	804	0.012	0.894	17.5528	16:0 3OH	0.54
3.5035	59266	0.009	0.893	17.8430	Sum In Feature 8	39.64
3.5342	582	0.009	0.893	17.9382	18:1 w5c	0.39
3.5529	10971	0.009	0.893	17.9963	18:0	7.34
3.5797	1234	0.011	0.893	18.0812	18:1 w7c 11-methyl	0.83
3.7517	1304	0.019	0.893	18.6277	19:0 iso	0.87
3.8096	1653	0.017	----	18.8116	unknown 18.815	----
3.8446	41251	0.009	0.894	18.9225	19:0 cyclo w8c	27.61
3.8695	998	0.015	0.894	19.0015	19:0	0.67
3.9118	5990	0.011	0.895	19.1386	18:1 2OH	4.01
4.0464	2199	0.010	0.897	19.5748	18:0 3OH	1.48
4.0939	1558	0.013	0.898	19.7286	20:2 w6,9c	1.05
4.1292	1844	0.011	0.898	19.8430	20:1 w7c	1.24
----	1163	---	----	----	Summed Feature 3	0.79
----	59266	---	----	----	Summed Feature 8	39.64



Fatty Acid Methyl Ester (FAME) analysis of CPD-03

Supplementary Table 2.

	Ochrobactrum sp. CPD-03
Genome Size (bp)	4,660,061bp (~4.6 Mb)
GC Content (%)	57.67 %
CDS	4566
rRNA	3
tRNA	51
No. of plasmid	0

Genome features of *Ochrobactrum* sp. CPD-03

Supplementary Table 3.

S No.	Compound Name	Gene Designation	KEGG Number	EC Number	Enzyme Name
1	Benzoate degradation	*pobA*	K00481	1.14.13.2	p-hydroxybenzoate 3-monooxygenase
		pcaG	K00448	1.13.11.3	protocatechuate 3,4-dioxygenase, alpha subunit
		pcaB	K01857	5.5.1.2	3-carboxy-cis,cis-muconate cycloisomerase
		pcaC	K01607	4.1.1.44	4-carboxymuconolactone decarboxylase
		dmpB, xylE	K00446	1.13.11.2	catechol 2,3-dioxygenase
		pcaD	K01055	3.1.1.24	3-oxoadipate enol-lactonase
		fadA, fadI	K00632	2.3.1.16	acetyl-CoA acyltransferase
		dmpB, xylE	K00446	1.13.11.2	catechol 2,3-dioxygenase
		fadJ	K01782	1.1.1.35/ 4.2.1.17/ 5.1.2.3	3-hydroxyacyl-CoA dehydrogenase / enoyl-CoA hydratase / 3-hydroxybutyryl-CoA epimerase
		fadA, fadI	K00632	2.3.1.16	acetyl-CoA acyltransferase
		gcdH	K00252	1.3.8.6	glutaryl-CoA dehydrogenase
		paaF, echA	K01692	4.2.1.17	enoyl-CoA hydratase
		paaH, hbd, fadB, mmgB	K00074	1.1.1.157	3-hydroxybutyryl-CoA dehydrogenase
		atoB	K00626	2.3.1.9	acetyl-CoA C-acetyltransferase
2	Aminobenzoate degradation	*amiE*	K01426	3.5.1.4	amidase
		bsdC	K16239	4.1.1.61	4-hydroxybenzoate decarboxylase subunit C
		paaF, echA	K01692	4.2.1.17	enoyl-CoA hydratase
3	Chloroalkane and chloroalkene degradation	*adhP*	K13953	1.1.1.1	alcohol dehydrogenase, propanol-preferring
		aldH	K00128	1.2.1.3	aldehyde dehydrogenase (NAD+)
		fdhA	K00148	1.2.1.46	glutathione-independent formaldehyde dehydrogenase
		NA	K01560	3.8.1.2	2-haloacid dehalogenase
4	Chlorocyclohexane and chlorobenzene degradation	*dmpB, xylE*	K00446	1.13.11.2	catechol 2,3-dioxygenase
		NA	K01560	3.8.1.2	2-haloacid dehalogenase
5	Xylene degradation	*dmpB, xylE*	K00446	1.13.11.2	catechol 2,3-dioxygenase
6	Nitrotoluene degradation	*nfsA*	K10678	1.5.1.34	nitroreductase
7	Ethylbenzene	*fadA, fadI*	K00632	2.3.1.16	acetyl-CoA acyltransferase
8	Styrene degradation	*amiE*	K01426	3.5.1.4	amidase
		dmpB, xylE	K00446	1.13.11.2	catechol 2,3-dioxygenase
9	Atrazine degradation	*ure*	K01427	3.5.1.5	urease
10	Dioxin degradation	NA	K00480	1.14.13.1	salicylate hydroxylase
11	Naphthalene degradation	NA	K00480	1.14.13.1	salicylate hydroxylase
		adhP	K13953	1.1.1.1	alcohol dehydrogenase, propanol-preferring
12	Polycyclic aromatic hydrocarbon degradation	NA	K00480	1.14.13.1	salicylate hydroxylase
		pcaG	K00448	1.13.11.3	protocatechuate 3,4-dioxygenase, alpha subunit

