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Cloning of Choline Dehydrogenase from *Escherichia coli*: Its Polynucleotide and Polypeptide Analysis

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ABSTRACT

Choline dehydrogenase catalyzes the oxidation of choline to glycine betaine via betaine aldehyde in glycine betaine biosynthesis. Glycine betaine is a compatible solute, able to restore and maintain osmotic balance of living cells under stress. In this study, choline dehydrogenase (*betA*) gene encoding for glycine betaine biosynthesis in *Escherichia coli* isolated from salted shark (*Scoliodon* sp.) was cloned and sequenced. The *betA* gene sequence reported in this study contains several base substitutions with that of reported sequences in GenBank, resulting in the altered amino acid sequences of the translated proteins.

Key words: *E. coli*, compatible solutes, glycine betaine, salt stress, *betA*

INTRODUCTION

Microorganisms are often exposed to the changes in environmentalparameters (Csonka, 1989; Lucht and Bremer, 1994). To cope with the environment stress, bacteria accumulate osmotically active compatible solutes, including: potassium, proline, glutamic acid, glutamine, α-aminobutyric acid, ectoine and betaine (Poolman and Glaasker, 1998). Among the compatible solutes, glycine betaine is a effective osmoprotectant (Wood *et al*., 2001). Glycinebetaine (N, N, N-trimethylglycine) is a quaternary ammonium compound that occurs naturally in a wide variety of plants, animals and microorganisms (Rhodes and Hanson, 1993). Numerous *in vitro* experiments have indicated that betaine acts as an osmoprotectant by stabilizing both the quaternary structure of proteins and cellular membrane against the adverse effects of high salinity and extreme temperatures (Gorham, 1995). Biosynthesis of betaine is catalyzed in a single step reaction by choline oxidase in soil bacterium, *Arthrobacter globiformis* (Ikuta *et al*., 1977). In higher plants, such as spinach (Rathinasabapathi *et al*., 1997), sugar beet and amaranth (Russell *et al*., 1998) betaine biosynthesis is catalyzed by choline monooxygenase in combination with betaine aldehyde dehydrogenase. In *E. coli*, the biosynthetic pathway for the production of glycine betaine from choline has been well characterized at the genetic level (Landfald and Strom, 1986). It has been shown that four genes encoding choline dehydrogenase (*betA*), betaine aldehyde dehydrogenase (*betB*), a putative regulator (*betI*) and a choline transporter (*betT*) are clustered in the *bet* operon (Andresen *et al*., 1988). The enzymes involved in the biosynthesis of betaine from bacteria and plants have been functionally characterized (Weretilnyk and Hanson, 1990; Gadda and McAllister-Wilkins, 2003).

Recently, bacterial glycine betaine synthesizing enzymes have become a major target in developing stress tolerant crop plants of economic interest. Previous studies reports the resistance towards salinity and low temperature in transgenic tobacco expressing the two *E. coli* genes *betA* and *betB* (Holmstrom *et al*., 2000), signifying the practical applications of choline dehydrogenase. Choline dehydrogenase (*betA*) of *E. coli* catalyses the first step in glycine betaine biosynthesis, the oxidation of choline. However, this enzyme also catalyses the second step, the dehydrogenation of betaine aldehyde to betaine (Landfald and Strom, 1986). Choline dehydrogenase catalyzes the four electron oxidation of choline to glycine betaine via a betaine aldehyde intermediate (Tsuge *et al*., 1980). Only nominal studies in characterization of choline dehydrogenase from *E. coli* have been reported to date. In this study, we report the characterization and structural analysis of choline dehydrogenase in *E. coli*. Moreover, the sequence analysis of choline dehydrogenase from our isolate shows several base substitutions with that of reported sequences in GenBank, resulting in the altered amino acid sequences of the translated proteins.

