Plant Archives Vol. 18 No. 2, 2018 pp. 1643-1651 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STRUCTURAL AND FUNCTIONAL *IN-SILICO* ANALYSIS OF TOXIN-ANTITOXIN PROTEINS IN PERSISTER CELLS OF *PSEUDOMONAS AERUGINOSA*

Ravi Kumar Chaudhary[1], Girraj Singh[1], Ram Naraian[2] and Siya Ram[1,*]

[1]School of Biotechnology, Gautam Buddha University, Greater Noida - 201 312 (U.P.), India.
[2]Department of Biotech., Faculty of Sci., Veer Bahadur Singh Purvanchal University, Jaunpur-222 003, (U.P.), India.

Abstract

Pseudomonas aeruginosa is an opportunistic as well as one of the most challenging hospitals and community-acquired pathogen producing toxin-antitoxin (TA). The production of TA is thought to regulate the multidrug tolerance and pathogenicity in bacterial pathogens. Recently, it has been well recognized that TA systems play a very crucial role in the formation of persister cells, which leads to recurrent chronic infections. Toxin-antitoxin proteins interact with RNA and protein molecules present in bacterial cell, which, consequently, halts the normal cellular process by inhibiting the molecules involved in transcription and translation as well as in other metabolic pathways. In this study, we have extensively assessed the homology modeling, protein interaction and functional relationship, along with the active site of TA proteins of *P. aeruginosa*. Our results represent the secondary structures of all TA proteins, which were highly conserved, and their sequence identity was between 88 to 100% through the BLASTp. Additionally, we compared the three dimensional (3D) models for all TA proteins through homology modeling that identified the HigA, HigB, ParD and ParE proteins as good models. Finally, the CASTp was utilized to identify the active site, which is generally specific for the binding of toxin and DNA molecules. The study suggests that all TA proteins found in *P. aeruginosa* have great potential function and responsible for antimicrobial drug tolerance and pathogenicity.

Key words : Persister cell, toxin-antitoxin, *Pseudomonas aeruginosa*, homology modeling.

Introduction

Development of persister cells employs bacterial toxin and antitoxin systems to confer multiple drug resistance and occurrence of recurrent and chronic infections (Lewis, 2010). The formation of persister cells is initiated by the environmental stress, phase of a bacterial cells and exposure of antibiotics (Lee and Lee, 2016; Helaine *et al*., 2015; Berneir *et al*., 2013). *P. aeruginosa* is a rare and opportunistic pathogen that cause both acute and chronic infections in human (Balasabramanian *et al*., 2013). Toxin-antitoxin (TA) systems in bacterial pathogens ate composed of toxin and of a cognate antitoxin, which plays a crucial role in persister cell formation (Verstraeten *et al*., 2015; Germain *et al*., 2013). Synthesis of toxins inhibits bacterial protein synthesis, cell wall synthesis, DNA replication, therefore in consequence of a slow bacterial growth (Page and Peti, 2016) and development of resistant persister. Although the exact role of TA systems is not clear, but recent findings suggest that these systems play an important role in stress management and antibiotic resistance (Van Melderen, 2010; Gerdes *et al*., 1986).

Bacterial system posses five categories of TA system, classifies on the basis of their mode of action. However, among five different classes, class II is known to be widely adopted by bacteria and archaea (Schuster and Bertram, 2013; Van Melderen, 2010). The class II TA system is chemically proteinous in nature and its antitoxin system interacts with their cognate toxin and nullifies them (Goeders and Van Melderen, 2014). Additionally, it also inhibits the expression of toxin through suppressing transcription of TA proteins, where it is governed by the interaction of toxin and the palindrome sequence of the promoter (Ramisetty and Santhosh, 2017; Masuda and

***Author for correspondence :** E-mail: s.ram@gbu.ac.in

Inouye, 2017).

With the advent of recent advances in sequencing technology, dealing with the massive amount of data is most possible in recent years; but unfortunately, many of the genomes are still not fully annotated (Mills *et al*., 2015; Sevin and Barloy-Hubler, 2007). Therefore, employing many of the genes and proteins with uncharacterized functions are still need to be characterized. Since, analyses and experimental methodologies have several limitations including cost and time. Therefore, an alternative approach has been developed, including web-based bioinformatics software to analyze the uncharacterised TA proteins (Oany *et al*., 2015). Thus, in this regard researchers, contemporaly have drawn their attention, for characterization of the active site of acetyltransfirase protein of *Shigella flexneri*.

For this objective, the computed atlas of surface topography of proteins (CASTp) has been executed, which provide online resource for location, delineation and measurement of concave surface regions of 3D protein structures (Ahmed *et al*., 2017; Oany*et al*., 2015). Moreover, the approaches of homology modeling and comparative proteomics have been extensively used to predict the 3D structure, binding sites and probable functions of TA proteins (Mahmud *et al*., 2016). As it is obvious protein-protein interactions (PPI) control imperative cellular process, such as regulation of transcription, signal transduction, post-translational modification etc. (Lee and Yaffe, 2016). However, the PPI network is a robust approach for understanding the molecular mechanisms associated with pathogenesis, functional annotation of gene function, etc., which can be detected through computational and experimental methods (Leplae *et al*., 2011).

Therefore, in the present investigation attempt to get some insight about TA proteins function, predicting the secondary and 3D structure along with comparative proteomics and catalytic sites.

Materials and Methods

The present study emphasized the homology modeling of five TA protein pairs (total 10 TA proteins), model quality assessment, physicochemical property analysis, protein interactions, and determination of the active site of TA proteins. All the above-mentioned, *In-silico* based analyses were conducted using web-based software and tools.

