# Structural modelling and functional analysis of *Pseudomonas* ACC deaminase: An *in silico* study

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## Abstract

The activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme and its impact on regulating ethylene concentrations and plant ACC are fundamental characteristic found in plant-associated rhizobacteria. ACC deaminase-producing bacteria, such as *Pseudomonas* mitigate the detrimental effects of high levels of ethylene and ACC in plant-microbe interactions, resulting in improved plant growth and development. Different species within the *Pseudomonas* genus are commonly found in plant microbiome worldwide. Their adaptation to the plant-associated environment makes several *Pseudomonas* strains highly promising for advancing novel sustainable biotechnological and agricultural solutions, particularly those exhibiting ACC deaminase activity. Thus, the present study conducted a comprehensive *in silico* analysis focusing on the structural and functional characteristics of ACC deaminase protein of *Pseudomonas* spp. The study explored correlations based on phylogenetic relatedness using both ACC deaminase enzymes and their respective genes across different *Pseudomonas* species and strains. This study also investigated physiochemical properties, CATH classification and STRING analysis using various bioinformatics tools. *Pseudomonas brassicacearum* was selected as the representative species from the *Pseudomonas* genus for the 3D modeling of ACC deaminase protein. The acidic ACC deaminase protein has an average molecular weight of around 25.30 kDa, with a high percentage of alpha helices in its secondary structure, demonstrating its thermal stability. This theoretical assessment of the structure and

function of ACC deaminase-producing *Pseudomonas* could assist researchers in understanding the ACC deaminase protein, potentially facilitating a better understanding of plant-microbe interactions, and aiding in the selection of highly efficient strains for various agricultural applications.

**Keywords:** *Pseudomonas,* ACC deaminase, *in silico* characterization, Ethylene

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## Introduction

The growing need for sustainable farming techniques necessitates the development of innovative approaches to improve plant growth and stress tolerance without relying on harmful pesticides and chemical fertilizers [1]. One potential substitute for these environmental damaging chemical compounds lies in utilizing plant-growth-promoting rhizobacteria (PGPR) [2]. These beneficial bacteria, commonly present in soils, have the potential to effectively enhance plant growth and overall plant health [3]. Among the key mechanisms utilized by PGPR is the activation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme, which subsequently regulates ethylene by metabolizing its immediate precursor, ACC [4].

Ethylene plays a vital role as an essential plant hormone that regulates the growth and development of plants, actively contributing to various developmental and physiological processes within them [5, 6]. Furthermore, ethylene plays a role in plants' responses to stress conditions, including those caused by abiotic and biotic stressors [7, 8]. Additionally, it participates in regulating interactions between plants and microbes [9, 10]. The ACC deaminase (*acdS*) genes show positive selection in microbial symbionts related to various leguminous plants worldwide [10], highlighting the importance of ACC deaminase enzyme in facilitating advantageous interactions between plants and microbes. Some rhizospheric bacteria possess the ACC deaminase (EC 4.1.99.4) enzyme, which can convert ACC into ammonia and α-ketobutyrate [11], thus regulating the production of ethylene. The activity of ACC deaminase is found in various gram-negative bacteria such as

*Cronobacter sakazakii*, *Mesorhizobium* sp., *Halomonas* sp., *Variovorax paradoxus*, *Burkholderia phytofirmans*, , *Methylobacterium fujisawaense*, and *Pseudomonas* sp. [12-14].

The *Pseudomonas* genus has a wide range of diversity, consisting of approximately 250 species commonly found in soil and plant microbiomes worldwide, with some of them demonstrating the ability to promote plant growth [15]. Due to their enhanced metabolic adaptability, biocontrol capabilities, rapid growth rate, capacity to thrive in diverse soil conditions, and direct interaction with plant hosts, various strains of *Pseudomonas* have proven important for the advancement of biotechnological and agricultural products [16]. However, the identification of highly efficient PGPR strains within the *Pseudomonas* genus remains a challenging process.

An effective approach for addressing this challenge is to select *Pseudomonas* strains capable of regulating the levels of plant hormones, particularly ethylene (a gaseous hormone) [6, 17]. The ability of bacteria to modulate plant ethylene levels is largely dependent on the expression of the ACC deaminase enzyme, responsible for breaking down the non-proteinogenic amino acid ACC [18], the immediate precursor of ethylene in all higher plants. The capability to metabolize plant ACC deaminase has been observed to improve the plant growth-promoting potential of several microbial strains, including those within *Pseudomonas* spp. Furthermore, numerous studies have demonstrated that *acdS* gene is commonly present in bacteria which have close association with their host plants, including members from both the α and β-proteobacteria classes [19].

Moreover, the plant microbiome, usually survive under stressful conditions. exhibits an increased abundance of bacteria containing the *acdS* gene, among which *Pseudomonas* is commonly found [20, 21]. Based on the literature cited above, it is essential to identify and characterize potential ACC deaminase-producing bacteria to develop effective bio-fertilizers, enhancing their potential for promoting plant growth and their ability for protecting plants against different environmental stresses. However, there are limited studies that conducted *in-silico* analysis on the function and structure of ACC deaminase proteins. Therefore, this study aimed to assess the role of the ACC deaminase in different *Pseudomonas* species through computational modeling. The current study used various *in-silico* modeling techniques to assess the primary, secondary, and

tertiary structures of the ACC deaminase protein, aiming to get a deeper knowledge of its function and underlying mechanism.

## Material and Methods

### Nucleotide and Amino acid Sequence Retrieval

The nucleotide sequences of the *acdS* gene and its corresponding protein sequences from various *Pseudomonas* species and strains were acquired from the NCBI database (https://www.ncbi.nlm.nih.gov/). A total of twenty-one ACC deaminase proteins and gene sequences were retrieved in FASTA format, subsequently, utilized for further *in-silico* investigations [22].

