Identification of response regulator genes of *Xanthomonas oryzae* pv. *oryzae* involved in the c-di-GMP signaling

Porwal Priyanshi and Pandey Alok*
Department of Biotechnology, School of Life Sciences and Biotechnology, Chhatrapati Shahu Ji Maharaj University (Formerly Kanpur University),
Kalyanpur, Kanpur – 208024, Uttar Pradesh, INDIA
*alokpandey@csjmu.ac.in

Abstract

Xanthomonas oryzae pv. oryzae (Xoo) is responsible for bacterial blight, a serious and destructive rice disease. Xoo genome analysis has indicated that this pathogenic bacterium encodes several proteins forming two-component signal transduction systems (TCSTSs). Two proteins, a sensor kinase and a response regulator form a classical TCSTS. Response regulators display a variety of functions including enzymatic activities. The Cyclic-di-GMP (c-di-GMP) participates in the signaling pathway operative in the members of Genus Xanthomonas. The present work aimed to identify the Xoo response regulators putatively participating in the c-di-GMP signaling.

Proteins having HD-GYP and EAL domains display c-di-GMP phosphodiesterase activity directed towards degradation of the c-di-GMP. Proteins having PleD (GGDEF) domain act as di-guanylate cyclases that participate in the synthesis of c-di-GMP synthesis. In silico analyses have been performed on the available genome of the BXO1 strain of Xoo to identify response regulator genes having HD-GYP/EAL/GGDEF domains. The present work has revealed the presence of several response regulator genes of Xoo that might be controlling the c-di-GMP-dependent signaling.

Keywords: *Xanthomonas*, Virulence, Two component system, c-di-GMP, Signaling

Introduction

Phytopathogens are known to cause loss of crop and food productivity. Crop plants are continuously threatened by various plant pathogens including viruses, phytoplasmas, bacteria, protists, fungi, nematodes, insects etc. Under these circumstances, food production is severely crippled, eventually leading to economic losses and food hunger issues. Therefore, effective management of plant disease is necessary to address the problem.

The study directed toward understanding interactions between plans and pathogens is required as the outcome will help devise novel methods to protect crop plants against plant pathogens. Recent advancements in genetics, molecular plant pathology and genomics have provided various tools to identify and characterize the virulence functions of phytopathogens. Interference in the virulence process might become one of the novel methods to control plant diseases.

Several plant pathogens are known to attack the rice (*Oryza sativa L.*) plants leading to the shortage of this leading staple food[15]. Infectious diseases drastically reduce rice productivity. The genus *Xanthomonas* is home to diverse plant pathogenic bacteria that can cause disease in more than a hundred monocot and dicot plant species[18]. One of the important members of this genus is *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) which is responsible for bacterial blight, a destructive disease of rice[16]. The problem of bacterial blight has been observed worldwide. The bacterial blight disease is also a serious problem in India that can reduce rice productivity drastically. High wind and rain associated with the monsoon season facilitate the spread of this pathogen[20].

Advancements in the field of genetics and genomics led to the characterization of various pathogenicity factors of *Xoo* that make this Gram-negative bacterium a successful pathogen. The Rice-*Xoo* has been established as a very important pathosystem[14]. Using this model pathosystem, several molecular players responsible for the *Xoo* pathogenesis have been identified[22,23]. Further, the availability of the genome of both rice and *Xoo* accelerated the discovery of the mechanism of the bacterial blight disease of rice at the molecular level[7,19]. *Xoo* virulence functions include surface polysaccharides/appendages, adhesins, several secretion systems, cell-cell communication systems, various nutrient uptake systems required for obtaining nutrients from host tissues; metabolic functions etc.