Homology modeling was performed through MODELLER (https://toolkit.tuebingen.mpg.de/#/tools/modeller) toobtain the 3D structures of TA proteins with default parameters against the PDB, while the suitable template was obtained by BLASTP search (https://blast.ncbi.nlm.nih.gov) (Altschu *et al*., 2005; Söding, 2004; Benson *et al*., 2000).

The model quality assessment was performed using QMEAN program of ExPASy server in SWISS-MODEL workspace (https://swissmodel.expasy.org/qmean/) (Benkert *et al*., 2010; Bordoli*et al*., 2008; Arnold *et al*., 2006). The 3D structures of TA proteins were visualized by using UCSF Chimera 1.5.3 and PyMOL (The PyMOL Molecular Graphics System, Version1.5.0.4, Schrödinger and LLC) (Pettersen *et al*., 2010; DeLano, 2002).

In a different observations physicochemical properties including the aliphatic and instability index, grand average of hydropathicity (GRAVY), isoelectric point (pI) and molecular weight were analyzed using Expasy tool Prot Param (http://web.expasy.org/protparam/) (Gasteiger *et al*., 2005). Moreover, to establish functional relationships with other proteins,protein interaction studies were analyzed using a STRING database to confirm the accurate functioning of TA protein residues interacting in diverse cellular environments (http://string-db.org/) (Szklarczyk *et al*., 2016; Szklarczyk *et al*., 2014). However, functional relationship of TA proteins among *P. aeruginosa* was analyzed by protein family (Pfam) database (https://pfam.xfam.org/) and all conserved patterns of toxin-antitoxin were highlighted as previously described (Bateman *et al*., 2004). Finally, the active sites of TA proteins were determined using computed atlas of surface topography of proteins (CASTp) server (http://sts.bioe.uic.edu/castp/) (Dundas *et al*., 2006).

Results and Discussion

Numerous web-based tools including BLASTp, MODELLER, STRING database and CASTp were used for the analysis of sequence identity, conserved domains for homology modeling, protein interactions with their functional relationships and site determination of TA proteins. Data related to the toxin and antitoxins found in *P. aeruginosa* are recorded in table 1. The study suggests that all TA proteins found in *P. aeruginosa* have a great functional potential of the TA proteins, which may be responsible for antimicrobial drug tolerance and pathogenicity of *P. aeruginosa* (Leplae *et al*., 2011). Similar observations regarding physicochemical properties of TA proteins in *P. aeruginosa* were also studied earlier (Chaudhary *et al*., 2017). Based on consensus predictions of the Pfam database of conserved domain database (CDD) and SUPERFAMILY, it can be

Table 1 : Details of protein family, homology showing with other microorganism and sequence identityof TA proteins found in *P. aeruginosa*.

Antitoxin/ Toxin	Name	Protein family	Sequence Identity	Microorganisms	Entry name	Score	E-value
TOX1	Hypothetical	No conserved domain	92%	*Achromobacter* sp	AJW29851.1	137	4e-41
TOX2	Hypothetical	No conserved domain	88%	*Klebsiella pneumoniae*	AKS10535.1	80.9	5e-19
T/AT1	Putative transcriptional regulator	MazE antitoxin	100%	*Bordetella bronchiseptica*	KCV24973.1	166	6e-52
T/AT2	Hypothetical protein	PIN superfamily	99%	*Xanthomonas gardneri*	WP_046934155.1	253	3e-85
RelB	DNA-damage-protein J	RelE/ParE family	99%	*Pseudomonas* sp.	AAT49350.1	187	4e-60
RelE	Type II TA system	COG3905	99%	*Pseudomonas* sp.	KJJ21186.1	135	8e-40
HigA	Antitoxin	Helix-turn-helix XRE	96%	*Pseudomonas* sp.	AAT49451.1	202	8e-66
HigB	Transcriptional regulator	ParE toxin superfamily	88%	*Pseudomonas xanthomarina*	WP_065983611.1	202	8e-66
parD	Antitoxin	ParD superfamily	99%	*Klebsiella pneumoniae*	WP_087638928.1	166	4e-52
parE	PIN domain-containing protein	ParE toxin superfamily	95%	*Klebsiella pneumoniae*	WP_087638929.1	201	2e-65

suggested that genes coding T/AT1 toxin and T/AT2 antitoxin proteins are located on the plasmid (pNOR-2000), which belongs to MazE antitoxin superfamily and PIN superfamily respectively. The MazE antitoxin is a known antidote of toxin MazF, that serves as a chromosomal addiction module, while PIN domain belongs to a large nuclease superfamily, It is strongly capable of both 5'-3' exonucleolytic activity and cleavage of bifurcated DNA strands in an endonucleolytic and structure-specific manner. However, Tox1 antitoxin and Tox2 toxin proteins synthesizing genes located on the plasmid as extrachromosomal, they do not have any conserved domain.

Based on the prediction of secondary structure of toxin-antitoxins found in *P. aeruginosa*; the toxin-antitoxin system can be categorized in two groups of TA systems located on plasmid includes tox1/ tox2 and at1/ at2. However, another group type II TA systems located in bacterial chromosomes include relBE, higBA and parDE. It is considered that relBE toxin-antitoxin is very important TA systems of *P. aeruginosa*, which are closely involved, in multidrug resistance and virulence in bacteria. The RelB toxin belongs to the RelE/ParE family and RelE toxin belongs to COG3905 superfamily. In addition, the RelE is a plasmid protein of stabilization system,while, COG3909 is a predicted transcriptome regulator. Moreover, the HigBA proteins belong to the helix-turn-helix XRE superfamily and ParE toxin superfamily respectively. Characteristically, HigA is an addiction module antidote protein, while HigB is RelE-like toxin of the type II TA system. In other hand, ParDE proteins belong to ParD superfamily and ParE toxin superfamily respectively; ParD is a well-known antitoxin while ParE is a plasmid stabilization protein. Once all five reported antitoxin proteins bound to their toxin component, they can bind DNA via N-terminus and inhibit the expression process of the operons that contain genes encoding TA system,which leads to antimicrobial tolerance and pathogenicity (Garcia-Pino *et al*., 2010; Anantharaman and Aravind, 2003). The predicted structure of all TA proteins contains motifs (β turn, γ turn and β hairpin), residue contacts (ligands, DNA/RNA and metal) and disulphide bonds(fig. 1a to 1d). All analyzed TA proteins showed diversified sequence identities as 82, 82, 100, 99, 99, 99, 96, 88, 99% in Tox1, Tox2, T/AT1, A/AT2, RelB, RelE, HigA, HigB and ParD respectively (fig. 2a to 2j). The secondary structures of the TA proteins are mostly conserved throughout the alignment (Garcia-Pino *et al*., 2010; Anantharaman and Aravind, 2003).

