International Journal of Agriculture, Environment and Biotechnology
Citation: *IJAEB*: **15**(03): 773-780, September 2022
DOI: 10.30954/0974-1712.03.2022.15

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

MOLECULAR BIOLOGY

Structure Analysis and Molecular Simulation Study of ACC Deaminase Mutants from *Pseudomonas* sp., an Endophyte in Reducing Abiotic Stress in Plants

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Paper No. 1053 **Received:** 11-05-2022 **Revised:** 27-08-2022 **Accepted:** 03-09-2022

ABSTRACT

Cellular stressors are abiotic or biotic conditions, such as drought, salinity, acidity, and infections, that induce plant damage or disease, as well as an increase in ROS and ethylene production. Endophytes are microorganisms that reside within plants and share an endosymbiotic relationship with their host to protect the plant from cellular stress. The bacterial endophytes under stress conditions produce ACC deaminase from the acdS gene to break down ACC, an ethylene precursor, which, in high concentrations, hinder and retard the plant's growth. ACC deaminase from *Pseudomonas* sp. (PDB ID: 1TYZ) was used for the mutation study to determine the possible effect of single amino acid substitutions using the Predict SNP tool. The mutant E295G (glutamic acid convert to glycine at position 295) was considered, and a simulation for 100ns was run on the E295G mutated ACCD docked with ACC (Compound CID: 535) using GROMACS 2019 version. The average values of the molecular simulation analysis were: MM-PSBA = -8.9047 kcal/mol, RMSD = 0.2093058013 nm, RMSF = 0.1089223565 nm, SASA = 149.3414 nm^2, RG = 1.961965 nm. This work indicates that enhancing the activity of the ACC deaminase enzyme from the bacterial endophytes would aid in mitigating stress in the plants.

HIGHLIGHTS

- Endophytic bacteria involvement in the management of abiotic/biotic cellular stress.
- Increasing enzymatic activity of ACC deaminase through point mutations.
- Managing cellular stress in plants through increased ACCD activity.

Keywords: ACC deaminase, Bacterial endophytes, Abiotic stress, Ethylene, *Pseudomonas* sp., Molecular simulation

Endophytes are microorganisms that reside within parts of a host plant (roots, stem, leaves, etc.) and possess an endosymbiotic relationship. They can be fungal or bacterial and have been found to provide mechanisms to counter cellular stress in their respective host plants. Abiotic stress factors, including drought, high salinity, and high acidity in the host plant, cause an increase in ethylene concentration beyond the optimum of around 0.05 µL/L, inhibiting plant growth. As a countermeasure, bacterial endophytes synthesize ACC deaminase from the *acd*S gene, which catalyzes the breakdown of ACC (1-aminocyclopropane-1-carboxylate), a precursor of ethylene. Several other mechanisms are employed in managing biotic stress factors, such as pathogens.

How to cite this article: Deeksha, S., Athreya, A., Lobo, E., Chandramohan, V., Sunil Kumar, C. and Sabat, S. (2022). Structure Analysis and Molecular Simulation Study of ACC Deaminase Mutants from *Pseudomonas* sp., an Endophyte in Reducing Abiotic Stress in Plants. *Int. J. Ag. Env. Biotech.*, **15**(03): 773-780.

Source of Support: None; **Conflict of Interest:** None

Commonly isolated endophytic bacteria genera include *Pseudomonas*, *Bacillus*, and *Microbacterium*, all of which have been successfully isolated from *Aloe vera* (Silva *et al.* 2020). From these, *Pseudomonas hibiscicola* and *Bacillus anthracis*, among other species, exhibited antimicrobial activity. The effect of drought was studied on *Capsicum annum* L., where bacterial endophytes *Bacillus, Achromobacter, Klebsiella,* and *Citrobacter* produced ACC deaminase to reduce stress in the plant (Marasco *et al.* 2012), . In the *Cicer arietinum* seedling, *Bacillus subtilis* BERA 71 showed increased ROS scavenging enzymes under high salinity to reduce the ramifications of high salt concentrations (Abd Allah *et al.* 2018). Maize was subjected to a pathogen, *Fusarium moniliforme*, and bacterial endophytes, namely, *Bacillus amyloliquefaciens* and *Bacillus subtilis*, upregulated pathogenesis-related genes in the host plant (Gond *et al.* 2015).

