IJBPAS, August, 2025, 14(8): 4344-4356

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANALYSIS OF SMALL GENETIC MODULES AS COMPONENTS OF TOXIN-ANTITOXIN SYSTEM IN *PSEUDOMONAS AERUGINOSA IN SILICO*

TUNGKHANG NU AND VAIPHEI ST*

1: Department of Biotechnology, School of Life Sciences, Mizoram University, Aizawl, Mizoram, India-796004

2: Associate Professor, Department of Biotechnology, School of Life Sciences, Mizoram University, Aizawl, Mizoram, India-796004

***Corresponding Author: Dr. S. Thangminlal Vaiphei: E Mail: mzut261@mzu.edu.in**

Received 20th April 2024; Revised 14th May 2024; Accepted 1st Sept. 2024; Available online 1st Aug. 2025

https://doi.org/10.31032/IJBPAS/2025/14.8.9293

ABSTRACT

The toxin-antitoxins (TA) modules are small genetic operons encoded on the extra chromosomal unit or chromosomal unit of a wide range of bacterial species. The toxin component targets cellular processes like DNA replication, protein translation, and cell wall production resulting in growth arrest of the host cells. However, the presence of antitoxin neutralizes the deleterious action of the toxin component by forming a toxin-antitoxin complex. TA modules have been implicated with several physiological processes such as bacterial survival mechanism against stress, apoptosis, growth arrest, gene regulation, biofilm formation and multidrug tolerance. *ParD/ParE* module that belongs to a type II toxin antitoxin system is highly abundant in plasmids and bacterial chromosomes. The positively charged toxin *ParE* can be neutralized by a negatively charged antitoxin *ParD* resulting in formation of a tight complex. In this study sequence of *parD* and *parE* were retrived from the genomic sequence database of *Pseudomonas aeruginosa* for bioinformatic evaluation and interaction *in silico*. *ParE* has several predicted functional partners, including *RHH_1* domain-containing protein with score 0.966 and uncharacterized protein with score 0.762. *ParD* also shows interaction with several predicted functional partners with score 0.966 and SnoaL-like domain-containing protein (0.668). Secondary structures exist as alpha helix, extended strand, beta turn and random

coil in both the toxin and antitoxin. Molecular docking of *ParD* antitoxin as ligand against *ParE* toxin shows involvement of Glu56 of *ParE* and Val47 of *ParD.* Valine suggests the involvement of hydrophobic residues for the interaction between the toxin and antitoxin components.

Keywords: Toxin; Antitoxin; ParD; ParE; *Pseudomonas*; TA systems

INTRODUCTION

Toxin-antitoxins (TA) systems are small genetic modules that are abundant in bacterial genomes consisting of two components, a stable toxin and its labile antitoxin. The toxins of TA system are mostly proteins, while the antitoxins are either proteins or small RNAs (sRNAs). If the supply of antitoxin stops, for instance under special growth conditions or by plasmid loss in case of plasmid encoded TA systems, the antitoxin is rapidly degraded and can no longer counteract the toxin **[20]**.

Bacterial TA modules are mostly involved in physiological processes such as apoptosis, growth arrest, gene regulation, and survival **[2, 8, 10, 12]**. TA modules are encoded on the extrachromosomal unit or chromosomal unit and consist of a toxin part and an antitoxin part. Extrachromosomal encoded TA modules are associated with plasmid stability and cell viability, whereas chromosomal encoded TA modules are associated with biofilm development, persister cell formation, growth arrest, and multidrug tolerance. The toxin component influences the host cell by inhibiting DNA replication, protein translation, and cell wall production, whereas the antitoxin component neutralises the deleterious action of the toxin component **[19]**. When the antitoxin or TA complex binds to the promoter of the TA operon, transcription is inhibited. During stress, the antitoxin is either degraded by intracellular proteases or its transcription is downregulated that leads to production of toxin. Thus, toxin binds to its target and inhibits essential cellular functions such as replication, transcription, translation, cell wall synthesis, and cell division, eventually leading to cell death or the persistent state. Stress endurance, phage resistance, mobile genetic element maintenance, gene regulation, biofilm development, programmed cell death, and persister cell production are all biological roles of the TA system **[3, 13, 18]**. *Pseudomonas aeruginosa* is a commensal pathogen that can cause a diverse array of infections such as pneumonia, bladder catheter infection, skin burn infection, urinary track infection and many more **[15]**. *Pseudomonas aeruginosa* can detect and respond to diverse environmental stimuli such as nutrient starvation and stress to increase its

fitness by altering the expression of numerous genes such as the toxin-antitoxin (TA) system.