The homology modeling is an essential part of the

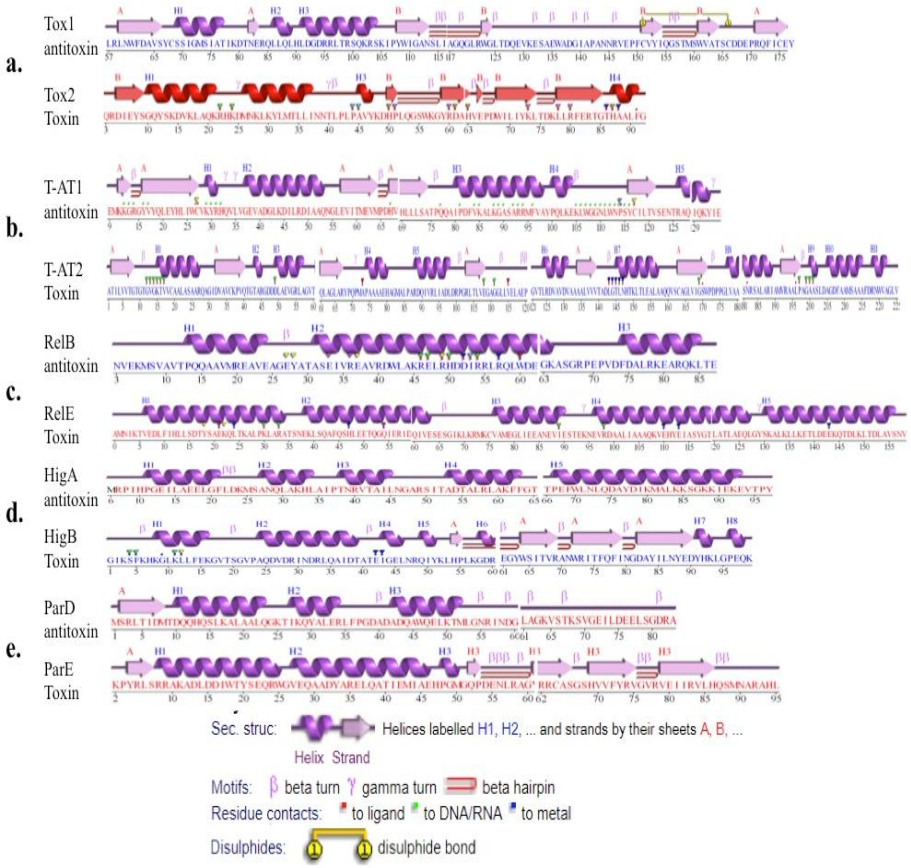

Fig. 1 : Secondary structure predictions of toxin-antitoxin proteins in *P. aeruginosa*. The TAsystems such as (a) tox1/tox2 and (b) t-at1/t-at2 are located on plasmid, while (c) relBE (d) higBA and (e) parDE are located on chromosome.

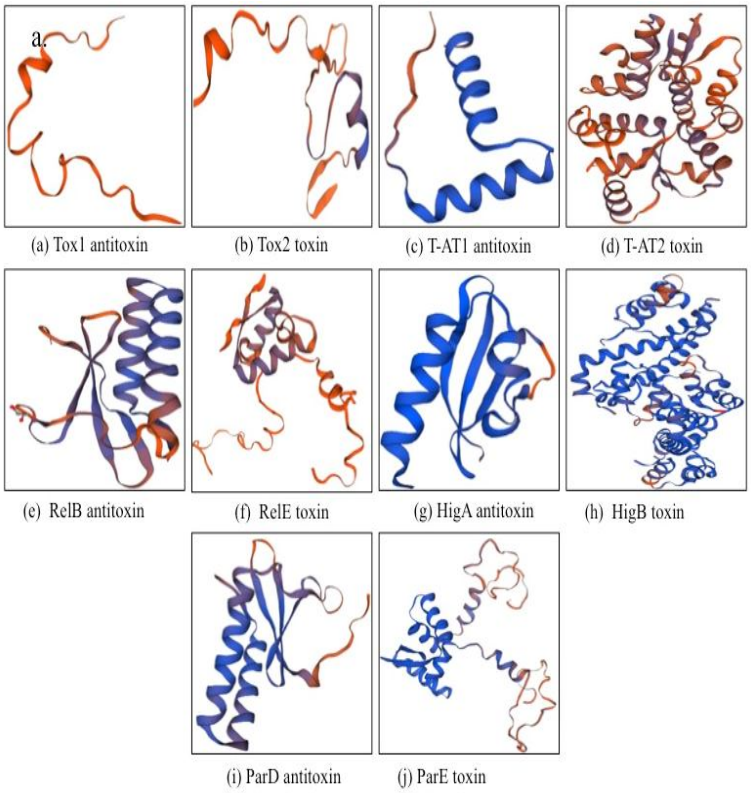

Fig. 2 : Predicted 3D models of TA proteins (a & b: tox1/tox2; c & d: t-at1/t-at2; e & f: relBE; g & h: higBA and I & j: parDE). The N-terminal end started with β sheet (Blue) and C-terminal end is coiled structure (Red).