Ethylene, a phytohormone involved in the regulation of senescence and the growth of plants, is synthesized from S-adenosylmethionine (SAM) via ACC as an intermediate through a ring formation mechanism. The enzymes, ACC synthase and ACC oxidase are pertinent to this synthesis pathway. Under biotic and abiotic cellular stress, there is an increase in ethylene production in the plant, whose concentration crosses a threshold, beyond which the hormone is rendered inactive, known as "stress ethylene" (Vaishnav *et al.* 2019). Ethylene displays more excellent activity at lower concentrations, as low as 0.05 µL/L (Glick *et al.* 2005). ACC deaminase is a PLP-dependent enzyme responsible for the breakdown of ACC in inhibiting the ethylene synthesis pathway. ACC deaminase produced by endophytic bacteria is synthesized by the *acd*S gene in response to increasing ethylene levels. It catalyzes the breakdown of the ACC intermediate to produce ammonia and alpha-ketobutyrate, reducing the concentration of ethylene (Kushwaha *et al.* 2020).

Mutations are often performed to inhibit the activity of the structure under consideration. For instance, the ACE2 enzyme present in SARS-CoV-2 underwent several point mutations in a promising attempt to inhibit its infectious activity for the development of inhibitors against SARS-CoV-2 (Dehury *et al.* 2021). Meanwhile, heme-copper oxidases from *Rhodobacter sphaeroides* underwent single amino acid substitutions, including N139D, which resulted in the elimination of proton pumping activity and an increase in steady-state activity rather than an inhibitory effect (Pawate *et al.* 2002).

In ACC deaminase, the effects of point mutations were approximated using the Predict SNP algorithm in order to identify particular residues that, when mutated, exhibit a non-deleterious/neutral effect on the activity of the enzyme. These structures were carried forward into molecular dynamics using GROMACS. In this article, we analyze the outcomes of a point mutation in ACC deaminase to increase and improve its enzymatic activity. ACC deaminase from *Pseudomonas* sp. ACP (PDB ID: 1TYZ), and discuss the positive effects of the same in the growth of crops or plants with stringent growth conditions.

MATERIALS AND METHODS

The ACC cyclopropane ring-opening process is a mechanism that is catalyzed by the pyridoxal 5′-phosphate (PLP) dependent enzyme, ACC deaminase. To commence the process, an internal aldimine between PLP and a lysine residue in the active site is converted to an external aldimine between PLP and the amino group of the substrate. Subsequent changes begin when the substrate's R-proton is removed.

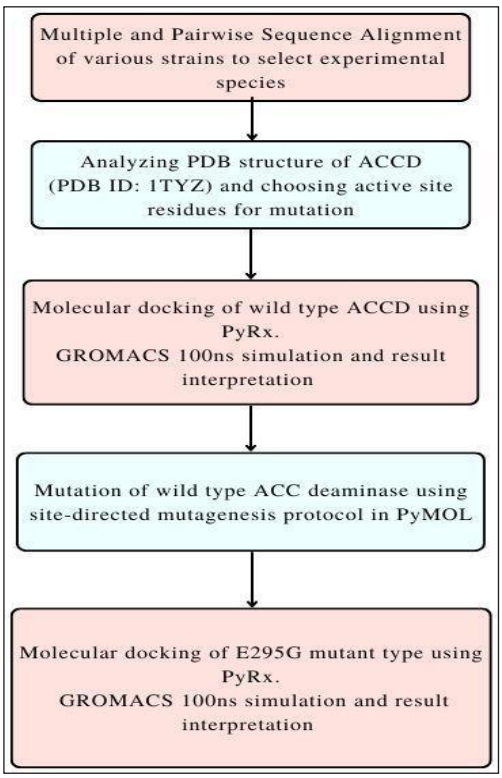

Fig. 1: Overview of Methodology

The final product retains the carboxyl group of ACC, and its R-hydrogen, which cannot be abstracted. As a result, ACC deaminase must begin the cyclopropane ring opening reaction without the availability of an R-carbanionic intermediate. Two potential methods for ring fragmentation have been suggested, both of which use the R-anion equivalent of vinylglycyl-PLP aldimine as a significant reaction intermediate. The first method is nucleophilic addition at the C beta methylene position to break the ring open. At the same time, the second involves a beta-proton abstraction that results in cyclopropane ring cleavage (Karthikeyan *et al*. 2004).