Classification of TA system:

Currently, there are eight classes of TA system according to their genetic structure and mode of action on the gene.

Type I

In type I TA module, an antisense RNA is the antitoxin that inhibits the translation from mRNA of the toxin. Toxins in type I TA modules are mostly small hydrophobic peptides that target the integrity of bacterial membranes, causing obstruction in membrane potential as well as cell division **[8]**.

Type II

Type II TA module is the most widely studied class of TA modules. Here both toxin and antitoxin are small proteins. For neutralization of toxin, the antitoxin forms a protein-protein complex with the toxin and acts as a tight-binding inhibitor **[7]**. For type II TA loci, it is possible to list a number of common traits that apply to most of the members belonging to the gene families *ccdAB, relBE, mazEF, parDE* and probably others **[8]**.

Type III

Type III TA systems consist of RNA antitoxin that directly interacts with toxin protein. These TA systems are made up of an RNA antitoxin that binds directly with the toxin protein. *ToxIN*, *tenpIN*, and *cptIN* are the three most well-known superfamilies of type III TA modules. The *toxIN* TA system in the plasmid of the plant pathogen *Pectobacterium atrosepticum* was the first type III TA system found. *ToxN* toxin has endoribonuclease activity and can create a macromolecular compound with the RNA antitoxin (*ToxI*). The interaction of three *ToxN* proteins with three *ToxI* monomers results in the formation of a trimeric *toxIN* complex, which inhibits *ToxN* toxin. The *toxIN* TA system has been implicated for its role in protection against infecting bacteriophages via abortive infection by supporting the altruistic suicide of the infected cell **[19]**.

Type IV

In type IV TA system, the antitoxin and toxin both are proteins and do not directly interacts with each other. The toxin target interaction is usually inhibited by the competitive binding of antitoxin to the toxin. The antitoxin protein prevents the activity of the toxin by binding to its substrate **[21]**.

Type V

In type V TA system the antitoxin is an enzyme, which does not directly bind to the RNA toxin but is capable of degrading mRNAs of the corresponding toxin. The new classification system reserves type V for antitoxins that enzymatically modify substrates other than the toxin **[21]**.

Type VI

The *socAB* operons in gram negative *Caulobacter vibroides* was first identified as a type VI TA module. The antitoxin *SocB* strongly binds with the β- sliding clamp, thus representing the elongation of replication. The antitoxin *SocA* acts as a proteolytic adaptor protein that bnds to the *SocB* toxin and shows protease- mediated degradation of the *SocB* toxin **[1]**.

Type VII

The newly classified type- VII modules involve antitoxins that are found to be enzymatically modifying the toxins. These enzymatic modifications are made through transient interactions, instead of primarily through binding as in the type II TA system **[22]**.

Type VIII

In the most recently described type VIII modules, the antitoxins are RNAs that repress the expression of their cognate RNA toxins either by acting as an antisense RNA or by mimicking a CRISPR RNA that recruits a Cas protein acting as a transcriptional repressor **[14]**.

Type II Toxin and Antitoxin: mode of action

In general, the type II antitoxins antagonize the activity of their toxins by blocking or masking the toxins active sites. For example, the *RelE* toxin, binding of the *RelB* antitoxin leads to displacement of a C- terminal α- helix essential for the *RelE* activity.