The extracellular polysaccharide and lipopolysaccharide are essential for optimum virulence displayed by *Xoo*[5,6,17]. The attachment and motility functions of *Xoo* are also very important for pathogenesis[4]. Various bacterial secretion systems including T1SS (type 1 secretion system), T2SS, T3SS, T4SS, T5SS and T6SS have been observed in the *Xoo*. These *Xoo* secretion systems are responsible for the secretion of numerous proteins, enzymes, or effectors that are required to establish successful interaction with its host rice eventually leading to disease[3].

Furthermore, several regulatory and signaling components of *Xoo* have been identified that perform a critical role in virulence factor production during infection[2,22]. Cyclic-di-GMP (c-di-GMP) participates in the signaling pathway that

https://doi.org/10.25303/2011rjbt1280131

is utilized by bacterial pathogens for adaptation to different environments[25]. In the form of a secondary messenger, c-di-GMP controls bacterial functions including biofilm formation, pathogenesis, host colonization, cell-cell signaling, cell division and differentiation[10,21]. The intracellular levels of c-di-GMP are controlled either by synthesis or degradation. Proteins having RpfG (HD-GYP) and VieA (EAL) domains act as c-di-GMP phosphodiesterase (degradation of c-di-GMP) while proteins having PleD (GGDEF) domain act as di-guanylate cyclase (synthesis of c-di-GMP)[8]. Both enzymes regulate the intracellular concentration of this important secondary messenger as well as c-di-GMP-mediated signaling events. The c-di-GMP metabolic and signaling genes have been identified and characterized in various pathogens.

Genome-level analysis of *Xoo* has indicated that this pathogenic bacterium can encode a large number of TCSTSs (Two-component signal transduction systems). This system is made of two partners, a sensor kinase (positioned in the cytoplasmic membrane) and a cytoplasmic response regulator[9,13]. TCSTSs facilitate the various arms of adaptation leading to pathogenesis in bacteria[24]. Response regulators display a variety of functions including enzymatic activities.

In the present work, we have identified genes of BXO1 (an Indian strain of *Xoo*) encoding for response regulators having enzymatic functions governing c-di-GMP signaling.

Material and Methods

We accessed the genome of *Xoo* strain BXO1 (GenBank CP033201) for the genes annotated for response regulator functions of two-component signal transduction systems[12]. Each gene and corresponding protein was searched for the presence of EAL, HD-GYP and GGDEF domains in the output domain of response regulators using conserved domain searches[11,26]. The presence of response regulator genes participating in c-di-GMP signaling in other *Xanthomonas* strains was confirmed by Blast analysis[1].

Results and Discussion

Our analysis revealed the presence of eight genes of *Xoo* strain BXO1 (an Indian strain) encoding for response regulator proteins possessing HD-GYP/EAL and GGDEF domains. Details for each gene along with *Xoo* locus ID have been provided.

BXO1_015540 (EAL domain-containing response regulator): BXO1_015540, a gene of 1827 bp, encodes a response regulator protein of 608 amino acids. Conserved domain search analysis revealed the presence of the EAL domain in the BXO1_015540 protein. This suggests that this gene might be controlling the level of secondary messenger c-di-GMP in *Xoo* by decreasing its amount.

Further, BlastX analysis indicated that BXO1_015540 protein is highly conserved across various strains of *Xoo* as well as in other related plant pathogenic bacterium including *X. citri* pv. *citri*, *X. oryzae* pv. *oryzicola*, *X. perforans*, *X. citri* pv. *malvacearum* etc. The gene indicates that this response regulator might have an important role in pathogen physiology and pathogenesis.

BXO1_013295 (HDOD domain-containing protein): We found another response regulator (BXO1_013295) of *Xoo* that might have enzymatic activity. BXO1_013295, a gene of 1116 bp, encodes a protein of 371 amino acids. Conserved domain search revealed a presence of HDOD (HD-related output domain) domain in the BXO1_013295 protein. HD domain is involved in the diverse signal transduction mechanism. The protein residues (1-134) display a DNA binding domain belonging to the NtrC family while protein residues (131-346) belong to the HDOD domain. The BXO1_013295 gene is conserved in the phytopathogens of the genus *Xanthomonas*. It would be interesting to find the function of the gene using a mutational approach.