The framework for the ring cleavage most consistent with our result is the nucleophilic addition process. However, clarification of the three-dimensional conformation is necessary to fully comprehend the precise manner of cyclopropane ring scission by ACC deaminase (Karthikeyan *et al.* 2004).

Sequence Alignment of ACCD from Multiple Strains

To select a suitable bacterial ACC deaminase for experimentation, multiple and pairwise sequence alignment was performed on the enzyme from three commonly isolated bacterial endophyte species. They included *Pseudomonas* sp. (UniProtKB: Q00740), *Bacillus* sp. (NCBI Reference Sequence: WP_113728387.1), and *Microbacterium* sp. (NCBI Reference Sequence: WP_211160350.1). Overall, the sequence similarity was found to be above 70%, and sequence identity was above 60%, indicating significant similarity between the three strains. Consequently, ACC deaminase from *Pseudomonas* sp. ACP was considered for further studies due to its crystalline structure in RCSB (PDB ID: 1TYZ).

Molecular Docking of Wild Type ACC Deaminase

ACC deaminase from the *Pseudomonas* sp. (PDB ID: 1TYZ) was selected, and its interaction with ACC (Compound CID: 535) was studied through PyRx. Blind docking and site-specific docking were performed with ACC deaminase as the macromolecule and ACC as the ligand. The significant sites included Ser78, the active site for the mechanism (Karthikeyan *et al.* 2004); Lys51, a PLP-bound cofactor involved; Tyr268 and Tyr294. These sites are essential to the interaction mechanism between ACC and ACC deaminase.

Mutating ACC deaminase

The primary objective was to perform a single amino acid mutation on the ACC deaminase enzyme in order to increase its activity, which indirectly aids in decreasing the effective stress on the plant by breaking down the high concentrations of ACC in the plant. To achieve this objective, the PredictSNP tool was used to estimate the effects of point mutations in ACC deaminase. PredictSNP is a consensus classifier that utilizes 8 prediction tools, namely MAPP, PANTHER, nsSNP Analyzer, PhD-SNP, PolyPhen-1, SIFT, PolyPhen-2, and SNAP, to provide accurate results (Bendl *et al.* 2014). Specific residues, including K51, K54, S78, T199, T202, and E295, that are pivotal to the mechanism