However, some antitoxins do not act upon their cognate toxins by blocking the active sites. For example, the *HigB/higA* TA system, the corresponding antitoxins bind to sites that are distant from the toxin sites. It was suggested that *HigA* antitoxin neutralizes the toxin activities by competing is binding to ribosome or RNA.

ParD/E TA system

Toxin-antitoxin modules, as encoded on the *parDE* operons are highly abundant in plasmids and bacterial chromosomes. *ParE* (93 amino acids) is the positively charged toxin, whereas *ParD* (75 amino acids) is the negatively charged antitoxin able to neutralize *ParE* toxin by forming a tight complex. In contrast to other TA systems that require the complex for full negative regulation of the operons, *ParD* alone is sufficient for autorepression **[5]**.

Distribution of *parDE* TA system

Evolutionary analysis indicated that type II TA systems are prone to move between microbial genomes through horizontal gene transfer, which may account for the surprisingly wide distribution and great numbers of type II TA systems in chromosomes of archea and eubacteria **[16]**.

TADB database (http://bioinfo-mml.sjtu.edu.cn/TADB/) that provides comprehensive information about Type II TA loci, currently contains *parDE* TA families across 128 species.

Biological role of parDE TA system

a) parE inhibits DNA gyrase

DNA-gyrase (EC 5.99.1.3) is one of the four topoisomerases found in bacteria. Topoisomerases are essential enzymes that regulate DNA topology and prevent chromosome entanglements **[4]**. Topoisomerases catalyze DNA supercoiling and relaxation and introduce and remove knots. These reactions require the passage of a DNA segment across a transient singlestrand or double-strand DNA break. Topoisomerase classification relies on the type of breaks these enzymes introduce into their DNA substrates, type I introducing single-strand breaks (Topo I and Topo III) and type II double-strand breaks (DSB) (DNA-gyrase and Topo IV). Unlike other bacterial topoisomerase, DNA gyrase introduces negative supercoils in an ATP-dependent reaction **[6]**. As a consequence, upon DNA-gyrase inhibition, relaxation of circular DNA molecules occurs. Activation of *ParE* causes inhibition of plasmid and chromosomal DNA replication. Similar to *CcdB*, *ParE* binds to DNA gyrase, resulting in formation of a gyrase-DNA complex that subsequently results in DNA breakage **[11]**. However, in *E. coli* a CcdB-resistant *GyrA* could still be inhibited by *ParE* **[17]**. Thus, it was suggested that *ParE* and *CcdB* inhibits DNA gyrase activity via different mechanisms, for instances, binding to different subunits of the DNA gyrase.

b) The Post-Segregational Killing Phenomenon

TA loci act by a mechanism called post-segregational killing (PSK) or addiction [9]. The molecular basis of PSK relies on the differential stability of the toxin and antitoxin. When a plasmid is not transmitted to a daughter cell, the antitoxin is rapidly degraded and the antitoxin pool is not replenished. The toxin is therefore released from it inhibition by the antitoxin, and may eventually kill the plasmid-free cell.

This work mainly focused on the structural prediction and interaction of *ParE* and *ParD* to form a stable TA complex using *Pseudomonas aeruginosa* as the model organism *in silico.*

METHODOLOGY

Identification and sequence retrieval

The nucleotide sequence and amino acid sequence of *ParE* toxin and its antitoxin *ParD* are retrieved (as FASTA format) from 'Pseudomonas Genome Database' server (http://www.pseudomonas.com).

Physiochemical properties

Physiochemical properties of the toxin-antitoxin proteins have been determined by ProtParam tool (http://web.expasy.org/protparam/) it computes various physicochemical properties such as molecular weight, sequence length, alipathic index, instability index, theoretical pI and average of hydropathicityon, GRAVY.

Protein-protein interaction analysis

STRING (http://string-db.org) quantitatively assimilated the protein- protein interactions network, ranked their significance or validity as targets and gene neighborhood of *parE* and *parD* (http://string-db.org). String database includes direct (physical) and indirect (functional) associations derived from various sources, such as genomic context, high-throughput experiments, (conserved) co-expression and the literature.

