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Full Length Research Paper

Molecular characterization of a gene capable of degrading trichloroethylene, an environmental pollutant

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Trichloroethylene (TCE) is a widely used organic solvent and metal degreasing agent, one of the most frequently detected groundwater contaminants and a potential health hazard. Our novel isolate, *Bacillus cereus* strain 2479 was capable of degrading TCE efficiently. The gene for TCE degradation was PCR amplified from genomic DNA of *B. cereus* 2479. The amplified gene was cloned into expression vector pUC I8 in the *E. coli* host XL1-Blue and expressed under the control of lac promoter and nucleotide sequence was determined. The sequencing results showed that this novel gene (designated as *tce1*, GenBank Accession No: GU183105) contained 342 bp long ORF encoding 114 amino acids with a predicted molecular weight 12.6 kDa and the theoretical pI value of the polypeptide is 5.17. *E. coli* expressing the *tce1* gene overproduces a polypeptide in the presence of the inducer Isopropyl-β-D-thiogalaocto-pyranoside which reacts immunologically to the polyclonal antibody against TCE inducible proteins of the strain 2479. The secondary structure of *Tce1* protein was predicted through internet resources with software, CLC Protein Workbench. The present study suggested that cloned gene product (*Tce1*) was capable of degrading TCE as verified chemically.

Key words: Trichloroethylene, *tce1*gene, molecular cloning, expression, bioinformatics analysis.

INTRODUCTION

Trichloroethylene (TCE) is an Environmental Protection Agency priority pollutant widely used as an industrial metal degreaser and cleaning of cotton, wool and other fabrics in textile industry (Storck, 1987). The contamination of drinking water supply with TCE is increasing in prevalence and concentration (Roberts et al., 1982). Studies on animal models indicate that TCE increases the risk of tumors and lymphoma (Miller and Guengerich, 1983). Exposure to TCE can affect the human central nervous system (CNS). A recent analysis of available epidemiological studies reports TCE exposure to be associated with several kinds of cancers in humans. Biotransformation of TCE to the potent carcinogen vinyl chloride (VC) by consortia of anaerobic bacteria (Vogel and McCarty, 1985) might have role for the aforementioned purpose. Although, TCE acts as potent air, water and soil contaminants, its effect in drinking water contamination and subsequent health hazard has been reported in great details. For these reasons, there is great interest in implementing processes to remove TCE from drinking water supplies. Bioremediation is one of the environment friendly means of degrading toxic chemicals. Therefore, extensive efforts have been made to study the biodegradation of TCE by bacteria. Many chlorinated organic compounds are known to persist in the environment because of their resistance to microbial attack. For example, TCE was observed to exhibit a half-life of 300 days in one aquifer. However, TCE was found to be co-metabolized by some ammonia-oxidizing bacterial species and by some bacteria able to grow on hydrocarbons such as methane (Oldenhuis et al., 1989) propane (Wackett et al., 1989) and isoprene (Ewers et al., 1990). It was demonstrated that oxygenation reaction by monooxygenase leads to the production of unstable epoxide intermediates (Fox et al., 1990; Little et al., 1988; Miller and Guengerich, 1983). In the case of aromatic inducer substrates for example, phenol or toluene (Harker and Kim, 1990) initial mono or dioxygenases of the degradative pathways may be

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responsible for the aerobic TCE degradation.

Several aromatic monooxygenase system, such as a toluene-4-monooxygenase in *Pseudomonas mendocine* KR-1 (Winter et al., 1989; Sheilds et al., 1989) a toluene-ortho-monooxygenase in *Pseudomonas cepacia* G4 (Winter et al., 1989) have been identified to co-oxidize TCE. To our knowledge, only one aromatic dioxygenase system, the toluene dioxygenase in *Pseudomonas putida* F1 has been shown to be involved in TCE degradation. Products generated by toluene dioxygenase from TCE include formic and glyoxylic acids (Nelson et al*.,* 1987). The toluene dioxygenase in *P. putida* F1 was identified as class IIB multicomponent dioxygenase comprising the large subunit of the terminal dioxygenase (*todC1*), which is the most important gene for TCE oxidation (Furukawa et al., 1994). Although no microbial growth on TCE as the sole carbon source has been reported yet, we were the pioneer in reporting a microorganism capable of growing on TCE as the sole carbon source (Dey and Roy, 2009) and we also reported first, *Bacillus cereus* group being used in biodegradation of trichloroethylene (Mitra and Roy, 2010).

In this study, we report the isolation, molecular cloning and characterization of the new gene, designated as *tce1* (GenBank Accession Number: GU183105) involved in trichloroethylene degradation from *B. cereus* strain 2479 belongs to dioxygenase family (Mitra and Roy, 2011a) by phylogenetic analysis. This gene sequence is different from known toluene dioxygenase or monooxygenase genes. Specific antibodies raised against the inducible proteins in *B. cereus* 2479 by TCE reacted with this Isopropyl-β-D-thiogalaoctopyranoside (IPTG) inducible protein synthesized in the recombinant *Escherichia coli* (pSM 101 containing *E. coli*) XL1-Blue.