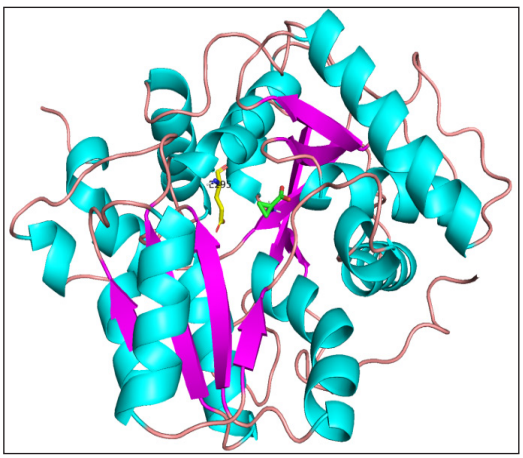

Fig. 2(a): Wild type ACC deaminase with glutamate at position 295

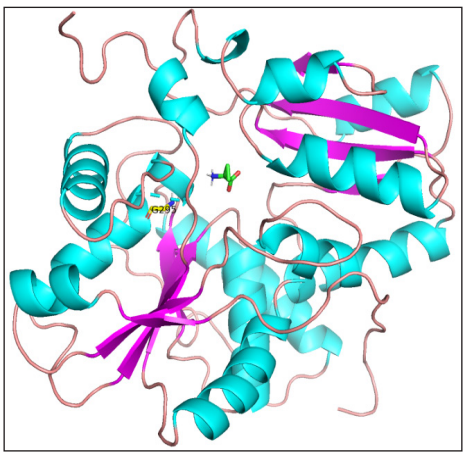

Fig. 2(b): Mutated ACC deaminase with glycine at position 295

(Karthikeyan *et al.* 2004), were considered, and single amino acid substitutions were carried out with the remaining 19 amino acids. While a majority of the substitutions proved to be harmful in nature, the substitutions T199S (threonine substituted by serine in the 199th position), E295G (glutamic acid substituted by glycine in the 295th position), E295S (glutamic acid substituted by serine in the 295th position), and E295T (glutamic acid substituted by threonine in the 295th position) were found to be neutral. Considering these results, the natural ACC deaminase structure was mutated at the positions mentioned above respectively, using the site-directed mutagenesis procedure in PyMOLver 2.5.2 (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.).

MD Simulations & Visualization

Once the wild type was mutated, the mutant models were optimized and cross-checked using the BIOVIA Discovery Studio Visualizer version 4.5 (Dassault Systèmes BIOVIA, BIOVIA DSV, 4.5, San Diego: Dassault Systèmes, 2019). Site-specific docking with ACC was performed for each mutated structure, and the T199S mutant produced binding energy of -4.3 kcal/mol, whereas the E295G mutant was found to have a binding energy of -4.9 kcal/mol. Therefore, the E295G mutant, in which glutamic acid is substituted with glycine at the 295th position, was chosen as the most favorable mutation.

Finally, the ACC-ACC deaminase complexes of the E295G mutant and wild type underwent an all-atoms MD simulation for 100ns utilizing the GROMACS 2019 version (Abraham *et al.* 2015).

The results were then plotted on a graph using Microsoft Excel for RMSD, RMSF, SASA, and Rg values. Additionally, the complexes were visualized on PyMOL.

RESULTS AND DISCUSSION

ACC oxidase has a significantly higher binding affinity to ACC when compared to ACC deaminase (Glick *et al.* 2005). To allow enzymatic activity of ACC deaminase compete with that of ACC oxidase under stress, this article postulates a mutation to increase the binding affinity of ACC deaminase with ACC.

The sequence and structure of ACC deaminase isolated from *Pseudomonas* sp. ACP (PDB ID: 1TYZ) were mutated at the 295th position, converting glutamate to glycine (ACCD-E295G), upon concluding significant similarity (above 70%) between ACC deaminase from *Bacillus* sp. and *Microbacterium* sp. for which crystalline structures were unavailable. The single amino acid mutation was performed on conserved residues and residues found to be pertinent to the catalyzing mechanism of ACC (Karthikeyan *et al.* 2004), and ACCD-E295G was selected. The basis of selection is that molecular docking of wild-type ACC deaminase with ACC demonstrated average binding energy of -4.2 kcal/mol. In comparison, the average binding affinity of ACCD-T199S and ACCD-E295G were -4.3 kcal/mol and -4.9 kcal/mol, respectively.

Upon performing the molecular dynamics simulation, the average values of the Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA), Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Solvent Accessible Surface Area (SASA), and the Radius of Gyration (Rg), were calculated and analyzed.

MM-PBSA

Molecular Mechanics Poisson-Boltzmann Surface Area, or MM-PBSA is a tool used in GROMACS to estimate relative binding energies, particularly