### Phylogenetic Analysis

Two phylogenetic trees were constructed to compare the evolutionary relationships among various *Pseudomonas* species and strains based on the *acdS* gene and its corresponding protein. MEGA11 software was used for generating both phylogenetic trees [23]. The analysis involved multiple sequence alignment of the retrieved acdS protein and gene sequences. The neighbor-joining method was utilized to construct these trees [24]. To assess the reliability of internal branches within both trees, 1000 bootstrap replicates were employed. The bootstrap technique plays a crucial role in result accuracy evaluation, where a bootstrap value of 70% or higher indicates strong support for the clade within the phylogenetic study, while a lower bootstrap value suggests a less definitive separation of the clade from others [25].

### Physiochemical Characterization

The Expasy ProtParam program (https://web.expasy.org/protparam/) was utilized to compute the physicochemical characteristics of the obtained acdS protein sequences. These characteristics included various parameters, including number and composition of amino acids, aliphatic and instability indices, and grand average of hydropathicity (GRAVY). The instability indices results provided insights into the stability of protein, while the aliphatic index value indicated the space occupied by aliphatic side chains within the protein structure. Additionally, GRAVY values for our proteins were determined by dividing the overall hydropathy value by the total number of residues [26].

### Secondary Structure Prediction

The PSIPRED web-based tool (http://bioinf.cs.ucl.ac.uk/psipred/) was utilized for secondary structure prediction, including helices, sheets, and turns [27]. In

this study, the PSIPRED algorithm predicted the secondary structure of the acdS protein sequences obtained from the *acdS* gene in *Pseudomonas brassicacearum* TY1210 strain. The PSIPRED algorithm operates on artificial neural networks as its fundamental framework. This approach aims to predict the secondary structure of amino acid sequence by investing information obtained from evolutionarily related proteins. Moreover, the SOPMA web-based server was utilized to predict the secondary structure of the acdS protein. The outcomes from both servers were analyzed to aid in the prediction of the protein tertiary structure.

**Tertiary Structure Prediction** Following secondary structure prediction, the tertiary structure of the *P. brassicacearum* TY1210 strain was predicted using I-TASSER server [28]. Initially, I-TASSER employs a multiple threading technique to recognize structural templates from the Protein Data Bank (PDB). Subsequently, it refines the 3D models by re-threading them through the protein function database to gain insights into the function of the target protein. The resultant model generated by I-TASSER was further analyzed and validated.

**Model Evaluation and Validation**

The quality of the I-TASSER model was evaluated through the SAVES SERVER (https://saves.mbi.ucla.edu/) using QMEAN and PROCHECK tools. The Saves server employed six programs to assess the model quality. The selection of the best model was based on the quality score provided by ERRAT, a method designed to identify inaccurately predicted areas within the protein structure, characterized by a random atom distribution. QMEAN evaluated both local and global quality aspects of a model focusing on crucial geometrical features within the protein structure. The PROCHECK function within the SAVES server allowed visualization of energetically permissible regions concerning backbone dihedral angles of amino acid residues in the acdS protein. The resulting Ramachandran plot, generated by PROCHECK server, facilitated an examination of the stereochemical quality inherent in the protein structure [29].

**Functional Analysis**

The prediction of functionally interacting proteins of the acdS protein from *P. brassicacearum* TY1210 strain was carried out using the STRING (https://string-db.org/). This platform utilized to investigate the interactions between acdS

deaminase proteins and other proteins. Numerous data regarding protein-protein interactions are available in this database sourced from experiments, textual analysis, and computer prediction techniques. Protein-protein interactions helps to understand cellular processes at the system level and play crucial role in annotating proteins structural, functional, and evolutionary characteristics. It encompasses both direct physical interactions and indirect functional relationships between proteins [30, 31].

**CATH Classification**

The acdS protein from the *P. brassicacearum* TY1210 strain was further analyzed through CATH classification. Within the CATH database, protein domains are hierarchically classified based on their folding patterns, sourced from protein structures submitted to the PDB. Domain identification and subsequent categorization within CATH involve a combination of manual and automatic processes [32].

**Results and discussion**

Beneficial bacteria producing ACC deaminase have the potential to enhance plant growth and tolerance against stresses, providing a sustainable solution to reduce reliance on excessive chemicals in agriculture [33]. In this study, various bioinformatics tools were used to assess the functional activity and structural attributes of the *acdS* gene in different *Pseudomonas* species. For this purpose, nucleotide and amino acids sequences of *acdS* gene and proteins were retrieved from the NCBI database, which provides extensive biological information including genomic data, literature resources, gene/protein sequences, and tools for data analysis [34]. Twenty-one different species and strains of *Pseudomonas* were retrieved, which include *P. putida* UW4 , *P. entomophila* PS-PJH, *Pseudomonas* sp. *6G5*, *P. putida* AM15, *P. brassicacearum* 3Re2-7, *P. fluorescens* A-RE-6, *P. fluorescens* A-RE-7a, *P. zarinae* SWRI108, *Pseudomonas* sp. St386 , *P. brassicacearum* NFM421, *P. ogarae* FR1, *P. asplenii* B21-058, *P. fuscovaginae* UPB0736, *Pseudomonas* sp. FP2262, *P. fluorescens* GL-RE-29, *P. thivervalensis* PLM3, *P. fuscovaginae* LMG, *P. bijieensis* L22-9, *Pseudomonas* sp. MPDS , *P. migulae* 8R6, and *P. brassicacearum* TY1210 (Table 1). The retrieved sequences were either partial or complete CDS sequences. Among all strains and species, *P. brassicacearum* TY1210 has stable protein, therefore,

further analysis was conducted on this strain.