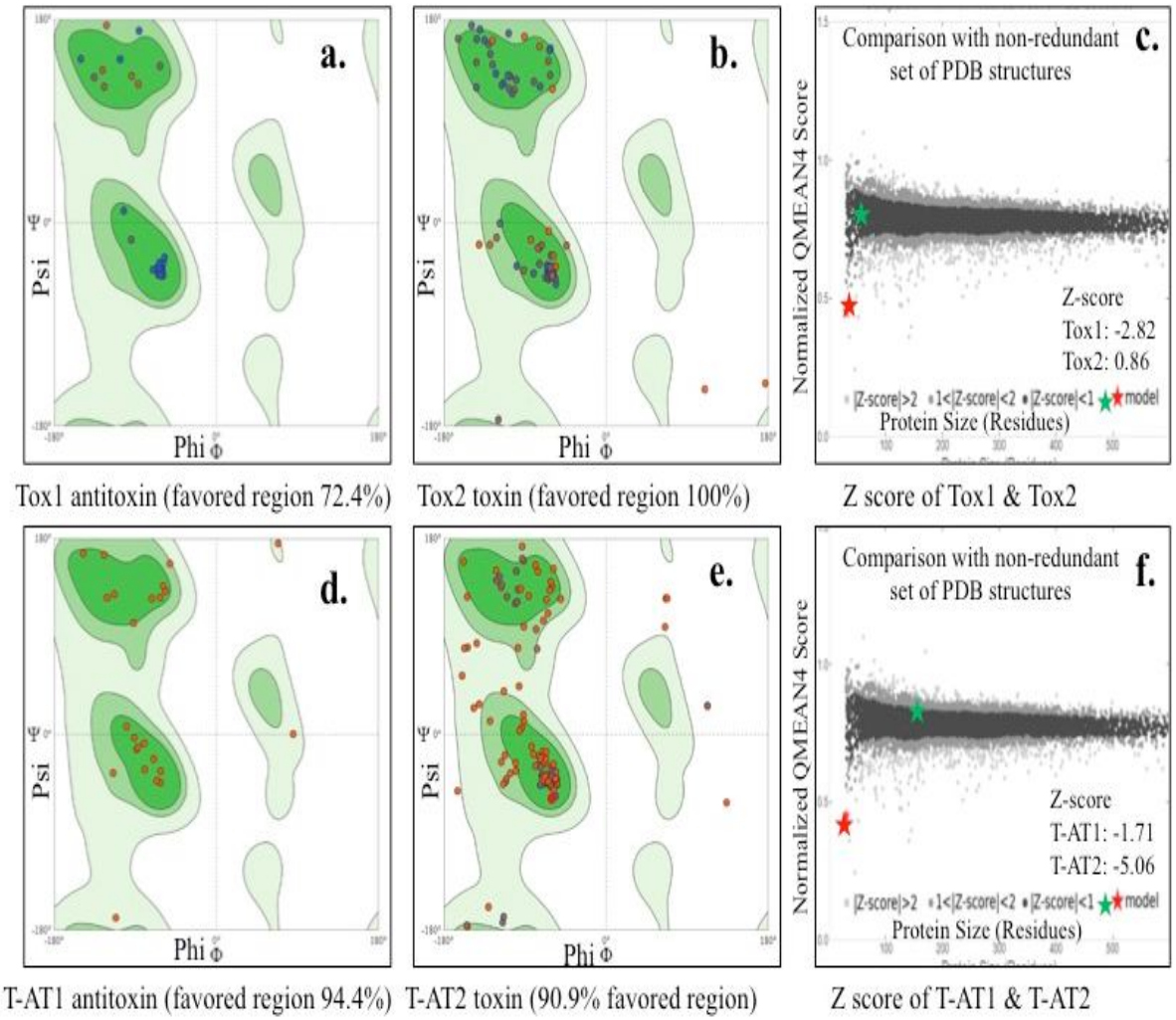

Fig. 3 : Ramachandran plots of modeled structures for plasmid based TA proteins(a: Tox1; b: Tox2; d: T-AT1; e: T-AT2) validated by QMEAN program. Graphical representation of Z-score value with non-redundant set of PDB (c: Tox1/Tox2 & f: T-AT1/T-AT2).

structural genomics in recent decade for comparative modeling of different unidentified structure with enormous tools (Chance *et al*., 2002; Vitkup *et al*., 2001). In the present study, our targeted TA proteins do not have any solved crystal structure. The homology models were compared through aliginment using templates such as 1ub4.1.C, 4q2u.1.A, 1ub4.1.C, 5wzf.1.A, 5cw7.1.F, 2an7.1.A, 3trb.1.A, 6f8s.1.B, 2an7.1.B, 3kxe.1.A for Tox1, Tox2, T-AT1, T-AT2, RelB, RelE, HigA, HigB, ParD, ParE, respectively. These templates were avalable in PDB. The homology modeling used to predict comparative 3D model of our TA proteins represent higher amount of similarity with our proteins.

In addition, the quality of the 3D models of all TA proteins of this study was checked by QMEAN server employing Ramachandran plot where percentage of total amino acid residues recorded in the most favored regions were as 72.4/100, 94.4/90.9, 92.2/83.6, 82.1/94.6 and 97.7/95.5 for Tox1/Tox2, T-At1/T-AT2, RelB/RelE, HigB/HigA and ParD/ParE respectively (figs. 3a, 3b, 3d, 3e, 4a 4b, 4d, 4e, 4g, 4h). Moreover, the 3D models of all TA proteins were also analyzed using dark grey zone through QMEAN resulted in QMEAN score as -2.82/0.86 (Tox1/Tox2); -1.71/-5.06 (T-AT1/T-AT2); -2.11/-5.68 (RelB/RelE); -0.66/-0.24 (HigB/HigA) and -4.95/-2.16 (ParD/ParE) (figs. 3c, 3f, 4c, 4f, 4i). As compared HigA, HigB, ParD and ParE models were found as good 3D models for TA proteins.