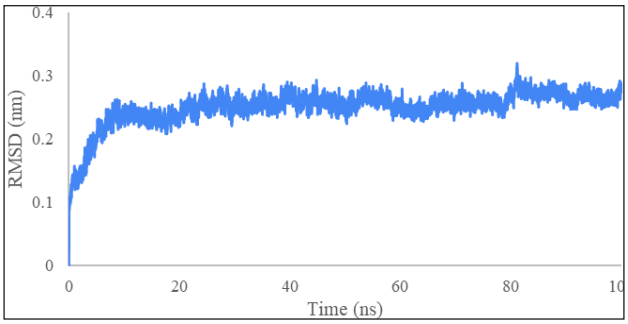

Fig. 3(a): RMSD graph of wild type ACCD docked complex

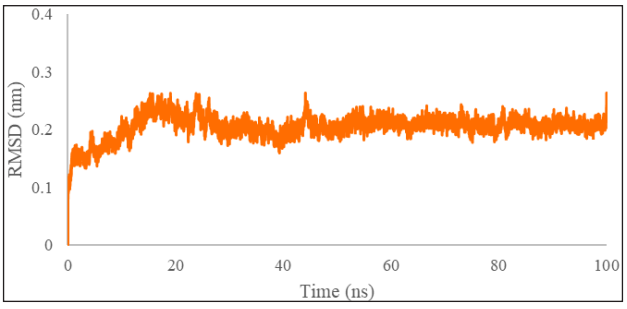

Fig. 3(b): RMSD graph of ACCD-E295G docked complex

in ligand-receptor interactions (Kumari *et al.* 2014).

Regarding the mutant ACCD-E295G, the sum of binding energies of several residues known to be involved in the catalysis of ACC, namely: Ser78 (-1.6767 kcal/mol), Tyr268 (-0.1546 kcal/mol), and Tyr294 (-7.0734 kcal/mol), was calculated, providing an average binding affinity of -8.9047 kcal/mol. On performing the same calculation with the wild-type ACC deaminase, MM-PBSA was 0.4814 kcal/mol, indicating that the mutant portrays a better binding affinity by +9.3861 kcal/mol.

RMSD

Root Mean Square Deviation, or RMSD, is used to measure the difference between initial and final structural conformations concerning the protein backbone, which indicates its stability. A smaller deviation between initial and final conformations indicates higher stability (Aier *et al.* 2016).

The range of RMSD for the wild type was 0.005 - 0.320 Å, while the range for the ACCD-E295G mutant was 0.005 - 2.636 Å. The average RMSD of the E295G mutant was calculated from 10 ns to 100 ns, and was found to be 2.093 Å, while the average RMSD of the wild type was 2.566 Å. Both graphs display increasing deviation until approximately 10 ns for the wild type and 20 ns for the mutant, after which the wild type stabilizes, while the mutant declines slightly, equilibrating after approximately 30 ns. Overall, the RMSD for the mutant remains more stable, with fewer fluctuations in deviation compared to the wild type, and maintains a lower average value. A lower RMSD value indicates more minor deviations, which correlates to a more stable protein structure in the mutant strain, with a lower RMSD by approximately 0.473 Å.

RMSF

Root Mean Square Fluctuation, or RMSF, provides an analysis of the fluctuation of regions of a structure from their mean structure, i.e. measuring the deviation from its reference position (Barnett *et al.* 2022). This indicates the flexibility of the residues in the protein structure.

The range of RMSF for the wild type was 0.612 - 6.183 Å, while the range for the ACCD-E295G mutant was 0.535 - 5.231 Å. The E295G mutant ACC deaminase displayed an average RMSF of 1.089 Å, while the wild type displayed an average RMSF of 1.222 Å. The RMSF values for the wild and mutant ACCD, respectively, regarding the specific residues, were: Ser78- 0.080 nm and 0.072, Tyr268- 0.119 nm and 0.078 nm, Tyr294- 0.082 nm and 0.060 nm, and the mutated residue at position 295 (wild type: Glu295; mutated type: Gly295)- 0.066 nm and 0.063 nm. Overall the flexibility of the residues pertinent to the mechanism of ACC deaminase, including the mutated residue, was lower in ACCD-E295G, indicating that the mutant makes comparatively stronger interactions with ACC at the binding site.

SASA

Solvent Accessible Surface Area, or SASA, provides the area of the molecule of interest which is accessible to a solvent by characterizing the surface of the molecule through Van der Waals forces of attraction with the hypothetical center of the molecule, determining the extent of protein folding and stability (Ausaf Ali *et al.* 2014).