Table 1: Gene and protein accession number of various species and strains of *Pseudomonas*

| **Strain name** | **Gene Accession No.** | **Protein Accession No.** |
|---|---|---|
| *P. putida* UW4 | AY823987.1 | AAV73804.1 |
| *P. entomophila* PS-PJH | FJ882923.1 | ACQ55296.1 |
| *Pseudomonas* sp. 6G5 | M80882.1 | AAA73153.1 |
| *P. putida* AM15 | EF011160.1 | ABJ91236.1 |
| *P. brassicacearum* 3Re2-7 | CP034725.1 | QEO78113.1 |
| *P. fluorescens* A-RE-6 | MW328592.1 | UET52172.1 |
| *P. fluorescens* A-RE-7a | MW328590.1 | UET52170.1 |
| *P. zarinae* SWRI108 | CP077086.1 | QXH97171.1 |
| *Pseudomonas* sp. St386 | AP021900.1 | BBP52783.1 |
| *P. brassicacearum* NFM421 | CP002585.1 | AEA68459.1 |
| *P. ogarae* FR1 | CP025738.1 | AUO47345.1 |
| *P. asplenii* B21-058 | CP087202.1 | UZE27194.1 |
| *P. fuscovaginae* UPB0736 | CP100603.2 | UUQ64316.1 |
| *Pseudomonas* sp. FP2262 | CP117443.1 | WLH48503.1 |
| *P. fluorescens* GL-RE-29 | MW328565.1 | UET52145.1 |
| *P. thivervalensis* PLM3 | CP022202.1 | AXA59978.1 |
| *P. fuscovaginae* LMG 2158 | LT629972.1 | SEI22525.1 |
| *P. bijieensis* L22-9 | CP048810.1 | QKS83047.1 |
| *Pseudomonas* sp. MPDS | CP054128.1 | QKJ34834.1 |
| *P. migulae* 8R6 | CP093428.1 | WGK88412.1 |
| *P. brassicacearum* TY1210 | KC430111.1 | AGJ52208.1 |

To compare the evolutionary relationships among various species and strains of *Pseudomonas*, two phylogenetic trees were constructed in MEGA11 [23]. One tree was based on the nucleotide sequences of the *acdS* gene, while the other tree used the amino acid sequences of the ACC deaminase protein from different *Pseudomonas* species. It was observed that *P. brassicacearum* TY1210 clustered closely with *Pseudomonas* sp. MPDS (Figure 1). This clustering was strongly supported by a high bootstrap value of 97, indicating significant acceptance within the cluster. The collected data strongly suggests a correlated expression of the *acdS* gene among different *Pseudomonas* species.

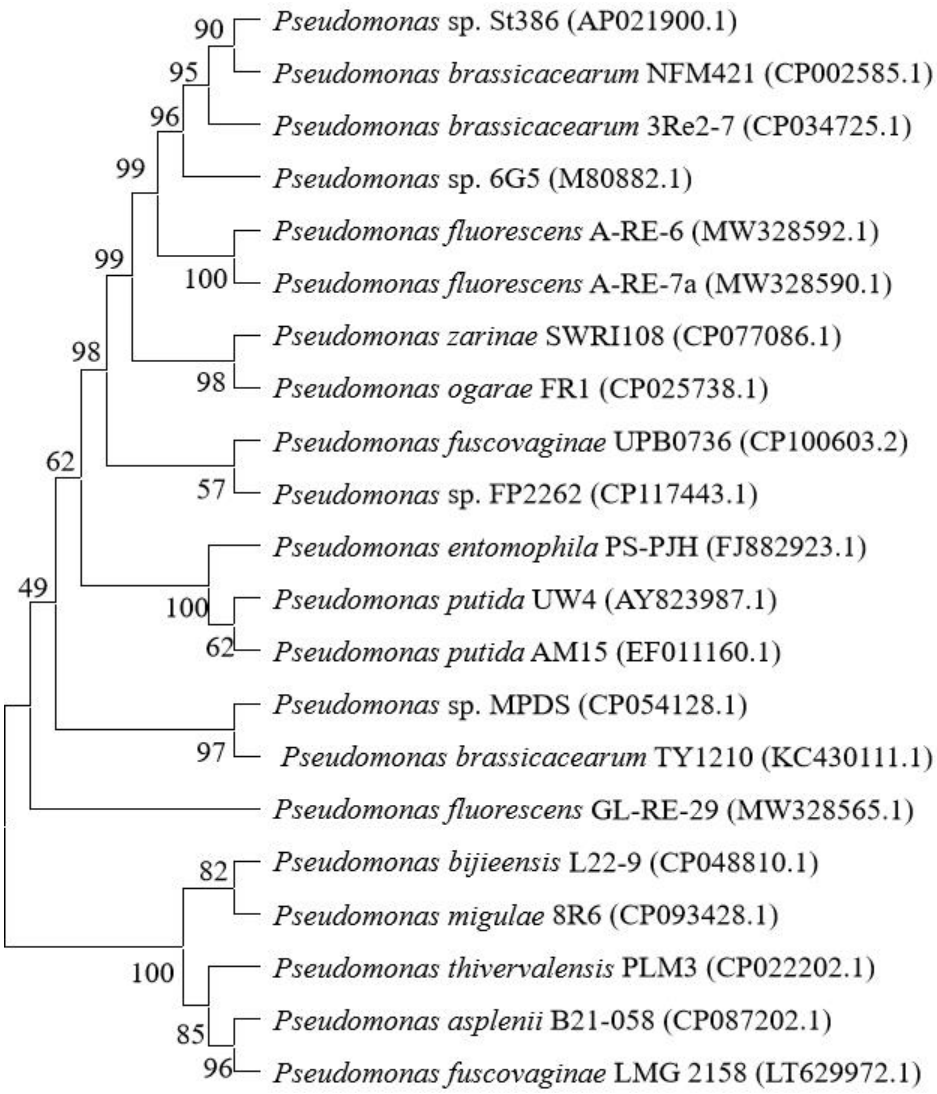


Figure 1: Phylogenetic tree of *acdS* gene of various species and strains of *Pseudomonas*

Similarly, the acdS protein tree showed that *P. brassicacearum* TY1210 clustered with *Pseudomonas* sp. MPDS, with 98% bootstrap value (Figure 2). The higher bootstrap value indicates strong support for the clade within the phylogenetic study. The computed data suggests a connection between the amino acid composition of acdS protein and gene. Previous study conducted by Nikolic et al., [35] reported similar clustering patterns among different *Pseudomonas* strains based on phylogenetic analysis of acdS genes. A similar trend was observed in the phylogenetic analysis of *acdS* genes among various PGPR, which included several *Pseudomonas* species [36].