MATERIALS AND METHODS

Bacterial strain

The strain 2479 was isolated from the soil of industrial belt, situated at Rajbandh (Durgapur, West Bengal, India) where the use of polychlorinated hydrocarbon (including TCE) is quite abundant. Strain 2479 was classified as *B. cereus* on the basis of its morphological and physiological characteristics and ribotyping (Dey and Roy, 2009; Mitra and Roy, 2010).

Chemicals and reagents

TCE (purity by GC analysis > 99%) was obtained from Merck Limited, India. Pyridine was obtained from Qualigen Fine Chemicals, India. Restriction endonuclease, Hind III, T4 DNA ligase, Isopropyl-β-D-thiogalaoctopyranoside, 5-Bromo4-Chloro-3-Indolyl-β-D-galactopyranoside (X-Gal) and Ampicillin were purchased from Sigma-Aldrich Chemical Co, USA. Vector pUC 18 was obtained from Helini, India.

Growth of organism

The isolate was grown in M9 medium (Na_2HPO_4-6 g; KH_2PO_4-3 g; NaCl-0.5 g; NH_4Cl-1 g; $MgSO_4$-0.002 M; $CaCl_2$ -0.0001 M; Glucose 0.2%; H_2O 1 L pH 7.0). In some experiments, TCE (0.2% v/v) replaced glucose as the sole carbon source. Incubation was done for 2 days with shaking at 31°C.

Amplification of *tce1* from genomic DNA

Genomic DNA of strain 2479 was isolated by Janarthanan and Vincent's method (2007). The quality and concentration of the extracted DNA was checked by 0.8% (wt/vol) agarose gel electrophoresis and measured by UV-VIS spectrophotometer (UV-1700 Pharma Spec, Shimadzu) at 260 and 280 nm respectively. The concentration of DNA was 1.5 mg/ml. The *tce1* gene of strain 2479 was amplified using toluene dioxygenase C1 (*todC1*) gene specific primers from *P. putida* F1 (Romine and Brockman, 1996) synthesized by Sigma-Aldrich Chemical Co, USA. Amplification reaction was performed with reagent supplied by Bangalore Genei, India as follows: Taq DNA polymerase 2 units, magnesium ion conc. of varying concentration of 1, 1 and 2 mM, 10X buffer (containing 100 mM Tris-HCl, pH 9.0; 500 mM KCl; 0.1% Gelatin), 0.2 mM each of the four dNTPs and 100 ng template DNA of strain 2479, 25 pmol each of the primers. The polymerase chain reaction was carried out with initial denaturation at 94°C for 5 min followed by 30 cycles of programmed temperature control: 1 min at 94°C, ½ min at 57°C, 1 min at 72°C with a 5 min final extension at 72°C using strain 2479 DNA as template. The primers were as follows:

Oligo- F: 5'-GCGAGATGAAGCGCTCTTTG-3'.
Oligo- R: 5'-GTATTGATACCTGGGAGGAGG-3'.

The amplified product was analyzed on agarose gel electrophoresis (1.2% wt/vol) and documented using a gel documentation system. PCR amplified product was sequenced using the dideoxynucleotide chain termination method (Chromous Biotech. Pvt. Ltd).

Accession numbers

The partial sequence of *tce1* gene was deposited at EMBL/GenBank/DDBJ database under the accession number GU183105 (454 bp). The 454 bp gene contained 342 bp open reading frame (ORF) encoding 114 amino acids (Accession No. ACZ57347).

Cloning and sequencing of PCR product

PCR amplified product of *tce1* was purified by Spin-Column (Sigma-Aldrich) and cloned into cloning vector pUC 18 at Hind III site. The cloned insert was transformed into *E. coli* XL1-Blue [*recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* (F'*proA*$^+$*B*$^+$ *lacI*^{q}*Z*Δ*M1*5Tn10)] and the recombinant *E. coli* was screened on Luria Bertani (LB) agar containing Ampicillin (50 µg/ml), IPTG (50 µM) and X-gal (40 µg/ml) plate for blue-white selection. From white colonies, the plasmid was isolated by alkaline lysis method (Sambrook and Russell, 2001). The isolated recombinant plasmid designated as pSM 101 was amplified using *todC1* gene specific primers as before.