Putative genes of *Ochrobactrum* CPD-03 involved in degradation of Aromatic and relative compounds. Information retrieved from KAAS annotation tool. [NA: Not available]

Supplementary Table 4.

S No.	Activity	Gene Designation	Enzyme Name
1	IAA production	**NA***	Indole-3-glycerol phosphate synthase
2	Siderophore production	*FhuB*	Ferric hydroxamate ABC transporter, permease component
		TonB	Ferric siderophore transport system, periplasmic binding protein
		FepC	Ferric enterobactin transport ATP-binding protein @ ABC-type Fe3+-siderophore transport system, ATPase component
		FepG	Ferric enterobactin transport system permease protein
		FepB	Ferric enterobactin-binding periplasmic protein
		FepC	Ferric enterobactin transport ATP-binding protein
		FhuD	Ferric hydroxamate ABC transporter, periplasmic substrate binding protein
		FhuC	Ferric hydroxamate ABC transporter, ATP-binding protein
		NA*	ABC transporter (iron.B12.siderophore.hemin) , permease component
		FhuA	Ferric hydroxamate outer membrane receptor
		EntS	Enterobactin exporter
		NA*	ABC-type Fe3+-siderophore transport system, permease component
		FhuB	Ferric hydroxamate ABC transporter, permease component
3	Flagellar protein	*FlhB*, *FliL*, *FliP*, *FliQ*, *FlhA*, *FliR*	Flagellar biosynthesis protein
		FliG, *FliN*, *FliM*	Flagellar motor switch protein
		MotA, *MotB*	Flagellar motor rotation protein
		FlgF, *FlgF*	Flagellar basal-body rod protein
		FliI	Flagellum-specific ATP synthase
		FlgB, *FlgC*, *FlgG*	Flagellar basal-body rod protein
		FliE	Flagellar hook-basal body complex protein
		FlgA	Flagellar basal-body P-ring formation protein
		FlgI	Flagellar P-ring protein
		FlgH	Flagellar L-ring protein
		FlaA, *FlaF*, *FliJ*	Flagellin protein
		FliF	Flagellar M-ring protein
		FliK	Flagellar hook-length control protein
		FlgE	Flagellar hook protein
		FlgK, *FlgL*	Flagellar hook-associated protein
		FlgD	Flagellar basal-body rod modification protein
		FlgJ	Flagellar protein [peptidoglycan hydrolase]
4	Chemotaxis	**NA***	Dipeptide-binding ABC transporter, periplasmic substrate-binding component
		RbsB	Ribose ABC transporter, periplasmic ribose-binding protein
		MalE	Maltose/maltodextrin ABC transporter, substrate binding periplasmic protein
		NA*	Dipeptide-binding ABC transporter, periplasmic substrate-binding component; Putative hemin-binding lipoprotein
		CheR	Chemotaxis protein methyltransferase (EC 2.1.1.80)
		motC	Chemotaxis protein
		MalE	Maltose/maltodextrin ABC transporter, substrate binding periplasmic protein
			Methyl-accepting chemotaxis protein
5	HCN production	**NA***	
6	Phosphate solublization		
7	ACC deaminase activity		
8	Ammonia production		