BXO1_000935 (EAL domain-containing protein): Our analysis revealed a second response regulator containing the EAL domain in the *Xoo*. The length of the BXO1_000935 gene is 1284 bp which encodes protein of 427 amino acids. An EAL domain was found in the protein as indicated by a conserved domain search study. This protein also contains a phosphoacceptor receiver domain. Therefore, upon receiving an environmental signal, the BXO1_000935 response regulator might be facilitating *Xoo* pathogenesis by altering the c-di-GMP levels in the *Xoo*. This protein is highly conserved in the members of *the Xanthomonas* genus.

BXO1_006885 (EAL and GGDEF domain-containing protein): Our genome analysis revealed another interesting response regulator protein of *Xoo* that has domains involved in both degradations as well as the synthesis of the c-di-GMP. The length of the BXO1_006885 gene is 1722 bp which encodes protein of 573 amino acids. Conserved domain search analysis revealed the presence of both EAL and GGDEF domains in the protein. Additionally, BXO1_006885 contains the usual phosphoacceptor receiver domain. The action of the BXO1_006885 protein might be critical for balancing the c-di-GMP levels leading to the optimum pathogenesis displayed by the *Xoo* on rice. Similar to other genes, this gene is present in the various strains belonging to the genus *Xanthomonas*.

BXO1_007710 (EAL domain-containing protein): We found a third response regulator of *Xoo* that contains the EAL domain. The length of the BXO1_007710 gene is 2070 bp which encodes protein of 689 amino acids. We observed the EAL domain in BXO1_007710 that suggests that the BXO1_007710 response regulator might be involved in the *Xoo* signaling leading to a reduction in the c-di-GMP levels. This protein also contains a receiver domain which is a characteristic feature of the TCSTSs. This response regulator is also conserved across the members of *the Xanthomonas* genus.

BXO1_008085 (GGDEF domain-containing protein/ diguanylate cyclase): The BXO1_008085 gene of *Xoo* encodes a response regulator protein. The gene length is 1245 bp and the corresponding protein length is 414 amino acids. Conserved domain search revealed a presence of GGDEF domain (responsible for diguanylate cyclase activity) in the BXO1_008085 protein. This protein also contains a receiver domain.

Based on the presence of the GGDEF domain, a function can be predicted for this protein. Our analysis suggests that the BXO1_008085 protein might be having c-di-GMP synthesis activity. Upon activation by an environmental stimulus, this protein might enhance the levels of c-di-GMP in *Xoo*. The BXO1_008085 gene is also highly conserved in the phytopathogens of the genus *Xanthomonas*.

BXO1_008145 (HD-GYP domain-containing protein): BXO1_008145, a gene of 987 bp, encodes a response regulator protein of 328 amino acids. Conserved domain search analysis revealed the presence of HD-GYP domain in this *Xoo* protein. Like the EAL domain, proteins having the HD-GYP domain display c-di-GMP phosphodiesterase activity. This suggests that the BXO1_008145 might also be involved in the reduction of c-di-GMP levels in *Xoo*.

BXO1_008750 (HD-GYP domain-containing protein): The BXO1_008750 gene of *Xoo* encodes a response regulator protein of 378 amino acids. The gene length is 1137 bp. Conserved domain search revealed a presence of HD-GYP domain in the BXO1_008750 protein. This analysis suggests that this gene also might be degrading the c-di-GMP. The BXO1_008750 gene is highly conserved in the phytopathogenic bacteria belonging to the genus *Xanthomonas*.