Expression of cloned gene

The recombinant *E. coli* XL1-Blue (pSM 101 containing *E. coli*) was over-expressed with different concentration of IPTG by inducing the lac promoter of the cloning vector. This recombinant strain was grown on LB, Ampicillin (50 µg/ml) medium; after 2 h of incubation at 37°C with shaking, when the absorbance reached 0.5 at 600 nm,

different conc. of IPTG (1.0, 1.5, 2.0 and 2.5 mM) were added in four culture flasks and one flask contained no IPTG. Incubation was at 37°C with shaking for 4 h. The cells were centrifuged at 10,000 g for 10 min and lysed by 1% SDS and PMSF (1 mM) and the extract was boiled for 5 min with Laemmli buffer (Laemmli, 1970) (1% SDS, 5% Mercaptoethanol, 0.05% Bromophenol blue in 25 mM Tris-HCl, pH 6.8 and 10% glycerol) and electrophoresed on 12% SDS-PAGE. To visualize the protein bands, the gel was stained by Coomassie blue R-250 (0.2%). Western-blot analysis was done on identical SDS-PAGE (without staining) using antibodies generated as follows:

Preparation of antibodies reacting to Tce1

The strain 2479 was grown separately in two conical flasks; one containing glucose (0.2%) as carbon source and other contained TCE (0.2%) as sole carbon source in minimal M9 medium. The SDS extracted proteins from bacterial cells grown on TCE containing M9 medium were injected into rabbits to obtain polyclonal antibodies. The titre of the antiserum was first determined by Dot-blot in different dilution (1:500; 1:1500; 1:2000) by using the spotted antigens (Mitra and Roy, 2011b). The polyclonal anti-serum was preadsorbed by nitrocellulose membrane (Sigma-Aldrich) on which total cellular proteins from glucose grown cells were immobilized. Thus the antibodies reacting to the common antigens found in both glucose grown and TCE grown cells were removed. The preadsorbed antiserum was found to react specifically with TCE induced protein in Western blot.

Western blot analysis

Following electrophoresis, the recombinant *E. coli* proteins (without staining) were electrotransferred onto nitrocellulose membrane (Sigma-Aldrich Chemical Co, USA.) for 2 h at 20 V. The membrane was blocked with 3% skimmed milk for 1 h at room temperature and probed for 1 h with 1:1000 diluted preadsorbed antiserum (raised against TCE inducible proteins). After washing with the buffer A (10 mM Tris-HCl pH-8.0; 0.9% normal saline; 0.2% Tween 20), the membrane was labeled with secondary antibody (alkaline phosphatase -conjugated goat anti-rabbit IgG, dilution 1:30,000) for 1 h at room temperature. After thoroughly washed with buffer A, the membrane was visualized with alkaline phosphatase substrates (5-bromo-4-chloro-3-indolyphosphate and nitroblue tetrazolium) (BCIP/NBT) in color development buffer-B (100 mM Tris-HCl with 5 mM Mg^{2+} at pH 9.5) and allowed to dry.

Measurement of TCE degradation by Fujiwara test

Fujiwara test performed to estimate the concentration of the polychlorinated hydrocarbon in the medium (Moss and Rylance, 1966). In the said test TCE was treated with pyridine in an alkaline environment. The observance of the red aqueous phase was determined at 470 nm by spectrophotometer. The absorbance of the red aqueous phase is proportional to the concentration of free TCE. If the test was performed without inoculation of the bacterium in the medium, the red upper layer was formed and it indicated that the medium contains the compound TCE. The same test was done with inoculation of the bacteria (capable of degrading trichloroethylene) and the absence of red color indicated that the medium contains no free TCE. Out of four TCE containing M9 media, three were inoculated each with recombinant *E. coli* (pSM 101 containing *E. coli*), non recombinant *E. coli, B. cereus* 2479 (positive control) and last one was not inoculated (negative control). These were incubated for 48 h at 37°C and Fujiwara test was performed. Optical density (OD_{470}) was measured by UV-VIS spectrophotometer (UV-1700 Pharma Spec, Shimadzu) at the beginning (just after inoculation) and after 12, 24, 36 and 48 h. The Fujiwara test was done repeatedly.

Bioinformatics analysis

The nucleotide sequence, deduced amino acid sequence and ORF encoded by *tce1* gene were analyzed and sequence comparison was conducted through database searches using the Gene Runner (version 3.01) software and BLAST program at NCBI http://www.ncbi.nlm.nih.gov) respectively. The molecular masses and the theoretical pI values of the polypeptide were predicted using the ProtParam tool (http://www.expasy.org/tools/protparam.html). Extensive structural analysis of the primary sequence of *Tce1* protein was performed at NPS@ (network protein sequence analysis: web server http://npsa-pbil.ibcp.fr) using novel software such as DPM, DSC, GOR4, PHD, PREDATOR, SIMPA96 and SOPM. The information regarding 'secondary structure prediction' thus obtained was supplemented by using PSIPRED software at the web server: http://bioinf4.cs.ucl.ac.uk. Physico-chemical properties were computed using the software package CLC-Workbench (Version 5.0). Kyte-Doolittle method (Kyte and Doolittle, 1982) method was used for obtaining the hydropathy profile of the protein using default parameters.

RESULTS

Cloning and characterization of *Tce1*

The ~600 bp PCR amplified product was obtained from *B. cereus* 2479 by using *todC1* gene specific primers (Figure 1A) and sequencing was performed with the PCR product. This gene is a new one and designated as *tce1*. To confirm the presence of insert, amplified product was inserted into plasmid, pUC 18 and then transformed into *E. coli*. XL1-Blue. Then recombinant plasmid pSM 101 was isolated and amplified by using the same two primers (OligoF and OligoR). In this PCR reaction the same ~600 bp amplified product was obtained as shown in Figure 1B.