Secondary and tertiary structure

The secondary structures were predicted using SOPMA server. It determines the percentage of helixs and turns.

Tertiary structure prediction was done by SWISS MODEL (https://swissmodel.expasy.org/) and the structures of the proteins are downloaded as pdb file format.

Validation of the crystal structures are done by using ERRAT (https://www.doe-mbi.ucla.edu/errat/) and PROCHECK (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/).

Conserved domain prediction

Conserved domains of the protein structures were predicted using the ConSurf server (https://consurf.tau.ac.il) by uploading the pdb files of the proteins. ConSurf is used for revealing functional regions in macromolecules by analysing the evolutionary dynamics of amino/nucleic acids substitutions among homologous sequences.

RESULTS

Collection of sequences

Amino acid sequence and nucleotide sequences of both the toxin and antitoxin protein (*ParE* and *ParD*) were retrieved from *Pseudomonas* genome database **(Table 1 and 2)**. Protein–protein BLAST of *parE* sequenced showed homology with *Acinetobacter baumnii* (WP-153069560.1) with query coverage 100% and *E.coli* (WP-163481393.1) with query coverage 100%. Protein BLAST of *parD* sequence showed homology with *Klebsiella pneumonia* (WP-172723439.1) with query coverage 96% and *E.coli* (MBE0969797.1) with query coverage 100%.

Table 1: Amino acid sequences from *Pseudomonas* genome database			
Protein	**Species**	**Amino acid sequence**	**Length**
parE	***Pseudomonas aeruginosa***	**MSLKWTRKAAADLDAIYDHYVVLIGPEKALKAVQDIVEQVKPL QQVANQGAGRPSEVPGVRTLTLERWPFSAPFRVKGKEIQILRID RVEITP**	**93**
parD	***Pseudomonas aeruginosa***	**MSTVVSFRADDALVAALDELARATHRDRPYHLRQALAQYLERQ QWQVAAIDEGLADANAGRLLEHIEIEKRWGLQ**	**75**

Table 2: Nucleotide sequence from *Pseudomonas* Genome database			
Protein	**Species**	**Nucleotide sequence**	**Length**
parE	***Pseudomonas aeruginosa***	**ATGAGCCTGAAGTGGACCCGCAAGGCGGCCGCCGACCTGGA CGCCATCTACGACCATTACGTCGTGCTGATCGGCCCGGAAA AAGCTCTGAAAGCCGTTC AGGACATCGTCGAGCAGGTGAAACCGCTGCAGCAGGTAGCC AACCAGGGGGCAGGGCGGCCCAGCGAGGTGCCAGGCGTAC GCACCCTGACCCTGGAGCG CTGGCCGTTCAGCGCCCCGTTTCGGGTTAAAGGCAAGGAAA TCCAGATTTTGCGCATCGACAGAGTCGAAATTACCCCCTGA**	**282**
parD	***Pseudomonas aeruginosa***	**ATGAGCACCGTAGTCTCGTTCCGCGCCGATGACGCCCTGGT CGCGGCCCTCGACGAACTGGCCCGCGCCACCCACCGCGACC GACCCTACCACCTGCGGC AGGCGCTCGCGCAGTACCTGGAAAGGCAGCAGTGGCAGGTC GCTGCCATCGATGAAGGCTTGGCCGATGCCAATGCCGGTCG CCTGCTGGAACACATCGA GATCGAGAAGCGCTGGGGGCTGCAATGA**	**228**

Physiochemical Properties

The physiochemical properties of toxin-antitoxin proteins have been determined by Expasy server 14. *In silico* Analysis of *parD/E* Toxin-Antitoxin Homolog's from the Genome of *Pseudomonas aeruginosa* (www.expasy.org) which showed that the positively charged toxin *parE* has pI of 9.45 with molecular weight of 10462.19 Da whereas its negatively charged antitoxin *parD* has pI of 5.48 with molecular weight 8568.64 Da **(Table 3)**.