MATERIALS AND METHODS

Bacterial strains and plasmids: *Escherichia coli* was isolated from salted shark (*Scoliodon* sp.) procured from retail fish markets in Cochin, Kerala, India in 2004 September. Microbial identification and biochemical characterization of *E. coli* was carried out as per [U.S. Food and Drug Administration (USFDA)]. *Escherichia coli* JM109 and plasmid pTZ57R/T (MBI Fermentas, Hanover, Maryland, USA) were used as transformation host and cloning vector, respectively.

DNA extraction and gene amplification: Genomic DNA extraction from *E. coli* was performed following the method of Ausubel *et al*. (1994). Plasmid DNA was recovered from the transformed clones by alkali lysis method (Sambrook and Russell, 2001). A pair of primers bAF (5′-CGTATGCAATTTGACTACATCATT-3′) and bAR (5′-GCATCATTTTTTCGCTCTCACCG-3′) were designed to amplify the *betA* gene. PCR amplification was carried out with 30 cycles of 1 min at 94°C, 1.5 min at 50°C and 2 min at 72°C. Additional extension was carried out for 5 min at 72°C using high fidelity PCR enzyme mix (MBI Fermentas).

Cloning and sequencing: The *betA* gene amplicon was purified using Perfectprep Gel Cleanup Kit (Eppendorf, Germany) and cloned into pTZ57R/T according to the manufacture's instructions. The pTZ57R/T-*betA* construct was transformed into *E. coli* JM109 (*recA*1, *endA*1, *gyrA*96, *thi*-1, *hsdR*17 (*rK-mk*+), e14-(*mcrA*-), *supE*44, *relA*1, Ä(*lac-proAB*)/F' [*traD*36, *proAB*+, *lac I*q, *lacZ*ÄM15]. Positive transformants were selected for PCR amplification with vector primers M13f-M13r (MBI Fermentas) and the clones with the correct insert as judged by size were sequenced on ABI PRISM 377 genetic analyzer (Applied Biosystems Inc., Foster City, CA, USA).

***In silico* sequence analysis:** The nucleotide sequences obtained were analysed with the available database sequences by BLAST analysis using the NCBI (http://www.ncbi.nlm.nih.gov). The sequences were aligned and clustered using CLUSTAL-X version 1.81 (Thompson *et al*., 1997). The output alignments were imported into the GeneDoc program (http://www.psc.edu/biomed/genedoc/) and BioEdit version 7.05 program (www.mbio.ncsu.edu/BioEdit/) to calculate the percent identities among the nucleotide and amino acid sequences. The molecular masses and the theoretical pI values of the polypeptides were predicted using the ProtParam tool (http://www.expasy.org/tools/protparam.html). The secondary structure prediction was performed using the PROTEAN program (DNASTAR, Inc, Madison, USA).

RESULTS AND DISCUSSION

Cloning and sequence analysis: Based on the sequence analysis, it was previously reported that the *betA* gene of *E. coli* codes for choline dehydrogenase (Lamark *et al.*, 1991). To date, only minimum reports on the characterization of choline dehydrogenase has been reported (Tsuge *et al.*, 1980; Gadda and McAllister-Wilkins, 2003). As the first step towards the functional characterization of choline dehydrogenase, in this study we cloned and characterized the *betA* gene from *E. coli* cells under the control of inducible promoter.

The choline dehydrogenase encodes the polypeptides comprised of 556 amino acids with the calculated molecular masses of 61848 Da., based on the *in silico* estimates. The *betA* gene coded for choline dehydrogenase was PCR amplified and is found to have the polynucleotides of 1671 bp length (Fig. 1). The *betA* amplicon was purified from agarose gel, ligated in pTZ57R/T cloning vector and transformed into *E. coli* JM109. Plasmid pTZ57R/T with *betA* gene insert was confirmed by nucleotide sequencing. The nucleotide sequence of *betA* gene was submitted to GenBank and have been given accession no. FJ823260.