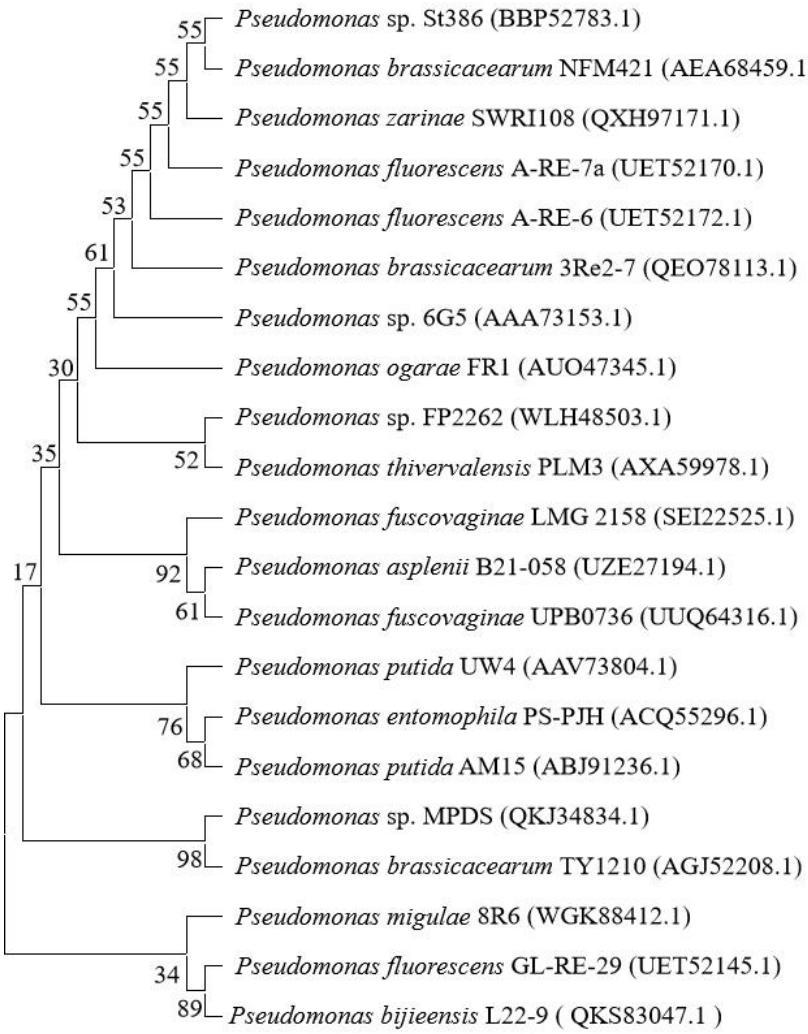


Figure 2: Phylogenetic tree of acdS protein of various species and strains of *Pseudomonas*

The physicochemical characterization of ACC deaminase proteins from various *Pseudomonas* species was conducted using the Expasy ProtParam tool (Table 2). The results indicated that almost all ACC deaminase proteins across different *Pseudomonas* species and strains consists of 338 amino acids, except for *P. brassicacearum* TY1210, which contains 234 amino acids. The molecular weight of the ACC deaminase in *P. brassicacearum* TY1210 was 25.30 kDa, while in other *Pseudomonas* species, it ranges from 36.80 to 37.01 kDa. All analyzed proteins exhibit instability indices above 40, indicating an unstable nature of ACC deaminase, except for the protein in *P. brassicacearum* TY1210, which exhibited an instability index value of 35.67, suggesting its stable nature. Studies reported that a protein is considered stable if its instability index is less than 40 [37].

Table 2: Physiochemical properties of acdS protein of various species and strains of *Pseudomonas*

| Strain name | No. of amino acids | Molecular weight (kDa) | pI | II | AI | GRAVY |
|---|---|---|---|---|---|---|
| *P. putida* UW4 | 338 | 36.87 | 5.43 | 40.22 | 84.23 | -0.187 |
| *P. entomophila* PS-PJH | 338 | 36.86 | 5.66 | 40.52 | 83.08 | -0.203 |
| *Pseudomonas* sp. 6G5 | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *P. putida* AM15 | 338 | 36.80 | 5.8 | 40.66 | 84.23 | -0.179 |
| *P. brassicacearum* 3Re2-7 | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *P. fluorescens* A-RE-6 | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *P. fluorescens* A-RE-7a | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *P. zarinae* SWRI108 | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *Pseudomonas* sp. St386 | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *P. brassicacearum* NFM421 | 338 | 36.87 | 5.43 | 42.2 | 84.53 | -0.183 |
| *P. ogarae* FR1 | 338 | 36.90 | 5.43 | 43.13 | 84.53 | -0.185 |
| *P. asplenii* B21-058 | 338 | 36.87 | 5.53 | 42.81 | 84.53 | -0.183 |
| *P. fuscovaginae* UPB0736 | 338 | 36.87 | 5.53 | 42.81 | 84.53 | -0.183 |
| *Pseudomonas* sp. FP2262 | 338 | 36.90 | 5.62 | 40.33 | 84.53 | -0.185 |
| *P. fluorescens* GL-RE-29 | 338 | 36.89 | 5.8 | 38.97 | 84.53 | -0.19 |
| *P. thivervalensis* PLM3 | 338 | 36.89 | 5.43 | 39.56 | 85.09 | -0.179 |
| *P. fuscovaginae* LMG 2158 | 338 | 36.94 | 5.66 | 42.81 | 84.53 | -0.194 |
| *P. bijieensis* L22-9 | 338 | 36.91 | 5.66 | 38.89 | 84.53 | -0.199 |
| *Pseudomonas* sp. MPDS | 338 | 36.93 | 5.53 | 39.06 | 85.38 | -0.174 |
| *P. migulae* 8R6 | 338 | 37.01 | 5.96 | 40.05 | 85.09 | -0.2 |
| *P. brassicacearum* TY1210 | 234 | 25.30 | 4.74 | 35.67 | 91.67 | -0.035 |