Table 3: Tabulate form of some physicochemical properties predicted by ProtParam						
Protein	**Molecular weight**	**Theoretical pI**	**Instability index**	**Aliphatic index**	**GRAVY**	**Amino acid sequence**
parE	**10462.19**	**9.45**	**49.74**	**103.76**	**-0.217**	**93**
parD	**8568.64**	**5.48**	**50.02**	**100.4**	**-0.397**	**75**

Protein- protein interaction analysis

The protein interaction network displays the proteins and their length and type of relationship with *ParE* and *ParD*. Based on the results, *ParE* has several predicted functional partners, such as *RHH_1* domain-containing protein with score 0.966 and uncharacterized protein with score 0.762. *ParD* also shows interaction with several predicted functional partners such as

uncharacterized protein with score 0.966 and SnoaL-like domain-containing protein; with score 0.668. The empty nodes suggest that 3D structure for the protein is not available.

Secondary and Tertiary structure prediction

The secondary structure prediction using SOPMA server revealed that the secondary structure exist in four states, Alpha helix, extended strand, Beta turn and Random coil in both the toxin and antitoxin **(Table 4)**.

The most reliable and acceptable method for predicting protein structure is homology modeling. Tertiary structure prediction was done by SWISS MODEL. For *ParE* template taken is "6xrw.1. A Plasmid stabilization system protein Chromosomal *ParDE* TA system from *P. aeruginosa ",* with GMQE score 95 and Identity 100. The BLASTp homology search was used to confirm the homology.

Table 4: Secondary structure prediction using SOPMA server

Protein	Alpha helix	Extended strand	Beta turn	Random coil
parE	**47.31%**	**8.60%**	**1.08%**	**43.01%**
parD	**80.00%**	**5.33%**	**2.67%**	**12.00%**

For *parD* the template taken is "6xrw.2.B Ribbon-helix-helix protein, CopG family Chromosomal *ParDE* TA system from *P. aeruginosa*", with GMQE score 91 and Identity 100. The BLASTp homology search was used to confirm the homology.

Tertiary structure validation was done by using PROCHECK and Errat **(Figure 1)**. Ramachandran plot obtained from PROCHECK shows scores above 90 % **(Figure 2)**. *ParE* showing 96.2% of residues were in the most favoured region and *ParD* showing 98.6% of residues were in the most favoured region which are consider as good model structure. Errat score for *ParE* is 91.667%, which fall under the average quality factor for lower resolutions.