The *in silico* sequence analysis of *betA* gene revealed a total of thirty-one base substitutions at the nucleotide level (Fig. 2) with that of the sequences deposited in the GenBank (accession nos. X52905 and M77738). However, only two of these changes translated into change of amino acids. The differences were observed at positions 133 and 452, which resulted in the amino acid substitution of valine with methionine and of threonine with valine residue, respectively (Fig. 3). No internal stop codons were observed due to the base substitution in the gene. Upon BLAST analysis it was found that the deduced amino acid sequence of *betA* was highly homologous to choline dehydrogenase of reported strains; 99% identity with *E. coli* (accession no. X52905) and 98% identity with *E. coli* (accession no. M77738). To authenticate the nucleotide variation in the *betA* sequence of our isolate, the gene was yet again PCR amplified from the genomic DNA with *Taq* DNA polymerase (Dynazyme II, Espoo, Finland). The underlying principle for this strategy was that feasible misincorporations of nucleotides in gene amplification might occur at different positions by using different DNA polymerases and PCR protocols. The PCR product was cloned in pDrive vector (Qiagen, Germany) and sequenced. The nucleotide sequence of the *betA* gene ligated in pDrive was the same as dogged using pTZ57R/T-*betA* cassette. This result confirms that the base divergence was conserved in the genome and not due to the external parameters.

Evaluation of the deduced amino acid sequence of *betA* gene with reported choline dehydrogenase sequences in the database revealed a maximum similarity. However, the sequence

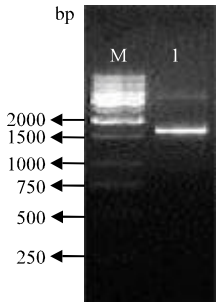

Fig. 1: Agarose gel electrophoresis of *betA* gene. Lane 1: *betA* amplicon; Lane M: DNA molecular weight marker