Over-expression of cloned *tce1* gene

In SDS-PAGE, one ~14.3 kDa recombinant protein was over-produced at 2 and 2.5 mM IPTG and less intense bands with 1 and 1.5 mM IPTG concentration but there was no such band with the negative control (without IPTG) (Figure 2A). In Western Blot studies, the over-expressed recombinant *E. coli* protein (14.3 kDa) specifically reacted with preadsorbed antiserum against TCE inducible protein (Figure 2B).

TCE degradation by pSM 101 containing *E. coli*

Fujiwara test was done to examine that the recombinant *E. coli* XL1-Blue (pSM 101 containing *E. coli*) could degrade TCE. Figure 4 shows OD value of red upper

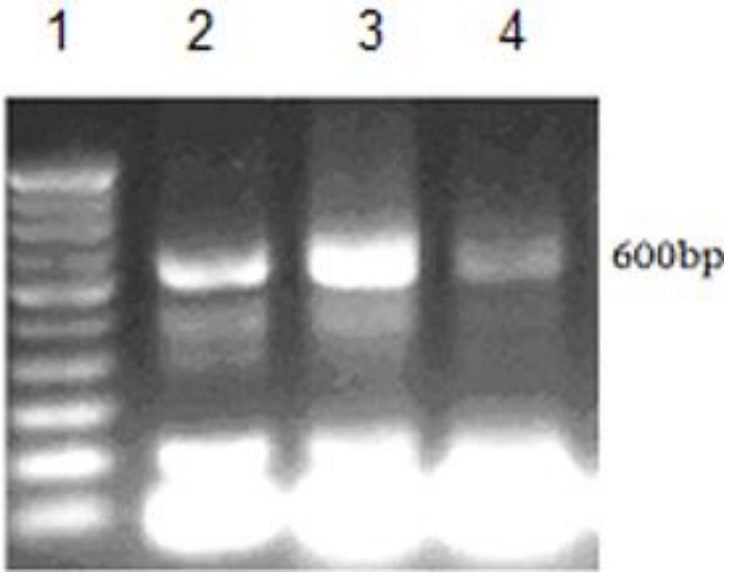

Figure 1A. 1.2% Agarose gel electrophoresis of polymerase chain reaction of *Bacillus cereus* 2479 genomic DNA using *todC1* gene specific primers. Presence of distinct bands of size 600 bp was observed with different magnesium ion concentration in the PCR mix: 2, 1.5 and 1 mM Mg^{++} in lane 2, 3, 4 respectively and lane 1 shows molecular weight marker, 100 bp ladder.

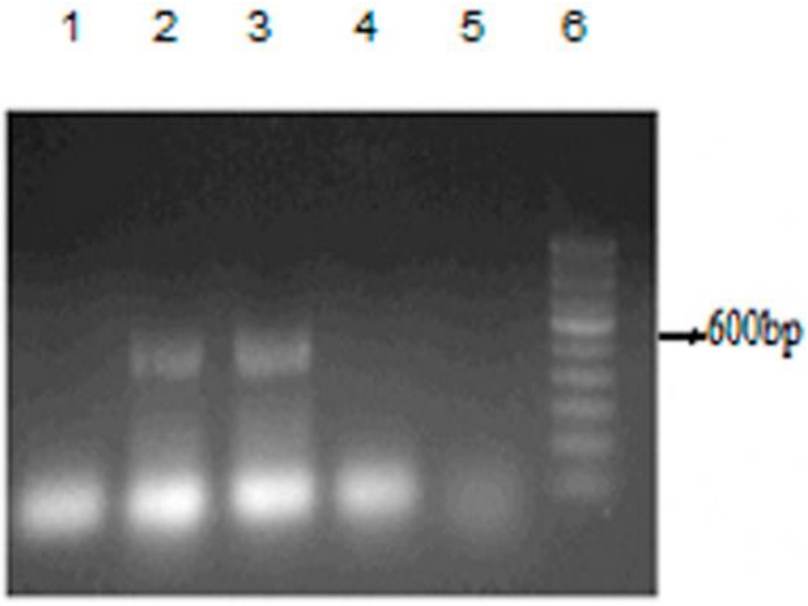

Figure 1B. PCR amplification with different templates: lane 1: genomic DNA of *E. coli*; lanes 2, 3: pSM 101; lane 4: pUC 18; lane 5: negative control (without any template) lane 6: shows 100 bp ladder.

layer gradually decreases with time (h) in case of recombinant *E. coli* and *B. cereus* 2479 during Fujiwara test. After 48 h OD reached 0.11 and 0.10 in recombinant *E. coli* and *B. cereus* 2479 respectively. It indicates that the medium contained no more free TCE. The plot shows no declination of OD value in the uninoculated medium as well as the non recombinant *E. coli* (without pSM 101) medium. It conferred that medium contained TCE.