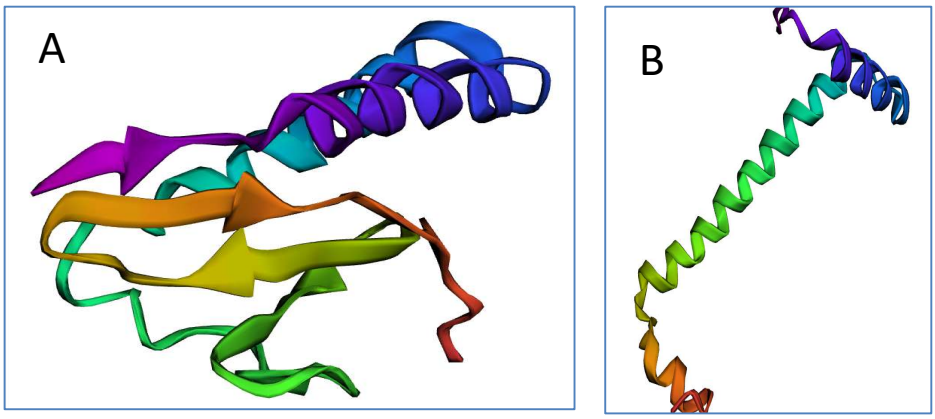

Figure 1: Tertiary structure of (A) *Par E* and (B) *ParD*

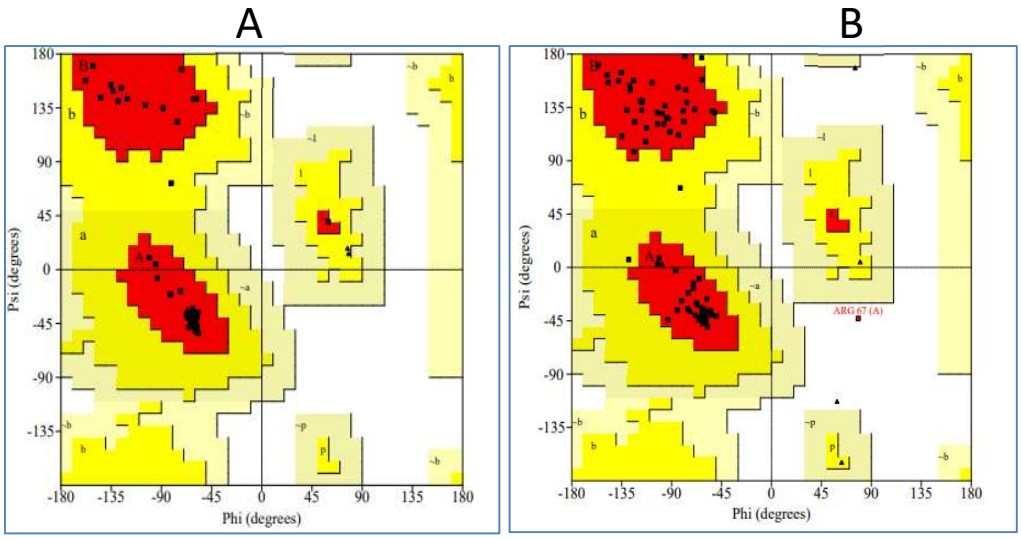

Figure 2: Ramachandran plot for (A) *ParD*: 96.2% residues in favoured region and (B) *parE*: 96.2% in favourable region

Conserved domain prediction

Conserved domains in the protein structures were predicted using ConSurf server. The resulting structure is shown in multicolor, the highly conserved region were colored red and least conserved region are colored blue **(Figure 3)**.

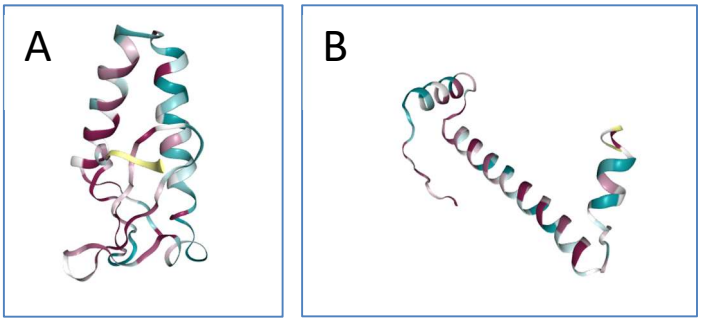

Figure 3: Conserved region prediction using ConSurf server. (A) Structure of *ParD* ; (B) structure of *parE*

Molecular docking with *ParE* toxin and *parD* antitoxin complex

The interaction between the toxin *ParE* and antitoxin *parD* was evaluated using online docking Patchdock server **(Figure 4A)**. The Patchdock protein-protein docking algorithm calculates the surface binding affinity of the receptor and ligand proteins using object recognition and image segmentation analysis. The toxin *ParE* as the receptor was docked against antitoxin *ParD* as the receptor and the results were analyzed. The patchdock results

were refined by using firedock server based on the global energy of the docked complex **(Figure 4B)**. Analysis of the docked complex was done by using Plymol software and one point of interaction was found in the complex (data not shown). The interaction between 56th amino acid (Glutamic acid) of *parE* and 47th amino acid (valine) of *parE*.