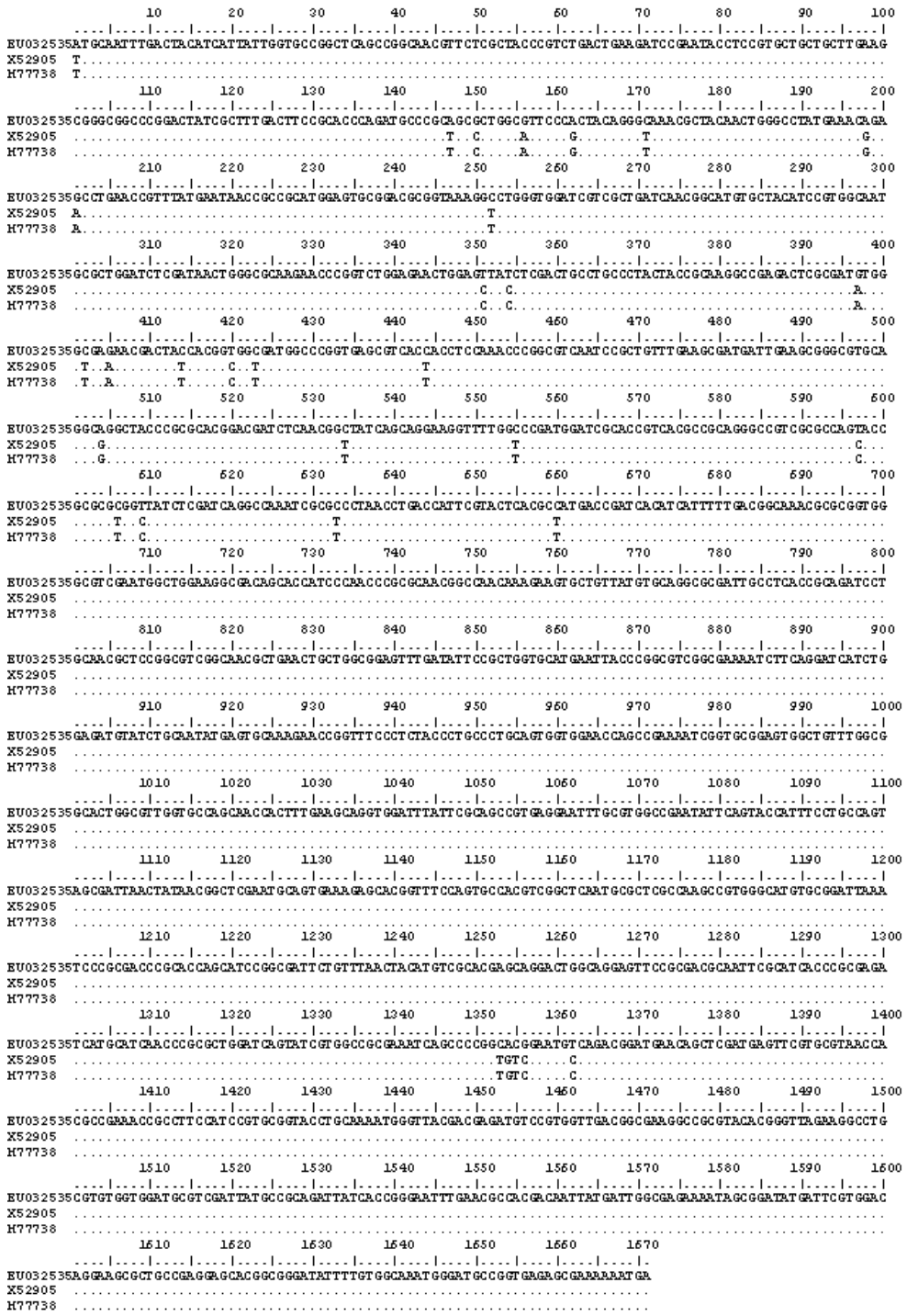

Fig. 2: BioEdit analysis of nucleotide substitutions of choline dehydrogenase (*betA*) in *E. coli* [FJ823260] (this study), with *betA* genes from *E. coli* strains [X52905 and M77738]

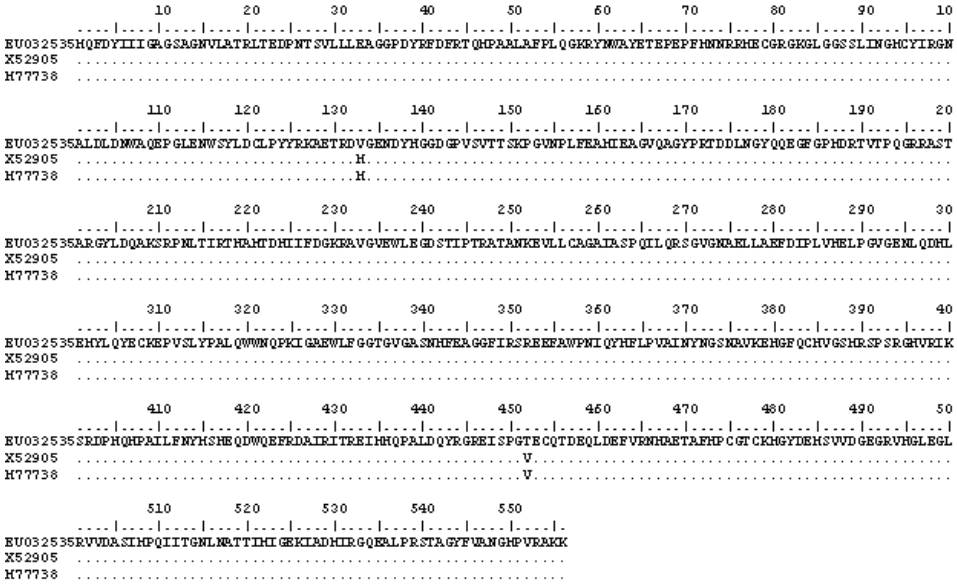

Fig. 3: BioEdit analysis of amino acid substitutions of choline dehydrogenase (*betA*) in *E. coli* [FJ823260] (this study), with *betA* genes from *E. coli* strains [X52905 and M77738]