The isoelectric point (pI) indicates the stable, neutral state of a protein, representing the pH value at which the protein surface maintains a net charge of zero. In this study, the pI values for all *Pseudomonas* species range between 4.74 and 5.96, indicating the relatively acidic nature of these proteins. When devising buffer systems for purifying recombinant proteins using the isoelectric targeting method, the theoretically estimated pI proves to be advantageous [38]. The aliphatic index is a metric indicating protein stability, demonstrating a direct association between high AI values and increased thermal stability in globular proteins [39]. AI values ranging between 83.08 to 91.67, suggest the thermostability of ACC deaminase across all *Pseudomonas* species and strains. The maximum AI (91.67) was

observed in *P. brassicacearum* TY1210 indicates its stable nature. On the other hand, the GRAVY index defines the hydrophilic or hydrophobic characteristics of proteins [40]. A low GRAVY value indicates a higher affinity of proteins for water, indicating their hydrophobic nature [41]. The GRAVY value for the ACC deaminase protein among all species and strains ranges between -0.035 and -0.19, indicating the non-polar and hydrophilic nature of this protein in *Pseudomonas*.

Proteins consist of polypeptide chains structured with 20 different amino acid residues. Each amino acid possesses unique characteristics that enable specific functions within a protein. The percentages reflecting polarity, charge, aromaticity, and aliphatic properties in proteins vary according to their roles and locations [42]. Phosphorylation, a pivotal process in signaling pathways, often targets two primary amino acid residues: Threonine and Tyrosine, due to their side chains containing hydroxyl groups that facilitate phosphate binding [43]. In this study, the ProtParam tool was employed to assess the percentage of all 20 amino acids. Results showed that the percentage of Ala, Cys, Glu, Asp, and IIe in the ACC deaminase of *P. brassicacearum* TY1210 was 9.83%, 2.1%, 9%, 5.1%, and 6.4%, respectively (Figure 3), while in other species and strains, these values were comparatively lower, indicating a different amino acid composition.

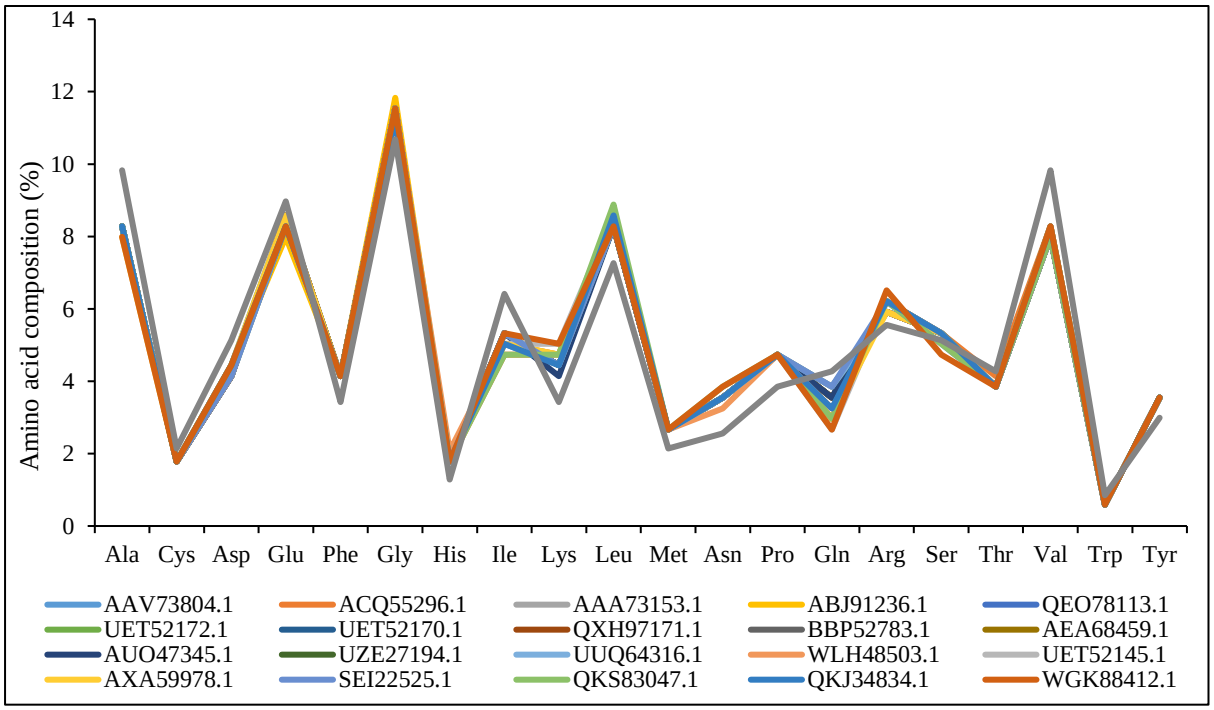

Figure 3: Amino acid composition of acdS protein of various species and strains of *Pseudomonas*

The secondary structure prediction of the ACC deaminase protein from *P. brassicacearum* TY1210 was conducted using two online web servers, PSIPRED [27] and SOPMA [44]. PSIPRED results indicated that the ACC deaminase protein contains all three secondary structure elements, i.e. coils, turns and helices (Figure 4A). However, this protein contains more alpha helices, suggesting its stable nature. Furthermore, SOPMA results indicated that alpha helices (42.31%) are more prevalent than coils (35.90%), followed by extended strands (15.38%) and beta turns (6.41%) as shown in Figure 4B. The presence of secondary structural arrangements in proteins suggests their folded state, indicating the stable nature of the protein. Research has shown that thermophiles, organisms thriving in high-temperature environments, tend to exhibit a larger portion of their protein residues in α-helical shape, enabling them to endure high temperatures [45]. In this context, the increased occurrence of the α-helical conformation further indicates the thermally stable nature of acdS protein.