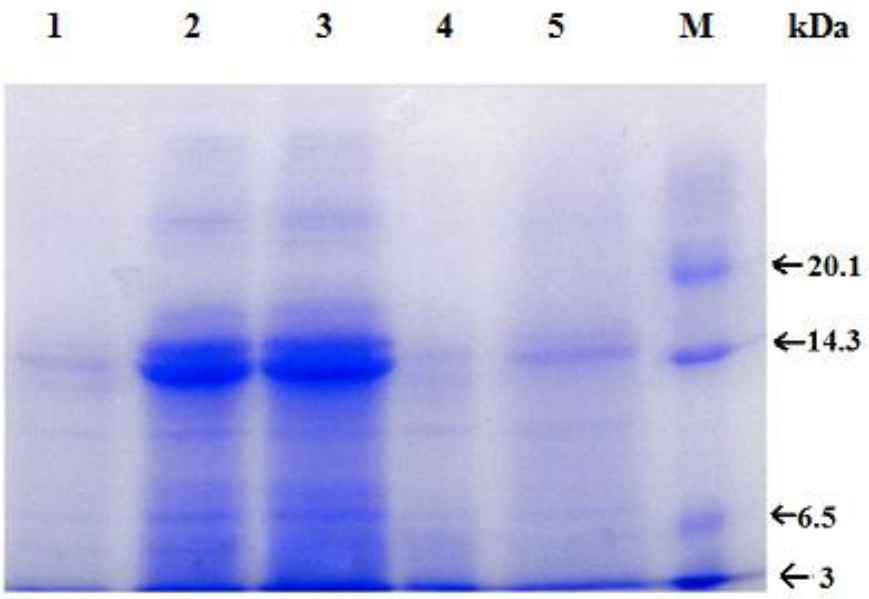

Figure 2A. SDS-PAGE profile of *E. coli* cells grown in LB, presence of IPTG: lanes 1, 2 and 3 cells grown at 1.0, 2 and 2.5 mM IPTG conc. respectively; lane 4 cells grown without any IPTG; lane 5 cells grown at 1.5 mM IPTG conc. lane 6 shows molecular weight marker.

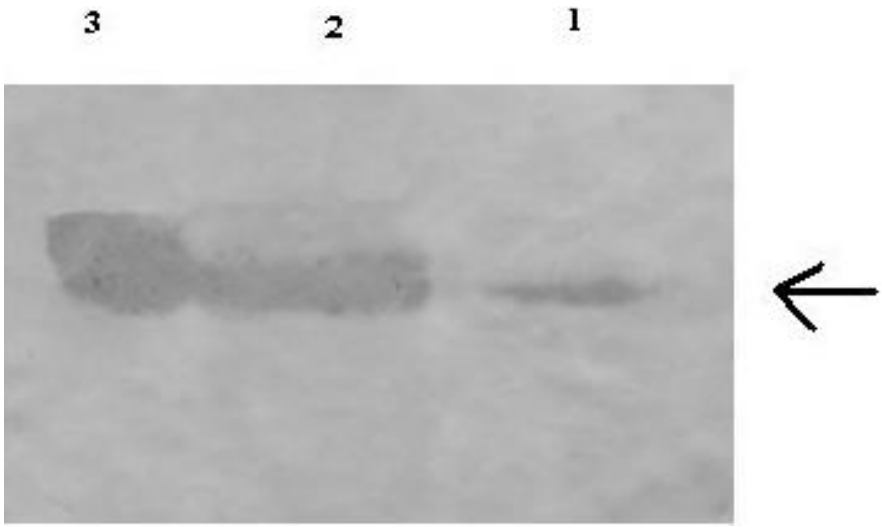

Figure 2B. In Western Blot: lanes 1, 2 and 3 shows specific reaction of anti-serum against TCE inducible protein with proteins from recombinant *E. coli* cells grown in presence of IPTG concentration at the rate of 1.0, 2, 2.5 mM respectively.

Nucleotide and amino acid sequence analysis

The partial sequence of *tce1* gene was deposited at EMBL/GenBank/DDBJ database under the accession No GU183105 (454 bp). The 454 bp sequence contained 342 bp open reading frame (ORF) encoding 114 amino acids (Accession No. ACZ57347) (Figure 5). The amino acid sequence was submitted to SWISS-PROT database with practical ProtParam procedures (http://www.expasy.ch/tools/protparam.html). Some physicochemical properties of deduced *Tce1* protein were as follows: molecular weight 12.6 kDa, molecular formula $C_{559}H_{886}N_{152}O_{165}S_8$, total number of atoms 1770, theoretical isoelectric point 5.17, aliphatic index 101.93,