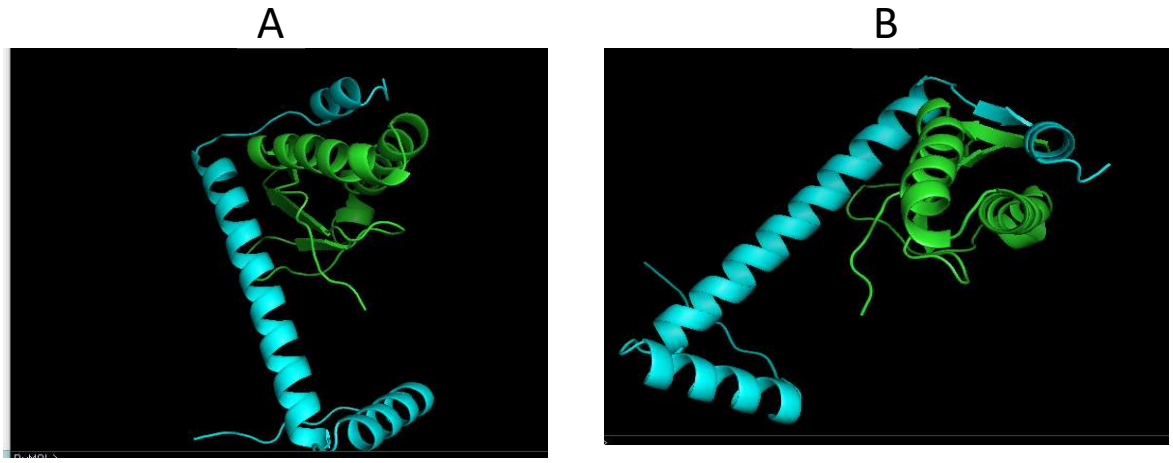

Figure 4: Complex fromation with patchdock (A) and firedock (B)

DISCUSSION

TA modules have been associated with bacterial survival, programmed cell death, and persistence under various unfavorable conditions which could be physical, chemical or nutrient depleted conditions. In this study, *ParE* and *ParD* toxin antitoxin system has been analyzed by employing *in silico* approaches that includes physicochemical property, secondary and tertiary structures of both the toxin and antitoxin protein. The molecular weight, theoretical pI, GRAVY score, instability index and aliphatic index are considered for the physicochemical properties. The theoretical pI was used to determine the charge of the protein based on their acidic or basic characteristics. GRAVY score was employed to determine the solubility and cytosolic nature of the protein. Instability index helped in predicting the stability of the peptide is stable and aliphatic index was employed to check the thermostablity of the peptide structures. Difference in the theoretical pI of *ParD* (5.48) and *ParE* (9.45) tends to form a complex.

The secondary structure prediction of both the toxin and antitoxin protein revealed alpha helices, beta turn, extended strand and random coils. The molecular docking of *ParD* antitoxin as ligand against *ParE* toxin as receptor showed only one interaction point between two proteins (glutamic acid at 56

position of *ParE* and valine at 47 position of *ParD*). This indicates that the resultant TA complex between toxin and antitoxin is not be very stable suggesting that several other factors may be involved in the regulation of this TA module. The presence of valine is a clear indication for the involvement of hydrophobic residues in the interaction between the toxin and antitoxin components.

CONCLUSION

From this study, *ParE* and *ParD* modules could be a part of toxin-antitoxin systems in *Pseudomonas aeruginosa* . The presence of such genetic modules is important for the survival of pathogens under environmental stress. In response to diverse environmental stimuli such as nutrient starvation and stress, the expression of genes such as the toxin-antitoxin (TA) system aids in improving the bacterial fitness.

REFERENCES

[1] Aakre, C.D., Phung, T.N., Huang, D., Laub, M.T. A bacterial toxin inhibits DNA replication elongation through a direct interaction with the β sliding clamp. Mol Cell. (2013) 52(5), 617-628.

[2] Buts, L., Lah, J., Dao-Thi M,H., Wyns, L., Loris, R. Toxin-antitoxin modules as bacterial metabolic stress managers. Trends Biochem Sci, (2005) 30 (12), 672–679.

[3] Chan, W.T., Moreno-Córdoba, I., Yeo, C.C., Espinosa M. Toxin antitoxin genes of the Gram- positive pathogen Streptococcus pneumoniae: so few and yet so many. Microbiol Mol Biol Rev, (2012) 76, 773–791.