Conclusion

We have identified a total of eight response regulator genes that might be regulating the levels of c-di-GMP in *Xoo*. The HD-GYP domain-containing proteins (BXO1_008145 and BXO1_008750) and EAL domain-containing proteins (BXO1_015540, BXO1_000935 and BXO1_007710) are responsible for degradation of c-di-GMP as they act as c-di-GMP phosphodiesterase. The BXO1_008085, a diguanylate cyclase containing GGDEF domain, is responsible for the synthesis of c-di-GMP. Interestingly, we observed the presence of BXO1_006885 containing both EAL and GGDEF domains indicating its ability to control both degradation and production of c-di-GMP.

The BXO1_013295 might also be displaying enzymatic function as the protein contains an HD-related output domain. It would be interesting to assign the function of each of these response regulators using a mutagenesis approach to evaluate the role of these genes in *Xoo* virulence on rice. Understanding virulence mechanisms will eventually help in devising novel methods that may protect rice crop plants from *Xoo*, a very devastating pathogen.

Acknowledgement

This work was supported, in part, by Centre of Excellence Grant, Uttar Pradesh Higher Education Department, Uttar Pradesh Government and C.V. Raman Minor Research Project Scheme Grant (Chhatrapati Shahu Ji Maharaj University) awarded to Alok Pandey, Principal Investigator.

References

1. Altschul S.F., Gish W., Miller W., Myers E.W. and Lipman D.J., Basic local alignment search tool, *J Mol Biol.*, **215(3)**, 403-410 **(1990)**

2. An S.Q., Potnis N., Dow M., Vorhölter F.J., He Y.Q., Becker A., Teper D., Li Y., Wang N., Bleris L. and Tang J.L., Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen *Xanthomonas*, *FEMS Microbiol Rev*., **44**, 1-32 **(2020)**

3. Buttner D. and Bonas U., Regulation and secretion of *Xanthomonas* virulence factors, *FEMS Microbiol Rev*., **34**, 107-133 **(2010)**

4. Das A., Rangaraj N. and Sonti R.V., Multiple adhesin-like functions of *Xanthomonas oryzae* pv. *oryzae* are involved in promoting leaf attachment, entry and virulence on rice, *Mol Plant Microbe Interact*., **22**, 73-85 **(2009)**

5. Dharmapuri S. and Sonti R.V., A transposon insertion in the *gumG* homologue of *Xanthomonas oryzae* pv. *oryzae* causes loss of extracellular polysaccharide production and virulence, *FEMS Microbiol Lett*., **179**, 53-59 **(1999)**

6. Dharmapuri S., Yashitola J., Vishnupriya M.R. and Sonti, R.V., Novel genomic locus with atypical G+C content that is required for extracellular polysaccharide production and virulence in *Xanthomonas oryzae* pv. *oryzae*, *Mol Plant Microbe Interact.*, **14**, 1335-1339 **(2001)**

7. Eckardt N.A., Sequencing the rice genome, *The Plant Cell*, **12(11)**, 2011-2017 **(2000)**

8. Hall C.L. and Lee V.T., Cyclic-di-GMP regulation of virulence in bacterial pathogens, *Wiley Interdisciplinary Reviews RNA*, **9(1)**, doi: 10.1002/wrna.1454 **(2018)**

9. Laub M.T. and Goulian M., Specificity in two-component signal transduction pathways, *Annu. Rev. Genet*, **41**, 121-145 **(2007)**

10. Liu C. et al, The global regulation of c-di-GMP and cAMP in bacteria, *mLife*, **3**, 42-56 **(2024)**

11. Marchler-Bauer A., Bo Y., Han L., He J., Lanczycki C.J., Lu S., Chitsaz F., Derbyshire M.K., Geer R.C., Gonzales N.R., Gwadz M., Hurwitz D.I., Lu F., Marchler G.H., Song J.S., Thanki N., Wang Z., Yamashita R.A., Zhang D., Zheng C., Geer L.Y. and Bryant S.H., CDD/SPARCLE: functional classification of proteins via subfamily domain architectures, *Nucleic Acids Res*., **45(D1)**, D200-D203 **(2017)**