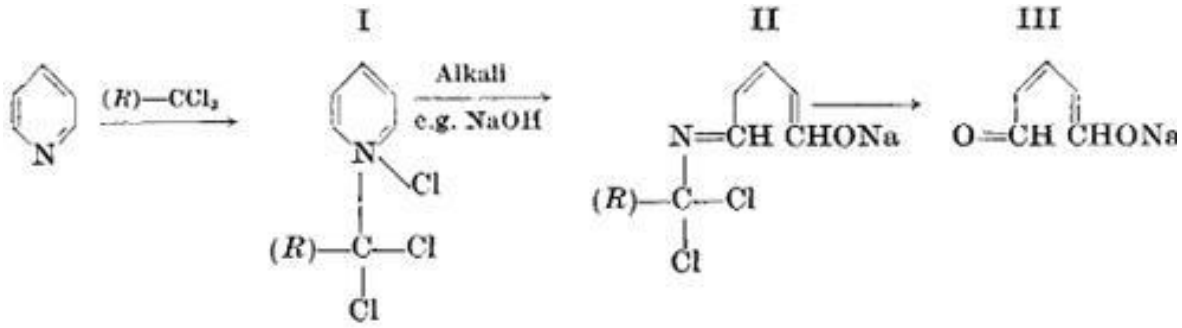

Figure 3. Reaction mechanism of Fujiwara test.

instability index 28.60, grand average of hydropathicity (GRAVY) 0.152.

Hydrophathy profile of *Tce1* protein

The hydropthy profile of TCE protein is presented in Figure 6 wherein the amino acids point score an averaged of nine residues individual scale is plotted against the position of amino acids in the sequence from N-terminal to C-terminal region. The plot shows that the protein possesses both hydrophobic and hydrophilic regions of which the later is dominating over the former. Hydrophobicity and hydrophilicity apparently show region specificity in that N-terminal portion is more hydrophobic and C-terminal portion is more hydrophilic. Hydrophilicity is more prominent than hydrophobicity as the score of the former reaches as high as -2.5 whereas the later is only +1.5 (Figure 6). The profile further identifies a region of about 25 residues long (from residue position 80 to 105) with very high hydrophilicity.

Secondary structure analysis of *Tce1* protein

Primary structure of proteins possesses multilayered information that helps to form functional structure of the proteins. Identification of local structures such as secondary structures and their sequences help to understand the fold topology of proteins. We used authentic web-based procedures (materials and methods section) to deduce the secondary structural characteristics of the protein whose result is presented in Figure 7. As seen in the figure that the protein is almost composed of right handed helix which constitutes about 85% of total secondary structures. Only about 5% of total secondary structures is constituted by beta-type (including turns) and rest 10% are of disorder type (random coil). Helical region are populated both in the N-terminal and C-terminal region of the sequence where as the middle portion of the sequence of *Tce1* protein possesses coil, turn and beta-structures.

DISCUSSION

Trichloroethylene a suspected carcinogen is the ground water contaminant (Richmond et al., 2001; Ma et al., 2002). It can readily be degraded by indigenous soil microbial population in presence or absence of toluene or phenol. One possible mechanism is toluene dioxygenase (TDO; EC 1.14.12) catalyzes the first reaction in the degradation of trichloroethylene or toluene by *P. putida* F1 (Yeh et al., 1977; Nelson et al., 1988). Nelson et al. (1987) and Wackett and Gibson (1988) showed that mutants of *P. putida* Fl in which toluene dioxygenase activity was absent failed to degrade TCE because *todC1* is the crucial gene for initial oxidation of TCE. The *todC1* gene product being capable of degrading TCE as shown in *P. putida* F1 (Romine and Brockman, 1996) was chosen for synthesizing the primers used in this study to amplify the novel gene (*tce1*) from *B. cereus* 2479. Toluene monooxygenase (*tmoA*) gene of *P. putida* F1 specific primers were also tried for amplification with our isolated DNA but no specific product was obtained. So we chose to concentrate on *todC1* gene for further research. The sequence of *tce1* showed homology to other known toluene dioxygenase gene on the basis of phylogenetic analysis. This is the first instance when *B. cereus* containing *todC1* gene can degrade TCE efficiently (Mitra and Roy, 2011). In this study, the theoretical molecular weight of the recombinant protein was 12.6 kDa. The molecular weight of the induced protein from pSM 101 containing *E. coli* was found to be 14.3 kDa. This discrepancy may be due to the expression of *tce1* gene sequence that was partial instead of the full-length coding region. The recombinant *E. coli* could degrade TCE efficiently was analyzed by Fujiwara test. When any 1:1:1-Trichloro compound is heated in presence of pyridine and aqueous caustic alkali, a red color is imparted to the upper layer. The reaction is extremely sensitive. The nature of the red material has not been elucidated, but the mechanism has been proposed (Moss and Rylance, 1966) (Figure 3). From the observations in Fujiwara test, it can be concluded that the compound, TCE was metabolized by the bacterium *B.*

