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Short Communication

PHYLOGENETIC ANALYSIS OF COLD SHOCK PROTEINS IN PSEUDOMONAS SPECIES

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ABSTRACT

Objective: The present study focuses on the determination of the relativity of the different types of cold shock proteins.

Methods: Our study was to determine the relationship among the types of CSPs. Three different strains of pseudomonas genus, *Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Pseudomonas putida* were chosen and molecular profiling was performed. The sequences thus obtained were subjected to multiple sequence analysis in ClustalW database. The molecular evolution and phylogenetic study have been carried out using phylodraw.

Results: The phylogenetic analysis has clearly revealed the evolutionary pattern of cold shock proteins in pseudomonas species and the current stress of mutation among the strains.

Conclusion: Phylogenetic analysis of cold shock proteins has clearly shown that important conserved sequences can be very useful to study the phylogeny of bacteria.

Keywords: Cold Shock Proteins, Phylogenetic Analysis, Pseudomonas, CspD

Microorganisms are known to survive under a wide range of environmental conditions ranging from hot springs to ice-cold Polar Regions and from salt brines to sulphur vents. However, the occurrence of microorganisms at near zero or subzero temperatures has always posed the riddle to biologists. Research regarding cold-adapted microorganisms not only help to understand the molecular mechanisms of cold adaptation, but also projects the significant and potential use of the organisms in various biotechnological, industrial and healthcare applications [1, 2]. Most often, bacteria have to adapt with more than one extreme condition at a given single time in natural environmental condition. Some of the places which are frigid viz. Antarctica with such adverse environment where bacteria have to be cold-tolerant as well as desiccation-tolerant [2, 3]. Moreover, bacteria dwelling at the bottom of the ocean ought to be salt-tolerant as well as pressure-tolerant. Interestingly, fewer known bio-molecules enable the microorganisms to cope up with more than one stress factor at a time. Microorganisms utilize bio-molecules and interlink the mechanisms involved in the process to different environmental stress factors for adaptation [2]. Among various bio-molecules which are responsible for developing cold resistance for microorganisms, the cold shock proteins (CSPs) play a significant role [4]. CSPs are protein domains of about 70 amino acids which have been found in prokaryotic and eukaryotic DNA-binding proteins. These so-called cold shock proteins are thought to help the cell to live in temperatures lower than optimum growth temperature, possibly by condensation of the chromosome and prokaryotic nucleoid organization [4]. CSP's are nucleic acid binding proteins and are well conserved in plants, animals and bacteria. Prokaryotes and eukaryotes exhibit cold shock response with the production of cold shock proteins to overcome detrimental cold shock effects. CSPs play a significance role in cell physiology and studies have been reported that they bind mRNA and regulate in ribosomal translation [4].

Temperature downshifts the cold shock response which has been studied in detail using model systems such as *Escherichia coli* and *Bacillus subtilis* [2, 6]. Acclimation phase (lag period of cell growth) was observed when an exponentially growing culture of *Escherichia coli* is shifted from 37 °C to 15 °C [2, 6]. This phase is characterized by the induction of cold shock proteins against a severe inhibition of general protein synthesis and leads to the growth resumption. Studies have shown that nine CSPA homologues were found in *Escherichia coli*, in which only CspA, CspB, CspG and CspI were found to be cold shock inducible [7-10]. Moreover, Xia and team have suggested that the functions of the CspA family members may overlap which can substitute for each other during cold acclimation [11].

In this study, a preliminary investigation was carried out to determine the relativity among various types of CSPs where complete molecular profiling was performed for the microorganism, necessitating different strains of the microorganism to be considered. For the purpose, three different strains of an Antarctic bacterium (pseudomonas genus) were chosen and studied. The strains used in this study were, *Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Pseudomonas putida* among which *Pseudomonas aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of significant clinical relevance.

The key word 'Cold Shock Proteins' yielded protein sequences from GenBank [URL: http://www.ncbi.nlm.nih.gov/Genbank/]; Synonyms to cold shock proteins were also collected. CSP coding proteins were chosen selectively from the protein sequences. The other proteins such as partial cold shock proteins were omitted from the analysis data. Total 225 protein sequences were collected from three different species, *Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas putida* and geographical locations such as China, India, Japan, Spain and USA were used for the analysis.

Multiple sequence alignment was performed using the software program CLUSTAL X (1.83) [12]. From the multiple sequence alignment, the guide tree was derived by using phylodraw software (Version 1.0). To justify the confidence of the clades, re-sampling method (bootstrap) was used with 100 trails.