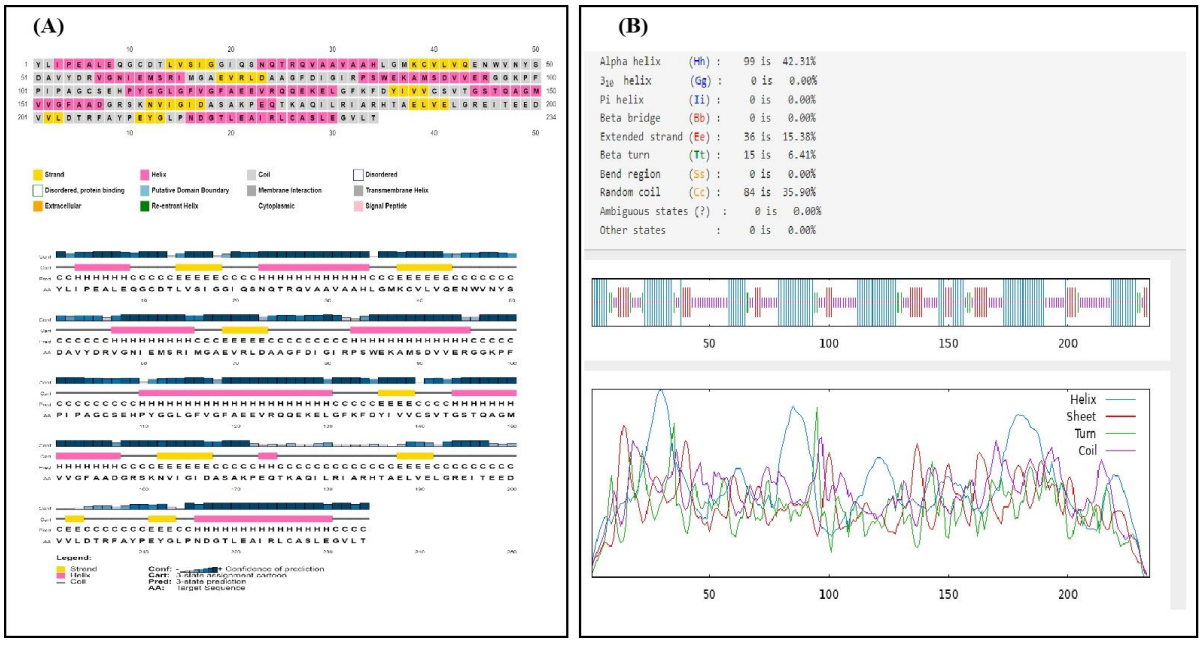


Figure 4: Secondary structure prediction of acdS protein of *P. brassicacearum* TY1210 (A) PSIPRED (B) SOPMA

The tertiary structure of the acdS protein of *P. brassicacearum* TY1210 was predicted using I-TASSER. The LOMET approach within I-TASSER identified structured templates from the PDB library. The significance of these templates was determined by their Z score, followed by selection based on threading alignment [46]. For predicting the tertiary structure of ACC deaminase,, templates with the highest Z scores were chosen. The 3D structure of ACC deaminase exhibits a C score of 1.12, a TM score of 0.87±0.07, and an RMSD value of 3.4±2.4 Å (Figure 5A). This model possesses a higher C score, indicating a high confidence level in this 3D structure. The TM score signifies the similarity level between two structures, and for this model, the score of 0.87 indicates correct topology. RMSD measures the average distance between all residues in two structures, emphasizing sensitivity to local errors. Both TM score and RMSD serve as standards for measuring structural similarity between two models [28].