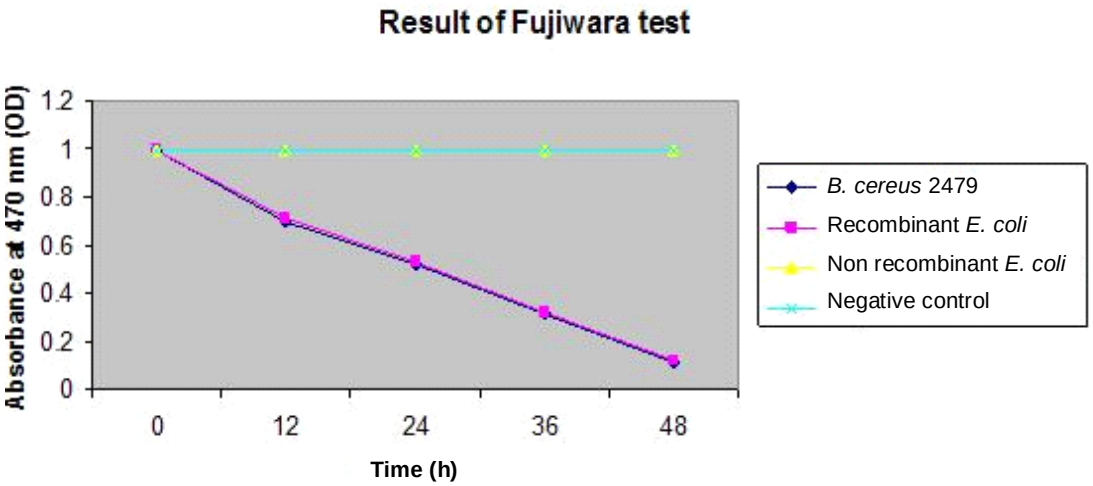

Figure 4. Optical density of *B. cereus*, recombinant *E.coli*, non recombinant *E.coli* and without any bacteria in the TCE containing M9 media recorded in different time (h) intervals, measured as OD_{470} (nm).

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CCG AAT ACT CGA CGC CAT TAT GTT TCG AGT CAC AAG TAA TGG CCC GCA AAT TTC
TTT TGG CTA TTG CGA CCT GGC ATC TGC GCC GGC GAA CTT TGA ACT AGG TCA AAT
TTA ATG GCA AAG TTA CTC GCT TTA GGT GAC TCC CAT CTC GAA GCC CTT AAA CTT
    M   A   K   L   L   A   L   G   D   S   H   L   E   A  L K    L
GCA GCT GAC TTG AAT CTT CTA GCT GTT GAT GAG GTT AGG TTT TGC ATC GTG CCT
 A   A   D    L N   L    L   A   V   D   E V   R   F   C   I   V   P
GGA GCT ACT GCA GTC GGG ATG CGC AAC CCT AAC TCG ATT ACC AAT GCG CTG ACC
G   A    T   A   V    G   M   R   N   P   N   S   I   T   N   A   L   T
CTG TTT CGC ACG GCG GCC TCC AGC ATG CAA GAT GCG ACG CAT ATT CTA GTA CAT
 L   F   R   T   A   A    S   S M   Q    D   A   T   H   I   L   V   H
CTT GGC GAA GTG GAC TGT GGT TTC GTA ATG TGG TGG AGA CAG CAA AAA TAT GGC
 L   G   E   V   D   C   G   F   V   M   W   W   R   Q    Q   K   Y   G
GAG CCG ATA GAA CAT CAA ATG CGT GAA TCG TTG GCC GCC TAC AGC GAC TTC ATT
 E   P   I   E   H   Q   M   R   E   S   L   A   A   Y    S   D   F   I
TTA GAA TTA CAA TCG ATG AAT T
  L   E   L   Q  S  M   N
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Figure 5. Nueclotide sequence and deduced amino acid sequence of *tce1* gene (Accession no. GU183105). The deduced amino acid sequences are shown in one letter code.

cereus strain 2479 and recombinant *E. coli*. No degradation was found in the control and non recombinant *E. coli* containing media (Figure 4). The structure and biochemical function of *Tce1* protein require further investigation.

Hydrophobic force is the dominant among all weak forces for protein folding. Hydropathy profile determination based on amino acids hydropathy scale would help in understanding region of protein forming hydrophobic domain, membrane spanning region,