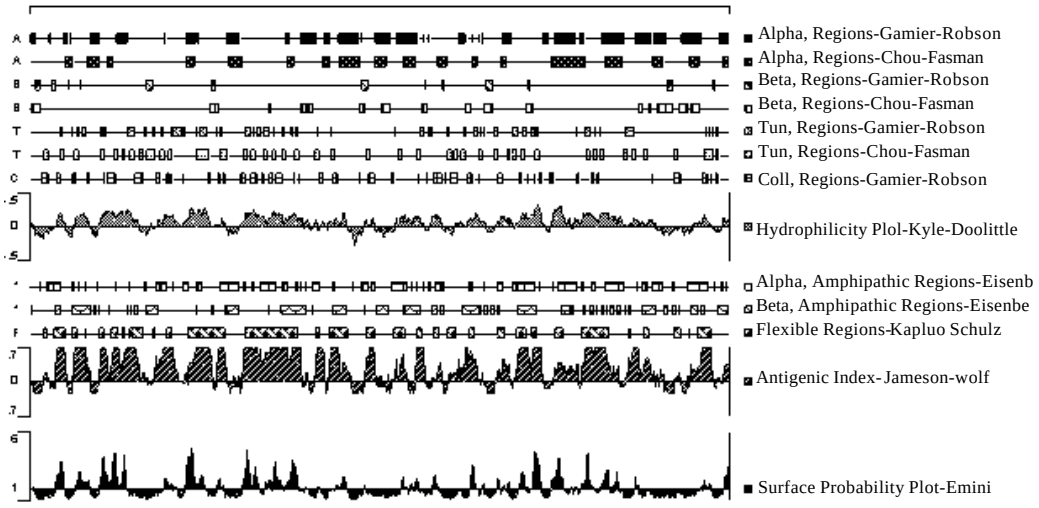

Fig. 4: Secondary structure prediction of choline dehydrogenase using PROTEAN

analysis of *betA* of our isolate showed several base substitutions with that of reported sequences, resulting in the altered amino acid sequences of the translated proteins.

Secondary structure prediction analysis: The secondary structure of choline dehydrogenase was predicted to have the alpha-helical structure with maximum hydrophilic molecules. The prediction analysis also revealed the presence of much acidic amino acids, regions with high antigenicity and very high backbone chain flexibility (Fig. 4). Upon analysis of *betA* protein, the

Fig. 5: Secondary structure analysis using PROTEAN. The analysis was performed using choline dehydrogenase from *E. coli* [FJ823260] (this study) and from *E. coli* strains [X52905 and M77738]

predicted charge at pH 7.0 was 15.45 with the isoelectric point of 5.52. Common amino acids include 58% glycine, 45% alanine, 41% glutamic acid, 40% leucine, 37% arginine, 33% praline and 30% each of isoleucine and aspartic acid.

The secondary structure prediction results also exhibited considerable similarity with the reported choline dehydrogenases from *E. coli* strains. Upon structural analysis, both Chou-Fasman and Garnier-Robson prediction of alpha helix structures showed a maximum similarity. These results suggested that the active domains of the enzyme from our isolate have the considerable identity with the database reports (Fig. 5).

In conclusion, this study represents the first instance in which the choline dehydrogenase in *E. coli* isolated from salted fish has been cloned and characterized in detail. Moreover, the determination of protein structure modification due to the nucleotide substitutions will certainly provide the basis for performing site-directed mutagenesis to improve the production and configuration of the osmolytes of biotechnological interest. This, in turn, has great potential in biotechnological applications aimed at stress tolerance in crop plants of economic interest.

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