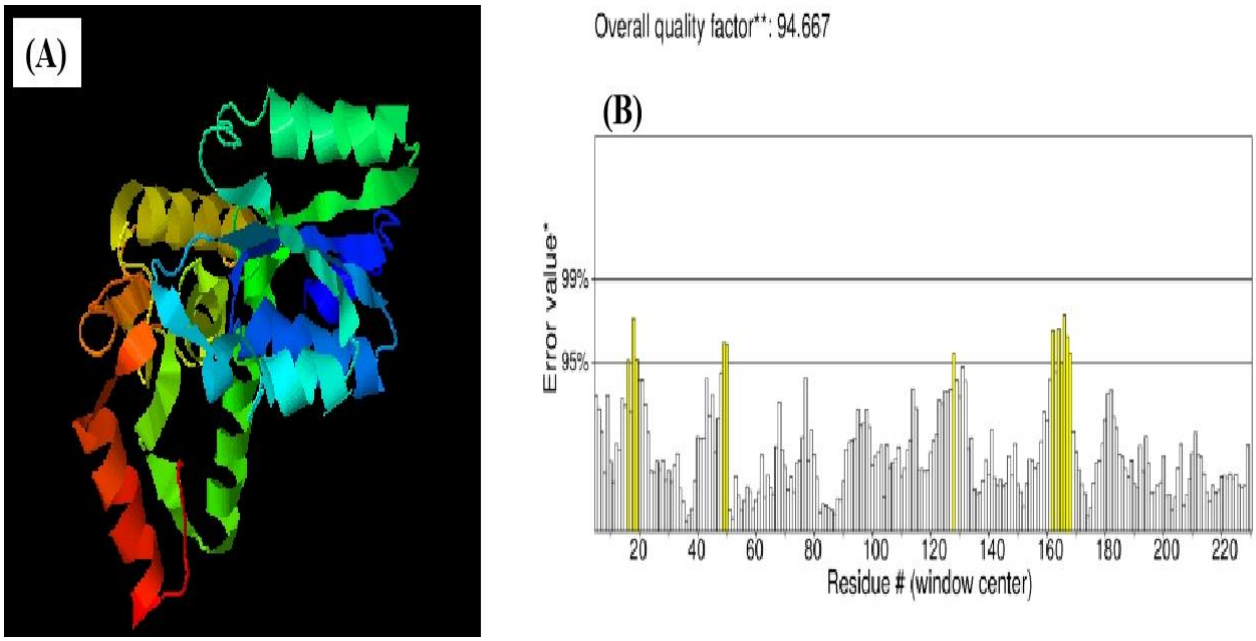


Figure 5: I-TASSER and ERRAT analysis of acdS protein of *P. brassicacearum* TY1210 (A) 3D model (B) ERRAT analysis

The SAVES server was used to assess the quality of the PDB model generated by I-TASSER. The ERRAT score of 94.66 indicates a high-quality 3D model (Figure 5B). The predicted 3D model of acdS protein was uploaded in .pdb format in the SWISS Model server to determine model quality and validation. The acdS protein

showed QMEAN4 scores of -3.37 (Figure 6).

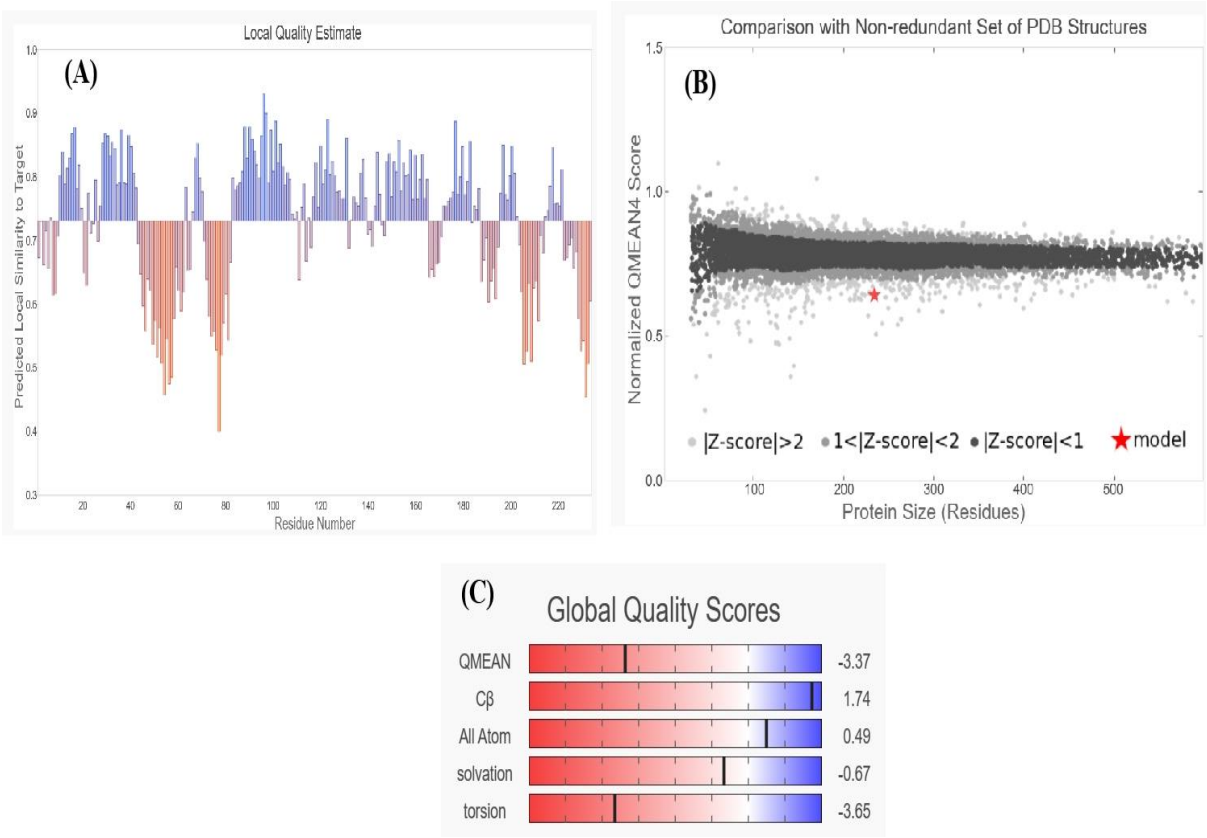


Figure 6: Quality assessment of acdS protein from *P. brassicacearum* TY1210 (A) Local quality estimate; (B) QMEAN 4 score; (C) Global quality score

The normalized QMEAN4 score, compared with a non-redundant set of PDB structures, was <1, meeting the criteria for a standard high-resolution structure [47]. The Ramachandran plot in Figure 6C illustrates that 76.8% of residues are in the most favored regions, 18.7% in additionally allowed regions, and 3% in generously allowed regions (Figure 7). However, a high-quality model is characterized by more than 90% of its residues positioned within the favored region [48]. Numerous studies conducted by different researchers have utilized similar *in silico* modeling techniques to predict 3D models of proteins of interest [49-51].