In a protein-protein analysis, the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database was used to map the network of all TA proteins. In this study, it was observed that RelB (PA0124)a 93 aa long protein interacts with its four predicted functional partners, namely PA0123 (Transcriptional regulator; 302 aa), PA0125 (Hypothetical protein; 75 aa), PA0126 (Hypothetical protein; 206 aa), PA0127 (Hypothetical

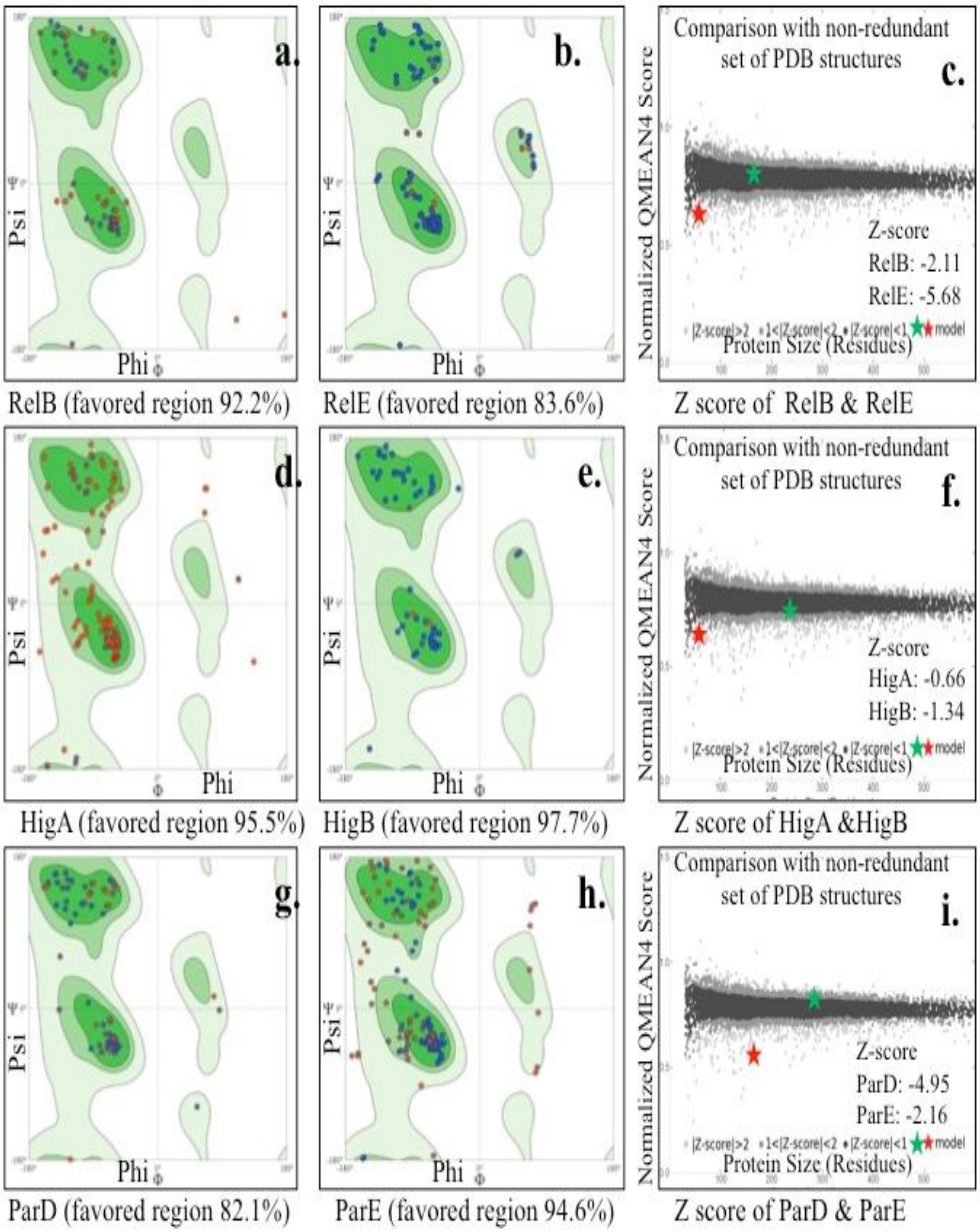

Fig. 4 : Ramachandran plots of modeled structures for chromosomal based TA proteins(a: RelB; b:RelE; d:HigA; e: HigB; g: ParD; h: ParE) validated by QMEAN program. Graphical representation of Z-score value with non-redundant set of PDB (c: RelB & RelE; f:HigA &HigB; i: ParD & ParE).

protein; 166 aa) (figure 5a). However, RelE (PA0125), 75 aa protein interacts with its six predicted functional partners PA0124 (Hypothetical protein; 93 aa), phoU (Phosphate uptake regulatory protein; 242 aa), phzM (Phenazine-specific methyltransferase; 334 aa), phzS (Hypothetical protein; 402 aa), PA0126 (Hypothetical protein; 206 aa) and PA0123 (Transcriptional regulator; 302 aa) (fig. 5b).