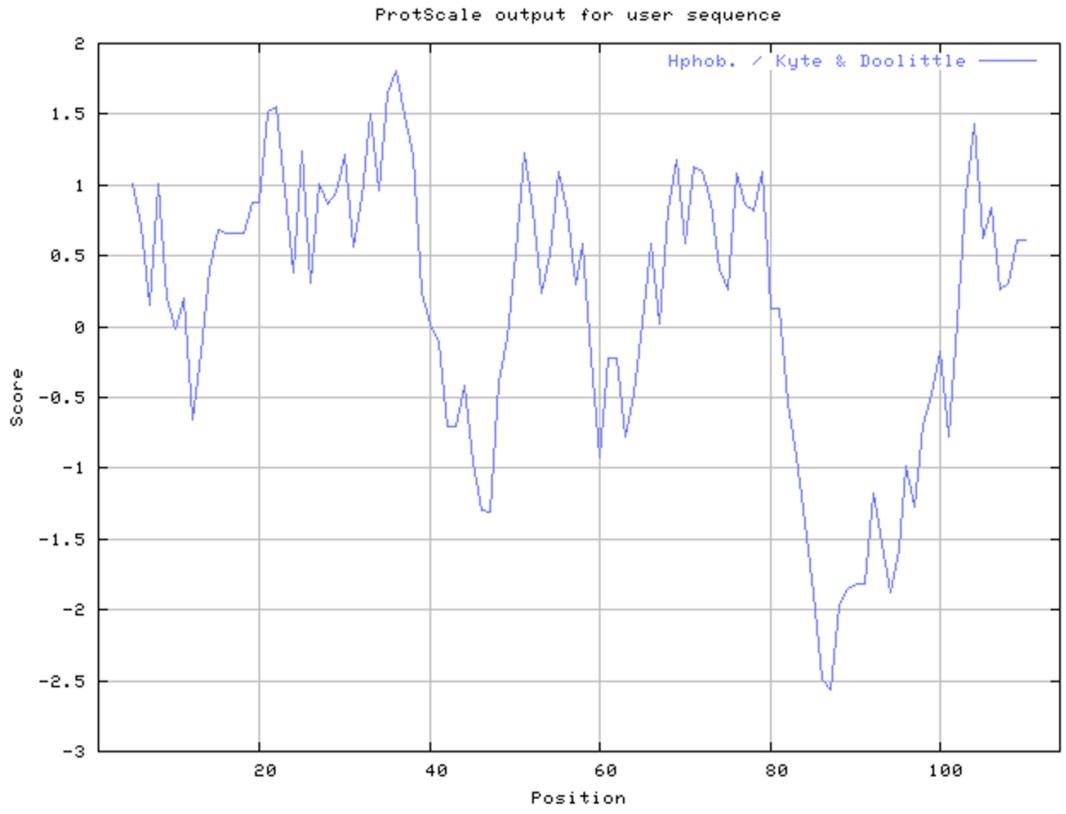

Figure 6. Hydrophobicity analysis of *Tce1* protein. using the scale Hphob. / Kyte and Doolittle, the horizontal axis indicates the location of amino acids and the vertical axis indicates hydrophobic percentile; hydrophobicity appears when percentile is higher than 0, while hydrophilicity appears when percentile is lower than 0.

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         |         |         |         |         |         |         |
MAKLLALGDSHLEALKLAADLNLLAVDEVRFCIVPGATAVGMRNPNSITNALTLFRTAASSMQDATHILV
ccHHHHHHHHHHHHHHHHHHHHHHHHHHHHHeeccHHHHtHHtccccchHHHHHHHHHHHHHHHHHHHH
HLGEVDCGFVMWWRQQKYGEPIEHQMRESLAAYSDFILELQSMN
hhhhhhhhhehhhhhhhhchhhhhhhhhhhhhhhhhhhhhhcc
Sequence length :   114
DPM :
   Alpha helix      (Hh) :    97 is  85.09%
   3₁₀ helix        (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :     3 is   2.63%
   Beta turn        (Tt) :     2 is   1.75%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :    12 is  10.53%
   Other states          :     0 is   0.00%
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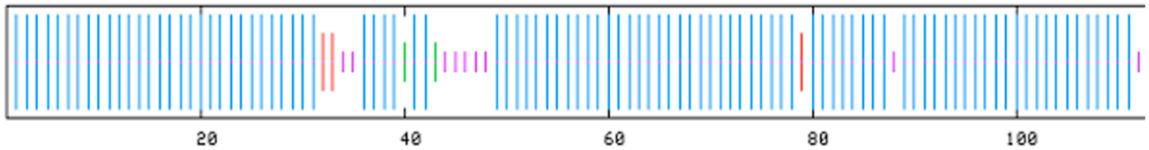

Figure 7. Secondary structure prediction of *Tce1* protein. Blue (longest): alpha helix; purple (shortest): random *coil*; red (medium): extended strand.

potential antigenic site and region that exposed on the protein's surface. Kyte and Doolittle method is the most popular one for determination of hydropathy profile which was used for the present study. Hydropathy profile analysis (Figure 6) shows presence of both hydrophobic and hydrophilic regions indication the possibility of balance interplay of all kind of weak forces for the formation of folded protein. Sequence region from 80 to 105 seems to form the surface of the protein and it also possesses strong hydrophilicity. Prediction of local order structure and their sequence from the primary sequence information help to understand folding topology, folding classes and other structural insights. Our analysis of secondary structural properties of the protein using authentic web server provides insightful observation in that the protein predominately composed of alpha-type structure with very little content of other structures indicating the possibility of formation of alpha-type domain. In this context it is widely known that the protein belong in the family dioxygenase whose hydroxylase component was shown to be oligomeric protein constituted either by alpha-type or alpha and beta-type structures (Butler and Mason, 1997). Thus it seems reasonable to think that our protein seems to possess former type hydroxylase properties.

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