Alignments were analyzed and phylogenetic relationships among the sequences were established using different procedures: Neighbor-Joining (NJ) [13], Fast Minimum Evolution (Fast ME) [14]. The final tree was displayed by using MEGA 3.1 [15], the nodes and clades of CSPs were traced out by visual examination.

Three different strains of pseudomonas were selected initially and the Phylogenetic tree was constructed based on the Mega software. Fig. 1 shows the Phylogenetic tree of Cold Shock Proteins in

pseudomonas species. The CSP sequences of pseudomonas aeruginosa were used as an out-group (3 and 4). The CspD subgroup of Cold Shock Proteins was found to be radiating from the root of the tree which suggested the divergence in CSPs. By the types of CSPs and the geographical location of the species, branching was speculated. Cold acclimation proteins were not related to any of the CSPs used and is found to be positioned separately in the tree which clearly indicated towards different Phylogenetic pattern [fig. 1].

The phylogenetic tree of cold shock proteins from pseudomonas species showed the grouping of 6 clades based on the identity in the query sequence. In the case of clade1, the most identical sequences of cold shock proteins from various strains were grouped and well conserved with amino acids that formed a group. Clade 1 splits into three lineages that consist of different subtrees. The top lineage with high identity contains a maximum number of sequences that confirmed the identical function of cold shock proteins. A change in the minimum number of amino acids directly correlated with the grouping of strains also showed less divergence from the ancestor. The other subtree also formed a different cluster with respective amino acids and mutation was observed in the sequence.

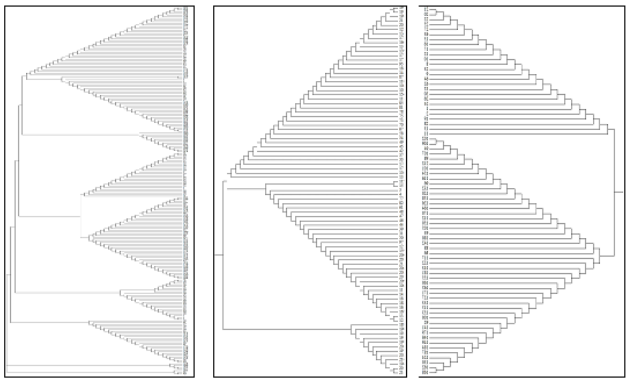

Fig. 1: Phylogenetic tree (phylogram) of cold shock proteins in pseudomonas species

In clade 1, small group members of cold shock proteins were also observed with the different evolution from the ancestor. In the case of clade 2, sequences with appropriate identity were combined and split into two subgroups. And the subtree was again split into two, based on the corresponding amino acid change. The subtree 1 with sequences of USA and Japan strains confirmed the evolution respective to the identity. Interestingly, subtree two was grouped with little divergence and formed a separate lineage from the ancestor. In the case of clade 3, the sequences from strains belonged to countries like USA, Japan, China and others and showed the conserved identity that grouped together. The tree showed the two sub-tress that split based on the amino acids variation. The tree pattern confirmed the evolutionary pattern with the ancestor. In the case of clade 4, the sequences from strains belong to mostly of USA and Japan showed the moderate identity that grouped together and started to diverge based on positive selection of amino acid mutation on cold shock protein. The clade four tree also showed the two sub-tress that split based on the amino acids variation. Clade 5 and 6 showed the divergence of cold shock proteins with the ancestor with less identity and change in amino acids. In the case of clade 5 with four sequences consists strain of WH6 and GB-1, confirmed the change in amino acids. In the case of clade 6, three sequences of short length 69 amino acids belong to strain SBW25 and S16 and confirmed the change in amino acids and also the role of cold shock proteins in the specific strains. Overall, our results suggested the evolutionary pattern of cold shock proteins in pseudomonas species and the current stress of mutation among the strains.

The results indicates that the phylogenetic tree could be reconstructed using more bootstrap values. The results also strongly suggest that other species of pseudomonas can be considered from different geographical areas to generalize the Phylogenetic tree. Phylogenetic hypotheses have become the framework for the choice of organisms in genomic analyses. Molecular biologists are using phylogenetic trees to guide their sampling of taxa for comparative research. Phylogenetic trees are useful in the fields of biology such as bioinformatics, systematic and comparative phylogenetics.

CONFLICT OF INTERESTS

We hereby declare that there is no conflict of interest

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