NA*: Not assigned in the genome of CPD-03

Putative genes of *Ochrobactrum* CPD-03 involved in Plant growth promoting activities and chemotaxis. Information retrieved from RAST annotation tool. [NA: Not available]

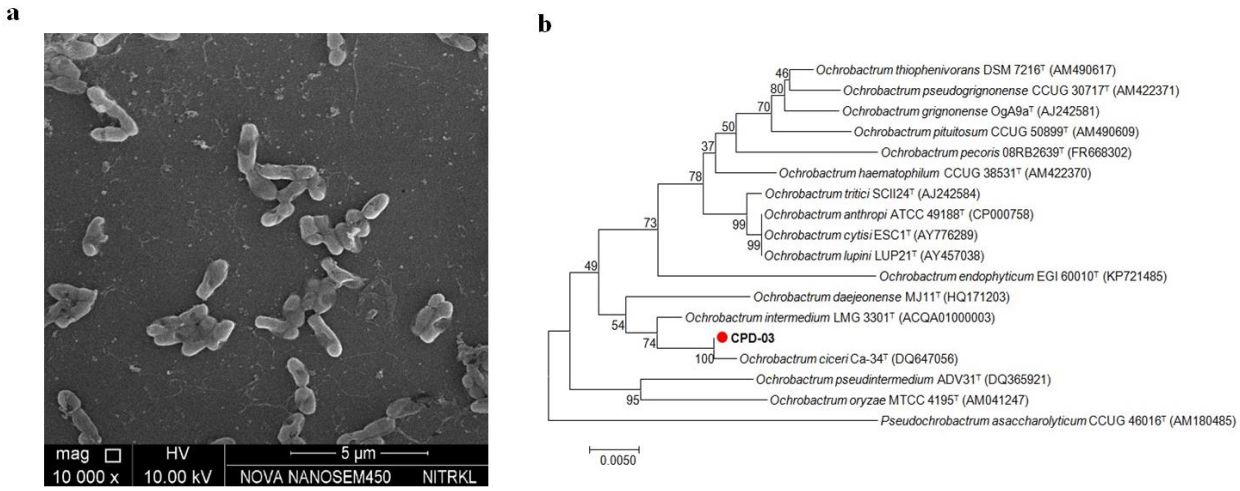


Fig.1. a. Scanning electron microscopy of CPD-03; b. phylogenetic tree of CPD-03 based on comparison of 16S rRNA sequence similarity of closely related *Ochrobactrum* sp. conducted in MEGA version 7.0