The range of SASA for the wild type was 138.736 - 164.349 nm^2, while the range for the ACCD-E295G mutant was 139.546 - 165.093 nm^2. The average

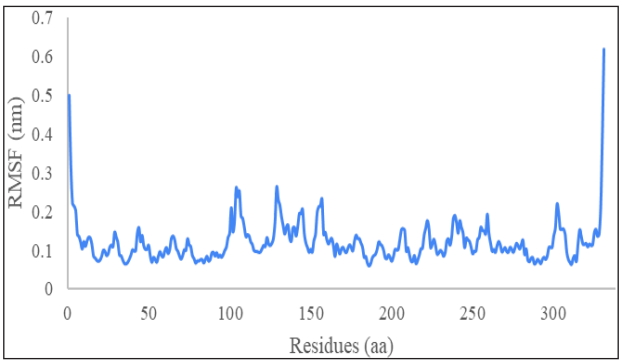

Fig. 4(a): RMSF graph of wild type ACCD docked complex

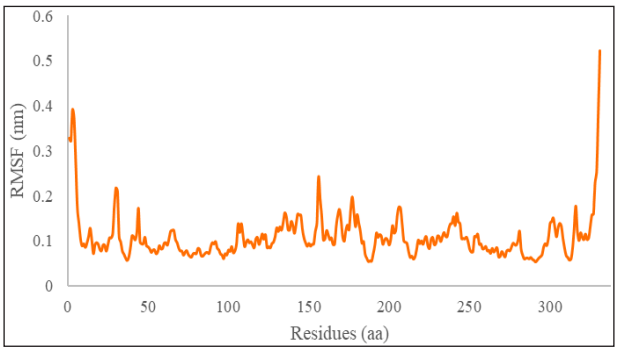

Fig. 4(b): RMSF graph of ACCD-E295G docked complex

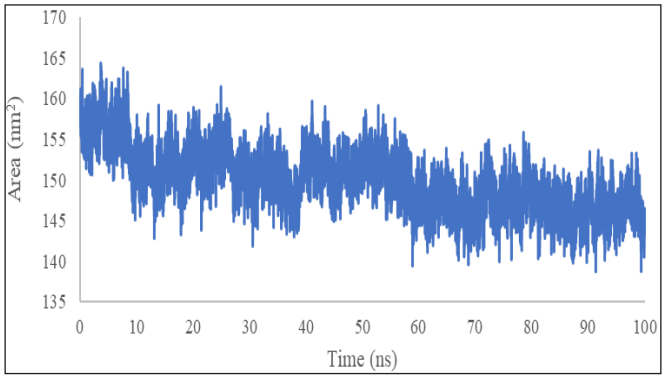

Fig. 5(a): SASA graph of wild type ACCD docked complex

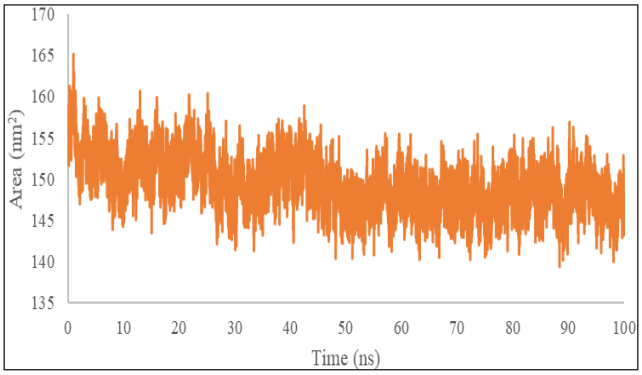

Fig. 5(b): SASA graph of ACCD-E295G docked complex

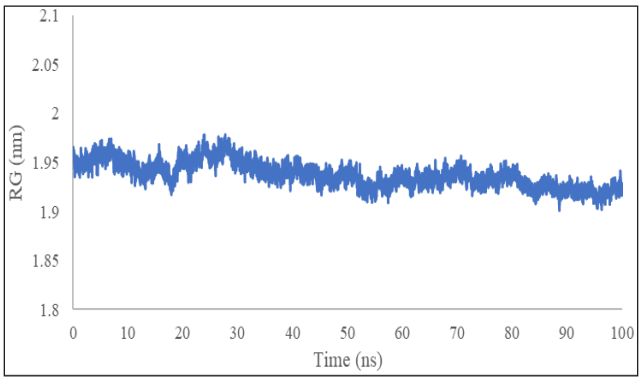

Fig. 6(a): Rg graph of wild type ACCD docked complex

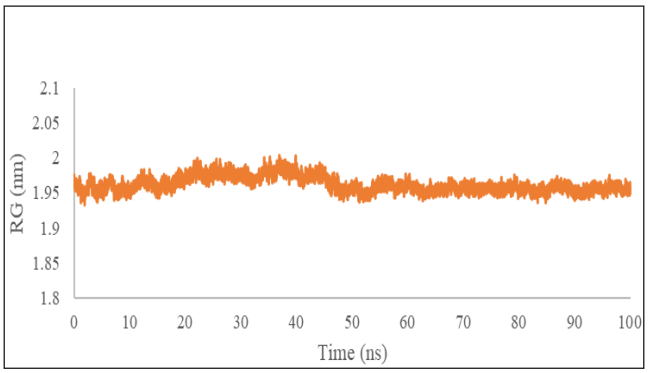

Fig. 6(b): Rg graph of ACCD-E295G docked complex

SASA for the E295G mutant was calculated and was found to be 149.341 nm^2. The wild type was analyzed for the same, and the average SASA was 149.925 nm^2. A higher SASA value correlates to better binding with the small molecule (ACC). From the results, it is observed that the mutant possesses only a slightly lower SASA by 0.584 nm^2 indicating no discernible change in stability compared to the wild type.

Rg

The radius of gyration, Rg, measures the change in protein structure concerning the overall size of the chain during molecular dynamics simulation (Jiang *et al.* 2019).

The range of Rg for the wild type was 1.901 - 1.938 nm, while the range for the ACCD-E295G mutant was 1.933 - 2.003 nm. The average Rg was calculated for the E295G mutant and the wild type over 100ns. The results were 1.962 nm and 1.938 nm, respectively. A lower radius of gyration is an indication of more fantastic folding and integrity of the protein structure. The mutant was shown to have a lower Rg by approximately 0.024 nm, which does not indicate any significant loss of compactness of the structure compared to the wild type.

CONCLUSION

A comparatively less-ventured domain in the study of endophytes and cellular stress is that of *in silico* and computational analyses, particularly regarding the mutation of structures with the intent of increasing their activity. To achieve this, all catalytically critical amino acid residues in ACC deaminase, specifically Lys51, Ser78, Tyr268, and Tyr294, were found to be conserved throughout the process of evolution