12. Midha S., Bansal K., Kumar S., Girija A.M., Mishra D., Brahma K., Laha G.S., Sundaram R.M., Sonti R.V. and Patil P.B., Population genomic insights into variation and evolution of *Xanthomonas oryzae* pv. *oryzae*, *Sci Rep*., **7**, 40694 **(2017)**

13. Mitrophanov A.Y. and Groisman E.A., Signal integration in bacterial two-component regulatory systems, *Genes Dev*., **22(19)**, 2601-2611 **(2008)**

14. Nino-Liu D.O., Ronald P.C. and Bogdanove A.J., *Xanthomonas oryzae* pathovars: model pathogens of a model crop, *Mol Plant Patho.*, **7**, 303-324 **(2006)**

15. Oerke E.C. and Dehne H.W., Safe guarding production – losses in major crops and the role of crop protection, *Crop Prot*., **23**, 275-285 **(2004)**

16. Pandey A. and Sonti R.V., Role of the FeoB protein and siderophore in promoting virulence of *Xanthomonas oryzae* pv. *oryzae* on rice, *J. Bacteriol*., **192**, 3187-3203 **(2010)**

17. Patil P.B. and Sonti, R.V., Variation suggestive of horizontal gene transfer at a lipopolysaccharide (*lps*) biosynthetic locus in *Xanthomonas oryzae* pv. *oryzae*, the bacterial leaf blight pathogen of rice, *BMC Microbiol*., **4**, 40 **(2004)**

18. Poplawsky A.R., Urban S.C. and Chun W., Biological role of xanthomonadin pigments in *Xanthomonas campestris* pv. *campestris*, *Appl and Environ Microbial*., **66(12)**, 5123–5127 **(2000)**

19. Salzberg S.L. et al, Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99^{A}, *BMC Genomics*, **9**, 204 **(2008)**

20. Sonti R.V., Bacterial leaf blight of rice: new insights from molecular genetics, *Current Science*, **73**, 206-212 **(1998)**

21. Romling U., Cyclic di-GMP signaling—Where did you come from and where will you go?, *Mol Microbiol.*, **120**, 564-574 **(2023)**

22. Timilsina S., Potnis N., Newberry E.A., Liyanapathiranage P., Iruegas-Bocardo F., White F.F., Goss E.M. and Jones J.B., *Xanthomonas* diversity, virulence and plant-pathogen interactions, *Nat Rev Microbiol*., **18(8)**, 415-427 **(2020)**

23. Timilsina S., Kaur A., Sharma A., Ramamoorthy S., Vallad G.E., Wang N., White F.F., Potnis N., Goss E.M. and Jones J.B., *Xanthomonas* as a model system for studying pathogen emergence and evolution, *Phytopathology*, **114(7)**, 1433-1446 **(2024)**

24. Tiwari S., Jamal S.B., Hassan S.S., Carvalho P.V.S.D., Almeida S., Barh D., Ghosh P., Silva A., Castro T.L.P. and Azevedo V., Two component signal transduction systems of pathogenic bacteria as targets for antimicrobial therapy: An Overview, *Front Microbiol*., **8**, 1878 **(2017)**

25. Valentini M. and Filloux A., Biofilms and cyclic di-GMP (c-di-GMP) signaling: lessons from *Pseudomonas aeruginosa* and other Bacteria, *J Biol Chem*., **291(24)**, 12547-12555 **(2016)**

26. Wang J., Chitsaz F., Derbyshire M.K., Gonzales N.R., Gwadz M., Lu S., Marchler G.H., Song J.S., Thanki N., Yamashita R.A., Yang M., Zhang D., Zheng C., Lanczycki C.J. and Marchler-Bauer A., The conserved domain database in 2023, *Nucleic Acids Res.*, **51(D1)**, D384-D388 **(2023)**.

(Received 25th January 2025, accepted 18th March 2025)