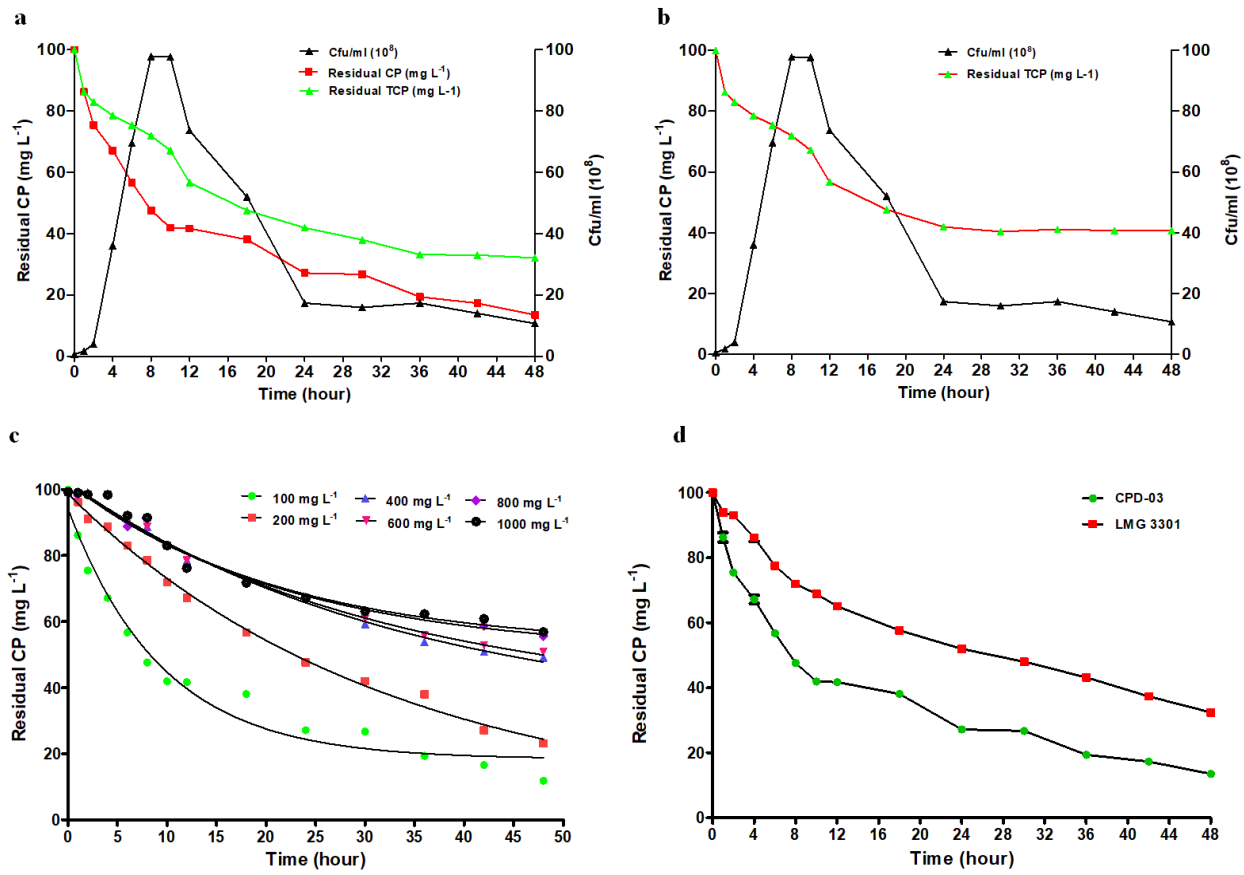


Fig.2. a. Degradation of CP (100 mg L^{-1}) and TCP (100 mg L^{-1}) by CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in a MSM supplemented molasses (0.1% v/v) showing formation of its intermediate TCP and its further degradation; b. degradation of TCP (100 mg L^{-1}) by CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in a MSM supplemented molasses (0.1% v/v); c. degradation of CP at various concentrations (100 mg L^{-1}, 200 mg L^{-1},400 mg L^{-1},600 mg L^{-1},800 mg L^{-1}, and 1000 mg L^{-1}) by CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}) in MSM supplemented molasses (0.1% v/v); d. comparision of CP (100 mg L^{-1}) degradation by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}) and LMG 3301^T (1.2-1.4x10^8 cells ml^{-1}). Data were represented in the mean of three replicates and analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