and were consequently selected as the primary active site residues in the present study. Mutating the enzyme to increase its activity was preferable since the concentration of the enzyme is maintained. This prevents other cellular processes from getting affected. The results obtained are consistent with the hypothesis that a single amino acid substitution, i.e., E295 → G295, could increase the binding affinity between ACC deaminase and ACC.

Molecular dynamics simulation with GROMACS

demonstrated an overall increase in stability and binding affinity concerning the mutant ACCD-E295G. The mutant displayed less deviation and fluctuation within the protein structure and a similar accessible surface area and radius of gyration over a 100 ns simulation. Additionally, the MM-PBSA of the wild-type ACCD was calculated to be 0.481 kcal/mol, while the same for the mutant ACCD-E295G was -8.905 kcal/mol, a significantly higher binding affinity.

As previously stated, ACC deaminase is a pivotal enzyme that breaks down ACC, which is present in high concentrations during periods of stress for the plant. In the presence of the mutated ACC deaminase, the plant could fight the stressors productively. This would effectively help plants grow in unfavorable stressful conditions and reduce the requirement to maintain stringent growth conditions of temperature, water content, etc. For instance, the plant, *Cuminum cyminum* is known to be difficult to grow under humidity and low temperatures. With the aid of endophytic bacteria that produce mutated ACC deaminase, the plant could tolerate the stresses and grow despite the conditions.

In summary, our results demonstrate the benefits of this point mutation in ACC deaminase through its higher binding affinity and higher stability, which is promising for developing plants with higher tolerance to cellular stress through endophytic mechanisms.

REFERENCES

Kumari, R., Kumar, R., Open Source Drug Discovery Consortium and Lynn, A. 2014. g_mmpbsa A GROMACS tool for high-throughput MM-PBSA calculations. *J. Chem. Inform. Model*, **54**(7): 1951-1962.

Aier, I., Varadwaj, P.K. and Raj, U. 2016. Structural insights into conformational stability of both wild-type and mutant EZH2 receptor. *Sci. Rep.*, **6**(1): 1-10.