On the other hand, HigA (PA4674) a hypothetical protein of 101 aa long length has interacted with its five predicted functional partners *viz*., pyoS5 (Pyocin S5; 498 aa), pchG (Pyochelin biosynthetic protein PchG; 349 aa), pare (DNA to poisomerase IV subunit B; 629 aa), pchC (Pyochelin biosynthetic protein PchC; 251 aa) and pchE (Dihydroaeruginoic acid synthetase; 1438 aa) (fig. 5c). However, the HigB protein (92 aa long) doesn't show any interactions with other proteins in the database. However, the *P. putida*, shows as a plasmid maintenance system killer protein and interacts with its four predicted functional partners Pput_4192 (XRE family plasmid maintenance system antidote protein; 99aa), Pput_4193 (Arsenate reductase-like protein; 118 aa), dapD (Tetrahydrodipicolinate N-succinyl transferase-like protein; 344 aa) and Pput_4190 (Na+/H+ antiporter; 548

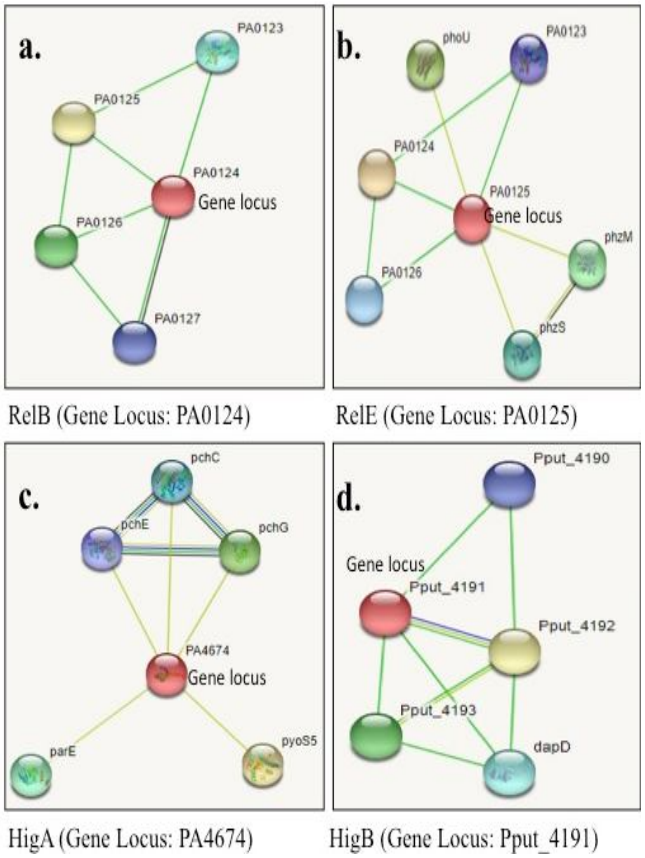

Fig. 5 : Protein-protein interaction analysis of TA proteins (a: RelB; b: RelE; c: HigA; d: HigB, shown in red color nodes) with other proteins shown in different color nodes (Green, Yellow, Blue, Violate and Light green), while Tox1/Tox2, T-AT1/T-AT2 and ParD/ParE didn't show any interactions with other proteins in STRING database.

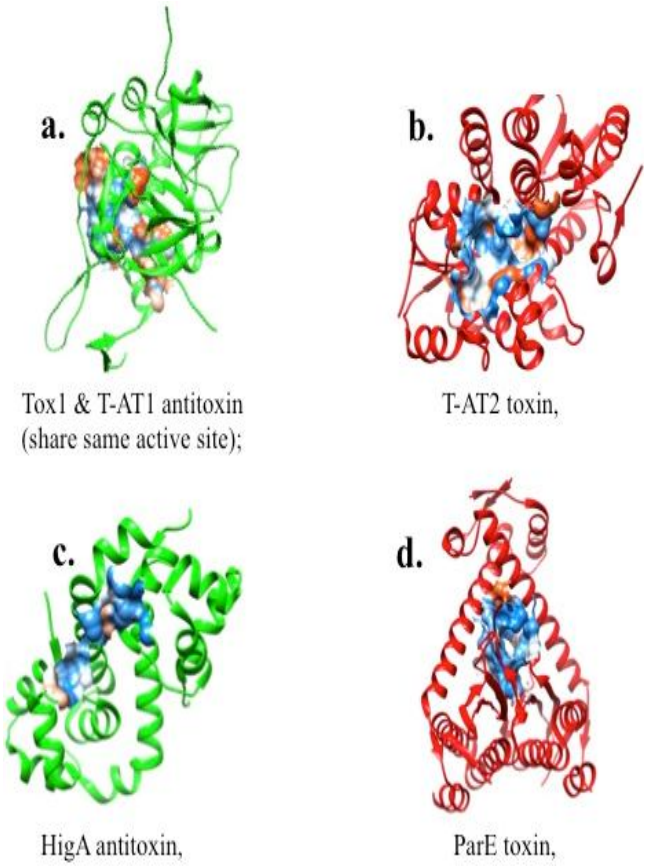

Fig. 6 : Active sites (spherical view, blue color) identification of five TA proteins, while Tox2, RelB, RelE, HigB and ParD TA proteins didn't show any active site in CASTp server.

aa) (fig. 5d). In this study, it is quite clear about the function of RelBE and HigBA proteins that they are TA proteins, which show interactions with distinct protein available in the database. In a similar observation, it has been reported that in the *P. putida* plasmid maintenance system killer protein interacts with its four predicted functional partners, including Pput-4192 (XRE family plasmid maintenance system antidote protein; 99aa), Pput-4193 (Arsenate reductase-like protein; 118 aa), dapD (Tetrahydrodipicolinate N-succinyl transferase-like protein; 344 aa) and Pput-4190 (Na+/H+ antiporter; 548 aa). Because of the above-mentioned observation, it is predicted that this protein might be a toxin protein in the cellular system of *P. aeruginosa*. Moreover, beyond expectations, it was observed that TA proteins Tox1/Tox2, T-AT1/T-AT2 and ParDE didn't show any interaction in the STRING database. Therefore, on the basis of the above-mentioned findings of this study, it is quite clear that RelBE and HigBA proteins that they are functionally TA proteins.