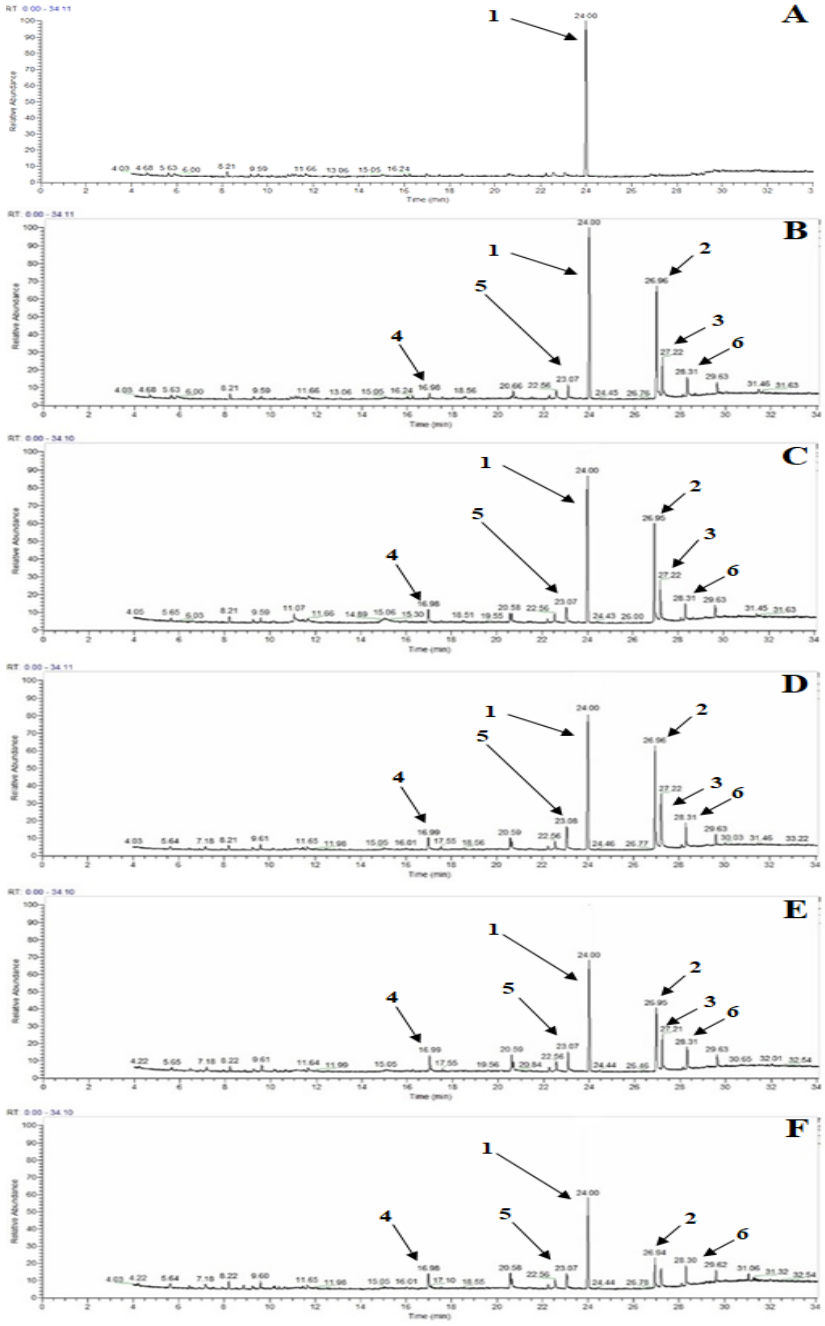


Fig.3 GC-MS chromatograms showing the metabolites formed during CP (100 mg L^{-1}) degradation by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}). A: 0 h, B: 2 h, C: 4 h, D: 6 h, E: 8 h, and F: 10 h respectively. 1: chlorpyrifos, 2: cis-Vaccenic acid, 3: Octadecanoic acid, 4: Benzoic acid, 4-hydroxyl 3-methoxy, 5: n-Hexadecanoic acid, 6: 9-Hexadecanoic acid, respectively.

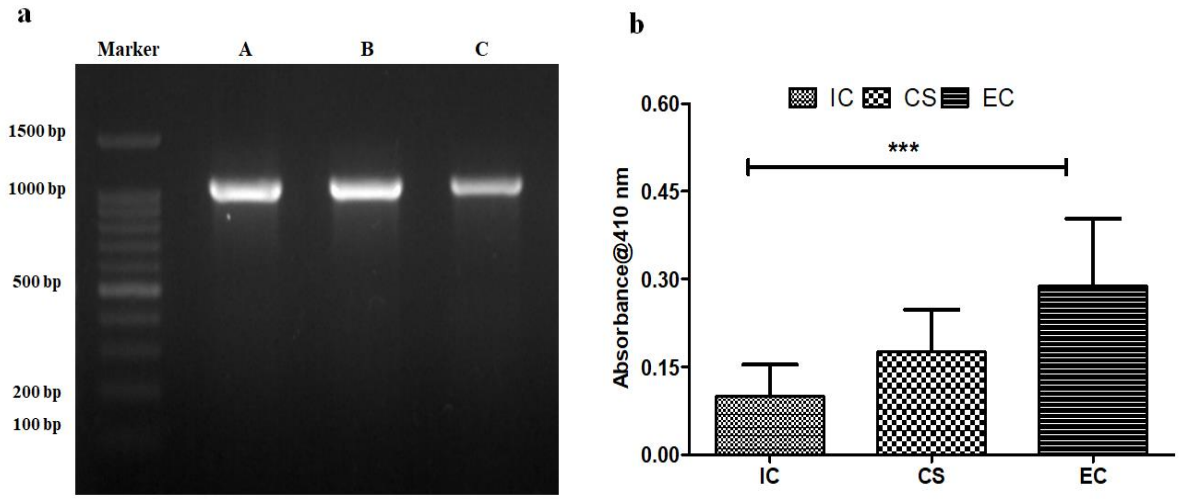


Fig.4. a. Amplification of ~1098 bp *opd* gene; b. enzyme activity of CPD-03 resting cell (1-1.5x10^8 cells ml^{-1}); where IC: intracellular, CS: cell suspension and EC: Extracellular. Data analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