Barnett, C., Senapathi, T., Bray, S. and Goué, N. 2022 Analysis of molecular dynamics simulations (Galaxy Training Materials). https://training.galaxyproject.org/training-material/topics/computational-chemistry/tutorials/analysis-md-simulations/tutorial.html Online; accessed Fri Jul 22 2022.

Ausaf Ali, S., Hassan, I., Islam, A. and Ahmad, F. 2014. A review of methods available to estimate solvent-accessible surface areas of soluble proteins in the folded and unfolded states. *Curr. Protein Pept. Sci.*, **15**(5): 456-476.

Glick, B.R. 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiology Letters*, **251**(1): 1-7.

Jiang, Z., You, L., Dou, W., Sun, T. and Xu, P. 2019. Effects of an electric field on the conformational transition of the protein: A molecular dynamics simulation study. *Polymers*, **11**(2): 282.

Bendl, J., Stourac, J., Salanda, O., Pavelka, A., Wieben, E.D., Zendulka, J., Brezovsky, J., Damborsky, J., 2014: PredictSNP: Robust and Accurate Consensus Classifier for Prediction of Disease-Related Mutations. *PLOS Computational Biology,* **10**: e1003440.

Karthikeyan, S., Zhou, Q., Zhao, Z., Kao, C.L., Tao, Z., Robinson, H., ... and Zhang, H. 2004. Structural analysis of Pseudomonas 1-aminocyclopropane-1-carboxylate deaminase complexes: Insight into the mechanism of a unique pyridoxal-5'-phosphate dependent cyclopropane ring-opening reaction. *Biochem.*, **43**(42): 13328-13339.

Vaishnav, A., Shukla, A.K., Sharma, A., Kumar, R. and Choudhary, D.K. (2019). Endophytic bacteria in plant salt stress tolerance: current and future prospects. *J. Plant Growth Regul.*, **38**(2): 650-668.

Kushwaha, P., Kashyap, P.L., Bhardwaj, A.K., Kuppusamy, P., Srivastava, A.K. and Tiwari, R.K. 2020. Bacterial endophyte mediated plant tolerance to salinity: growth responses and mechanisms of action. *World J. Microbiol. Biotechnol.*, **36**(2): 1-16.

Silva, C.F., Vitorino, L.C., Mendonça, M.A.C., Araújo, W.L., Dourado, M.N., Albuquerque, L.C. ... and Souchie, E.L. 2020. Screening of plant growth-promoting endophytic bacteria from the roots of the medicinal plant Aloe vera. *S. Afr. J. Bot.,* **134**: 3-16.

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S. ... and Daffonchio, D. 2012. A drought resistance-promoting microbiome is selected by root system under desert farming. *PloS One,* **7**(10): e48479.

Abd_Allah, E.F., Alqarawi, A. A., Hashem, A., Radhakrishnan, R., Al-Huqail, A. A., Al-Otibi, F. O. N., ... and Egamberdieva, D. 2018. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact*, **13**(1): 37-44.

Gond, S.K., Bergen, M.S., Torres, M.S. and White, J.F. Jr. 2015. Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. *Microbiol. Res.,* **172**: 79–87.

Dehury, B., Raina, V., Misra, N. and Suar, M. 2021. Effect of mutation on structure, function and dynamics of receptor binding domain of human SARS-CoV-2 with host cell receptor ACE2: a molecular dynamics simulations study. *J. Biomol. Struct. Dyn.*, **39**(18): 7231-7245.

Pawate, A.S., Morgan, J., Namslauer, A., Mills, D., Brzezinski, P., Ferguson-Miller, S. and Gennis, R.B. 2002. A mutation in subunit I of cytochrome oxidase from *Rhodobacter sphaeroides* results in an increase in steady-state activity but completely eliminates proton pumping. *Biochem.*, **41**(45): 13417-13423.

Mark James Abraham, Teemu Murtola, Roland Schulz, Szilárd Páll, Jeremy C. Smith, Berk Hess and Erik Lindahl. 2015. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX, 19-25. https://doi.org/10.1016/j.softx.2015.06.001