In an ultimate observation of the study, the identification and characterization of functional sites on protein were interestingly performed. For this purpose, CASTp server was used to analyze the active sites of all distinguished proteins. In consequence, variable observations regarding active sites were recorded in fig. 6a to 6d. The solvent surface areas/volume of the active site found were 609.162/448.926 (Tox1& T-AT1), 414.632/301.579 (T-AT2), 130.854/143.636(HigA) and 55.096/480.985 (ParE). In the majority of cases, for the class II an antitoxin has two domains, first is toxin-binding domain found in the C- terminal and second is DNA-binding domain found in the N- terminal region (Brown *et al*., 2009; Santos-Sierra *et al*., 2002; Bernard and Couturier, 1991).

Conclusion

We demonstrated the homology modeling, protein interaction and functional relationship, along with the active site of TA proteins of *P. aeruginosa*. Our study

shows that HigA, HigB, ParD and ParE TA proteins as good models, which were compared through aliginment against templates such as 3trb.1.A, 6f8s.1.B, 2an7.1.B, 3kxe.1.A, avalable in PDB. Therefore, according to the interpretations, it can be concluded that functional proteins and their binding site, can help in understanding the biological role that fulfills with the assistance of a clearly expressed structure and annotation. Consequently, the above findings confirm that the functions of the target proteins are 'toxin-antitoxin' which works as type II TA systems in *P. aeruginosa*. Hopefully, this comprehensive study over this track might produce a significant breakthrough that leads to impending research, referencing the Toxin-antitoxin system regulating the drug resistance persister cell generation.

Acknowledgements

The financial support provided by the Department of Science and Technology (DST) Government of India, New Delhi (Project N0: SR/FT/LS-91/2011) is highly acknowledged.

References

Ahmed, M. S.,M. A. Islam, M. M. Hossain and M. Nasreen (2017). An *In-Silico* aproach for characterization of an acetyltransfarase protein from *Shigella flexneri* serotype 5b (strain 8401). *J. Proteomics Computational. Biol.*, **3(1)** : 6.

Altschul, S. F., J. C. Wootton, E. M. Gertz, R. Agarwala, A. Morgulis, A. A. Schäffer and Y. K. Yu (2005). Protein database searches using compositionally adjusted substitution matrices. *FEBS J.*, **272(20)** : 5101-5109.

Anantharaman, V. and L. Aravind (2003). New connections in the prokaryotic toxin-antitoxin network: relationship with the eukaryotic nonsense-mediated RNA decay system. *Genome Biol.*, **4(12)** : R81.

Arnold, K., L. Bordoli, J. Kopp and T. Schwede (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics*, **22(2)** : 195-201.

Balasubramanian, D., L. Schneper, H. Kumari and K. Mathee (2012). A dynamic and intricate regulatory network determines *Pseudomonas aeruginosa* virulence. *Nucleic Acids Res.*, **41(1)** : 1-20.

Bateman, A., L. Coin, R. Durbin, R. D. Finn, V. Hollich, S. Griffiths Jones, A. Khanna, M. Marshall, S. Moxon, E.L. Sonnhammer and D. J. Studholme (2004). The Pfam protein families database. *Nucleic Acids Res.*, **32** : D138-D141.

Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, B. A. Rapp and D. L. Wheeler (2000). GenBank. *Nucleic Acids Res.*, **28(1)** : 15-18.

Benkert, P., M. Biasini and T. Schwede (2010). Toward the estimation of the absolute quality of individual protein structure models. *Bioinformatics*, **27(3)** : 343-350.

Bordoli, L., F. Kiefer, K. Arnold, P. Benkert, J. Battey andT. Schwede (2008). Protein structure homology modeling using SWISS-MODEL workspace. *Nat. Protoc.*, **4(1)** : 1-13.

Bernard, P. and M. Couturier (1991). The 41 carboxy-terminal residues of the miniF plasmid CcdA protein are sufficient to antagonize the killer activity of the CcdB protein. *Mol. Gen. Genet.*, **226(1-2)** : 297-304.

Bernier, S. P., D. Lebeaux, A. S. DeFrancesco, A.Valomon, G. Soubigou, J. Y. Coppée, J. M. Ghigo and C. Beloin (2013). Starvation, together with the SOS response, mediates high biofilm-specific tolerance to the fluoroquinolone ofloxacin. *PLoS genetics*, **9(1)** : e1003144.

Brown, B. L., S. Grigoriu, Y. Kim, J. M. Arruda, A. Davenport, T. K. Wood, W. Peti and R. Page (2009). Three dimensional structure of the MqsR: MqsA complex: a novel TA pair comprised of a toxin homologous to RelE and an antitoxin with unique properties. *PLoS pathogens*, **5(12)** : e1000706.

Chance, M. R., A. R. Bresnick, S. K. Burley, J. S. Jiang, C. D. Lima, A. Sali, S. C. Almo, J. B. Bonanno, J. A. Buglino, S. Boulton and H. Chen (2002). Structural genomics: a pipeline for providing structures for the biologist. *Prot. Sci.*, **11(4)** : 723-738.

Chaudhary, R. K., G. Singh and S. Ram (2018). Homology modeling and *In-silico* structural annotation of the toxin-antitoxin systems in *P. aeruginosa*. *Indian Res. J. Genet. & Biotech.*, **10(1)** : 180-188.

DeLano, W. L. (2002). The PyMOL molecular graphics system. *http://www. pymol. org*.

Dundas, J., Z. Ouyang, J. Tseng, A. Binkowski, Y. Turpaz and J. Liang (2006). CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues. *Nucleic Acids Res.*, **34** : W116-W118.