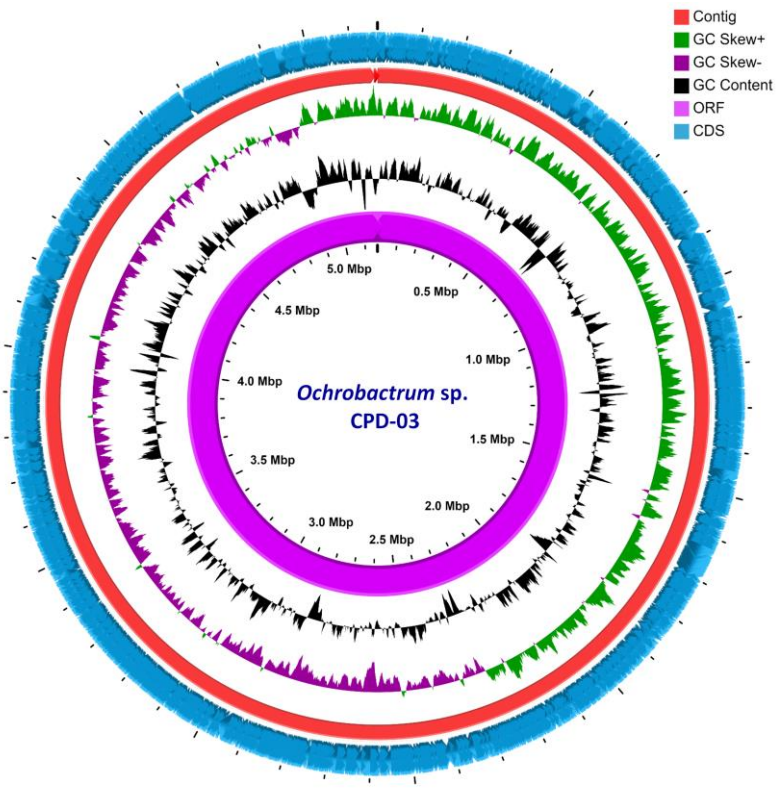


Fig. 5 Genome map of *Ochrobactrum* sp. CPD-03

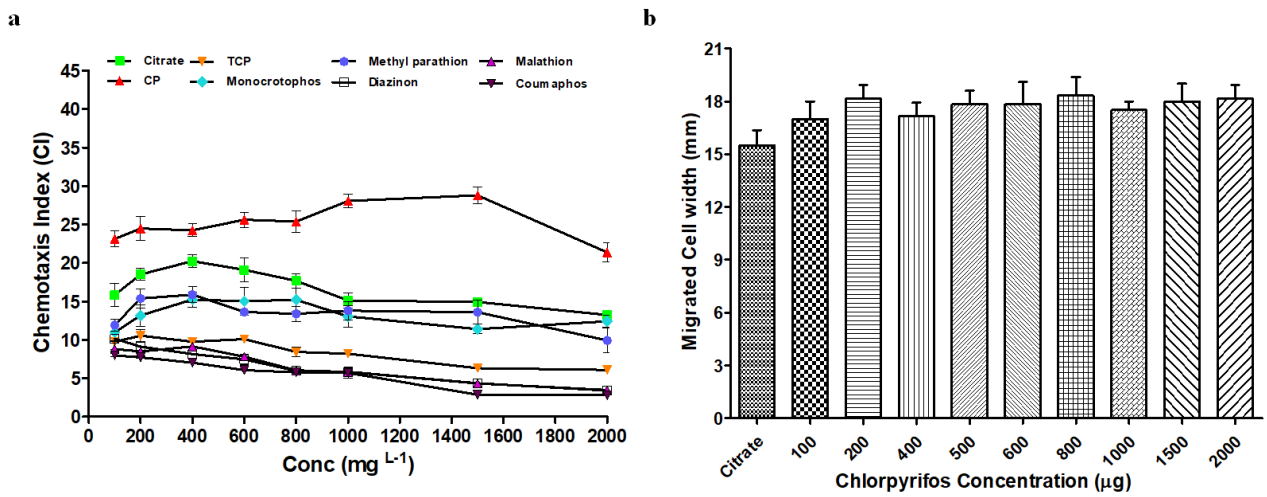


Fig.6 a. Chemotaxis index of CPD-03 resting cells CPD-03 (1-1.5x10^8 cells ml^{-1}) towards CP, TCP, monocrotophos, methyl parathion, coumaphos, diazione and malathione as competitive attractant at various concentrations (from 200 mg L^{-1} to 2000 mg L^{-1}). Values are presented as arithmetic means and error bars indicate standard deviations based on three independent replicates. b. migrated cell width of chemotactic movement by CPD-03 towards various concentration of CP (100 mg L^{-1}, 200 mg L^{-1}, 400 mg L^{-1}, 600 mg L^{-1}, 800 mg L^{-1}, 1000 mg L^{-1}, 1500 mg L^{-1} and 2000 mg L^{-1}) along with citrate (0.5%) as positive control. Data analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level