[4] Collin, F., AKarkare, S., Maxwell, A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. Appl Microbiol Biotechnol, (2011) 92(3), 479-97

[5] Davis, T.L., Helinski, D.R., Roberts, R.C. Transcription and autoregulation of the stabilizing functions of broad-host-range plasmid RK2 in *E.coli*, *agrobacterium tumefaciens and Pseudomonas aeruginosa.* Mol. Microbial, (1992) 6, 1981-1994

[6] Gellert, M., Mizuuchi, K., O'Dea, M.H., Nash, H.A. DNA gyrase: an enzyme that introduces superhelical turns into DNA.Proc Natl Acad Sci USA, (1976) 73(11),3872-6.

[7] Geoders, N.,Van, Melderen, L. Toxin-antitoxin systems as multilevel interaction systems. Toxin (basel), (2013) 6 (1), 304-24

[8] Gerdes, K., Christensen, S.K., Løbner-Olesen. A Prokaryotic toxin-antitoxin

stress response loci. Nat Rev Microbiol, (2005) 3 (5), 371–382.

[9] Gerdes, K., Rasmussen, P.B., Molin, S. Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. Proc Natl Acad Sci USA, (1986) 83,3116-3120

[10] Hayes, F., Van, Melderen, L. Toxins-antitoxins: Diversity, evolution and function. Crit Rev Biochem Mol Biol, (2011) 46 (5), 386–408.

[11] Iang, Y., Pogliano, J., Helinski, D.R., Konieczny, I. ParE toxin encoded by the broad-host-range plasmid RK2 is an inhibitor of Escherichia coli gyrase. Mol Microbiol, (2002) 44,971-979.

[12] Kim, D.H, Kang, S.M., Park, S.J., Jin, C., Yoon, H.J., Lee, B.J. Functional insights into the Streptococcus pneumoniae HicBA toxin-antitoxin system based on a structural study. Nucleic Acids Research, (2018) 46 (12), 6371–6386.

[13] Klimina, K.M., Poluektova, E.U., Danilenko, V.N. Bacterial toxin–antitoxin systems: properties, functional significance, and possibility of use. Appl Biochem Microbiol, (2017) 53,494–505

[14] Li, M., Gong, L., Cheng, F., Yu, H., Zhao, D., Wang, R. *et al*. Toxin-antitoxin RNA pairs safeguard CRISPR-Cas systems. Science, (2021) 372(6541), eabe5601.

[15] Moradali, M.F., Ghods, S., Rehm, B.H. *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival and persistence.Front Cell Infect Microbiol, (2017) 7, 39.

[16] Ramisetty, B.C., Santhosh, R.S. Horizontal gene transfer of chromosomal type II toxin-antitoxin systems of *Escherichia coli.* FEMS Microbial Lett, (2016) 363,fnv238

[17] Roberts, R.C., Strom, A.R., Helinski, D.R. The parDE operon of the broadhost-range plasmid RK2 specifies growth inhibition associated with plasmid loss. J Mol Biol, (1994) 237,35-51.

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

[19] Singh, G., Yadav, M., Ghosh, C., Rathore, J.S. Bacterial toxin-

antitoxin modules: classification, functions, and association with persistence. Current Research in Microbial Sciences, (2021) *2*, 100047.

[20] Unterholzner, S.J.**,** Poppenberger, B., Wilfried, Rozhon, W. Toxin-antitoxin systems: Biology,identification, and application. Mob Genet Elements, (2013) 3(5), e26219.

[21] Wang, X., Lord, D.M., Cheng, H.Y., Osbourne, D.O., Hong, S.H., Sanchez-Torres, V. *et al*. A new type V toxin-antitoxin system where mRNA for toxin GhoT is cleaved by antitoxin GhoS. Nat Chem Biol, (2012) 8, 855–861

[22] Wang, X., Yao, J., Sun, Y.C., & Wood, T.K. Type VII toxin/antitoxin classification system for antitoxins that enzymatically neutralize toxins. Trends in Microbiology, (2021) 29(5), 388-393.