Garcia-Pino, A., S. Balasubramanian, L. Wyns, E. Gazit, H. De Greve, R. D. Magnuson, D. Charlier,N. A.van Nuland and R. Loris (2010). Allostery and intrinsic disorder mediate transcription regulation by conditional cooperativity. *Cell*, **142(1)** : 101-111.

Gasteiger, E., C. Hoogland, A. Gattiker, M. R. Wilkins, R. D. Appel and A. Bairoch (2005). Protein identification and analysis tools on the ExPASy server. *InThe proteomics protocols handbook* (pp. 571-607). Humana press.

Goeders, N. and L. Van Melderen (2014). Toxin-antitoxin systems as multilevel interaction systems. *Toxins*, **6(1)** : 304-324.

Gerdes, K., P. B. Rasmussen and S. Molin (1986). Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. *Proc.Nat. Acad. Sci.*, **83(10)** : 3116-3120.

Germain, E., D. Castro-Roa, N. Zenkin and K. Gerdes (2013).

Molecular mechanism of bacterial persistence by HipA. *Mol. Cell*, **52(2)** : 248-254.

Helaine, S., A. M. Cheverton, K. G. Watson, L. M. Faure, S. A. Matthews and D. W. Holden (2014). Internalization of *Salmonella* by macrophages induces formation of nonreplicating persisters. *Science*, **343(6167)** : 204-208.

Lee, M. J. and M. B. Yaffe (2016). Protein regulation in signal transduction. *Cold Spring Harbor Perspectives in Biology*, **8(6)** : a005918.

Lee, K. Y. and B. J. Lee (2016). Structure, biology, and therapeutic application of toxin–antitoxin systems in pathogenic bacteria. *Toxins*, **8(10)** : 305.

Leplae, R., D. Geeraerts, R. Hallez, J. Guglielmini, P. Dreze and L. Van Melderen (2011). Diversity of bacterial type II toxin–antitoxin systems: a comprehensive search and functional analysis of novel families. *Nucleic Acids Res*., **39(13)** : 5513-5525.

Lewis, K. (2010). Persister cells. *Annu. Rev. Microbiol.,* **64** : 357-372.

Mahmud, S., S. Ahmed, M. Mia, S. Islam and T. Rahman (2016). Homology modelling, bioinformatics analysis and *In-silico* functional annotation of an antitoxin protein from *Streptomyces coelicolor* A3 (2). *J. Proteomics Computational Biol*., **2(1)** : 7.

Masuda, H. and M. Inouye (2017). Toxins of prokaryotic toxin-antitoxin systems with sequence-specific endoribonuclease activity. *Toxins*, **9(4)** : 140.

Mills, C. L., P. J. Beuning and M. J. Ondrechen (2015). Biochemical functional predictions for protein structures of unknown or uncertain function. *Comput. Struct. Biotechnol. J*., **13** : 182-191.

Oany, A. R., M. S. Ahmed, N. Jahan, M. A. Latif, S. Mahmud, M. A. Hossain, F. Akter, H. H. Rakib and M. S. Islam (2015). Homology modeling and assigned functional annotation of an uncharacterized antitoxin protein from *Streptomyces xinghaiensis*. *Bioinformation*, **11(11)**: 493.

Page, R. and W. Peti (2016). Toxin-antitoxin systems in bacterial growth arrest and persistence. *Nat. Chem. Biol*., **12(4)** : 208.

Pettersen, E. F., T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng and T. E. Ferrin (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.*, **25(13)** : 1605-1612.

Ramisetty, B. C. M. and R. S. Santhosh (2017). Endoribonuclease type II toxin–antitoxin systems: functional or selfish?. *Microbiology*, **163(7)** : 931-939.

Santos-Sierra, S., C. Pardo-Abarrio, R. Giraldo and R. Dýìaz-Orejas (2002). Genetic identification of two functional regions in the antitoxin of the parD killer system of plasmid R1. *FEMS Microbiol. Lett.*, **206(1)** : 115-119.

Schuster, C. F. and R. Bertram (2013). Toxin–antitoxin systems are ubiquitous and versatile modulators of prokaryotic cell fate. *FEMS Microbiol. Lett.*, **340(2)** : 73-85.

Sevin, E. W. and F. Barloy-Hubler (2007). RASTA-Bacteria: a web-based tool for identifying toxin-antitoxin loci in prokaryotes. *Genome Biol.*, **8(8)** : R155.

Söding, J. (2004). Protein homology detection by HMM–HMM comparison. *Bioinformatics*, **21(7)** : 951-960.

Szklarczyk, D., J. H. Morris, H. Cook, M. Kuhn, S. Wyder, M. Simonovic, A. Santos, N.T. Doncheva, A. Roth, P. Bork and L. J. Jensen (2016). The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. *Nucleic Acids Res.*, **45** : D362-D368.

Szklarczyk, D., A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, K. P. Tsafou and M. Kuhn (2014). STRING v10: Protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.*, **43(D1)** : D447-D452.

Verstraeten, N., W. J. Knapen, C. I. Kint, V. Liebens, B. Van den Bergh, L. Dewachter, J. E. Michiels, Q. Fu, C. C. David, A. C. Fierro and K. Marchal (2015). Obg and membrane depolarization are part of a microbial bet-hedging strategy that leads to antibiotic tolerance. *Mol. Cell*, **59(1)** : 9-21.

Vitkup, D., E. Melamud, J. Moult and C. Sander (2001). Completeness in structural genomics. *Nat. Struct. Mol. Biol.*, **8(6)** : 559-566.

Van Melderen, L. (2010). Toxin–antitoxin systems: why so many, what for?. *Curr. Opin. Microbiol*., **13(6)** : 781-785.