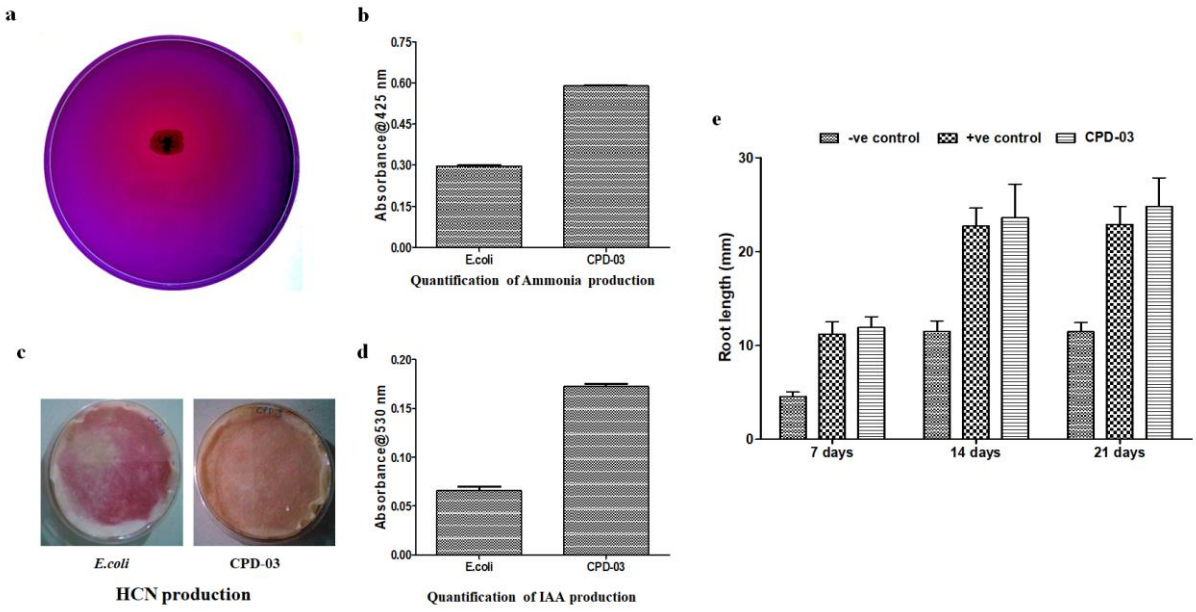


Fig.7(a-e). a. Phosphate solubilization on Pikovskaya's agar supplemented with tricalciumphosphate by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}); b. quantification of ammonia production; c. HCN production and d. quantification of IAA production by CPD-03 resting cells (1-1.5x10^8 cells ml^{-1}); e. Effect of root length upon CPD-03 inoculation on rice seed; -ve control: culture free, +ve control: *Bacillus* sp., lab stock for positive PGPR activity. *E.coli* was used as a negative control. Data analysis were performed using ANOVA with the Prism8. All were tested at the $p<0.005$*** significance level