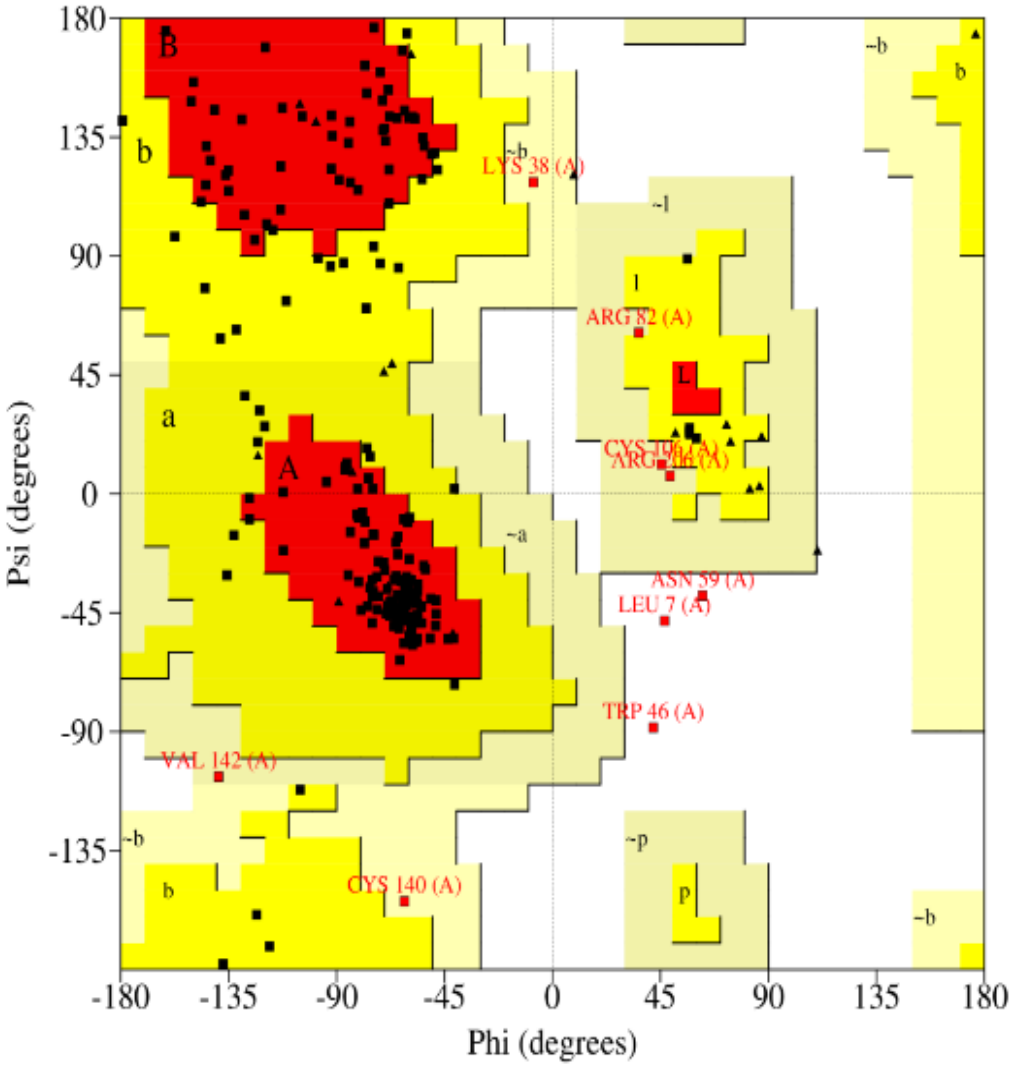


Figure 7: Ramachandran plot analysis of acdS protein of *P. brassicacearum* TY1210

The acdS protein of the *P. brassicacearum* TY1210 was classified using the CATH database, which showed 38 matching domains. The hierarchical classification system assists in predicting the evolutionary relationships and structural characteristics of a protein [52]. In the present study, the classification was predicted using the 1TZK matching domain, which showed 7.0 x $10^{-48}$ E value. This protein is categorized as "Alpha Beta" in Class 3, indicating its fundamental structural fold (Table 3). At the Architecture level, it presents a "3-Layer(aba) Sandwich" (Architecture 3.40), delineating the organization of its secondary structures. Moreover, at the Topology level, it displays a "Rossmann fold" (Topology 3.40.50), defining its precise configurations and relationships

within this architecture. However, it does not show any classification at Homologous Superfamily 3.40.50.300. This suggest that the protein may have experienced substantial evolutionary changes, causing it to deviate from other proteins in the same superfamily, thereby complicating its classification [53]. Moreover, CATH also identifies the functional protein family that aligns with "1-aminocyclopropane-1-carboxylate deaminase (3.40.50.1100/FF/53)".

Table 3: CATH classification of acdS protein of *P. brassicacearum* TY1210

| **Level** | **CATH code** | **Description** |
|---|---|---|
| C | 3 | Alpha Beta |
| A | 3.4 | 3-Layer(aba) Sandwich |
| T | 3.40.50 | Rossmann fold |
| H | 3.40.50.1100 | - |

The functional analysis identified nine different protein interactions for *P. brassicacearum* TY1210 within the protein-protein interaction network, observed through STRING analysis (Figure 8). Results indicated that *P. brassicacearum* TY1210 acdS interacts with AEA68040.1, AEA69700.1, AEA69701.1, AEA68458.1, AEA68460.1, AEA68263.1, AEA69826.1, PqqB, and PqqB-2. However, the closet interaction was observed with AEA68040.1, a putative 1-aminocyclopropane-1-carboxylate deaminase, having a 0.954 score. Previous computational studies on functional annotation have involved similar assessments such as identifying protein primary, secondary and tertiary structures, exploring protein-protein interactions, and identifying structural motifs [51, 54]. The results indicated the presence of ACC deaminase activity in Pseudomonas sp., offering insights into its potential interactions, functions, or biological pathways associated with this activity. However, it is necessary to thoroughly examine these predictions and conduct further validation experiments to confirm the actual functional relationships of this protein.

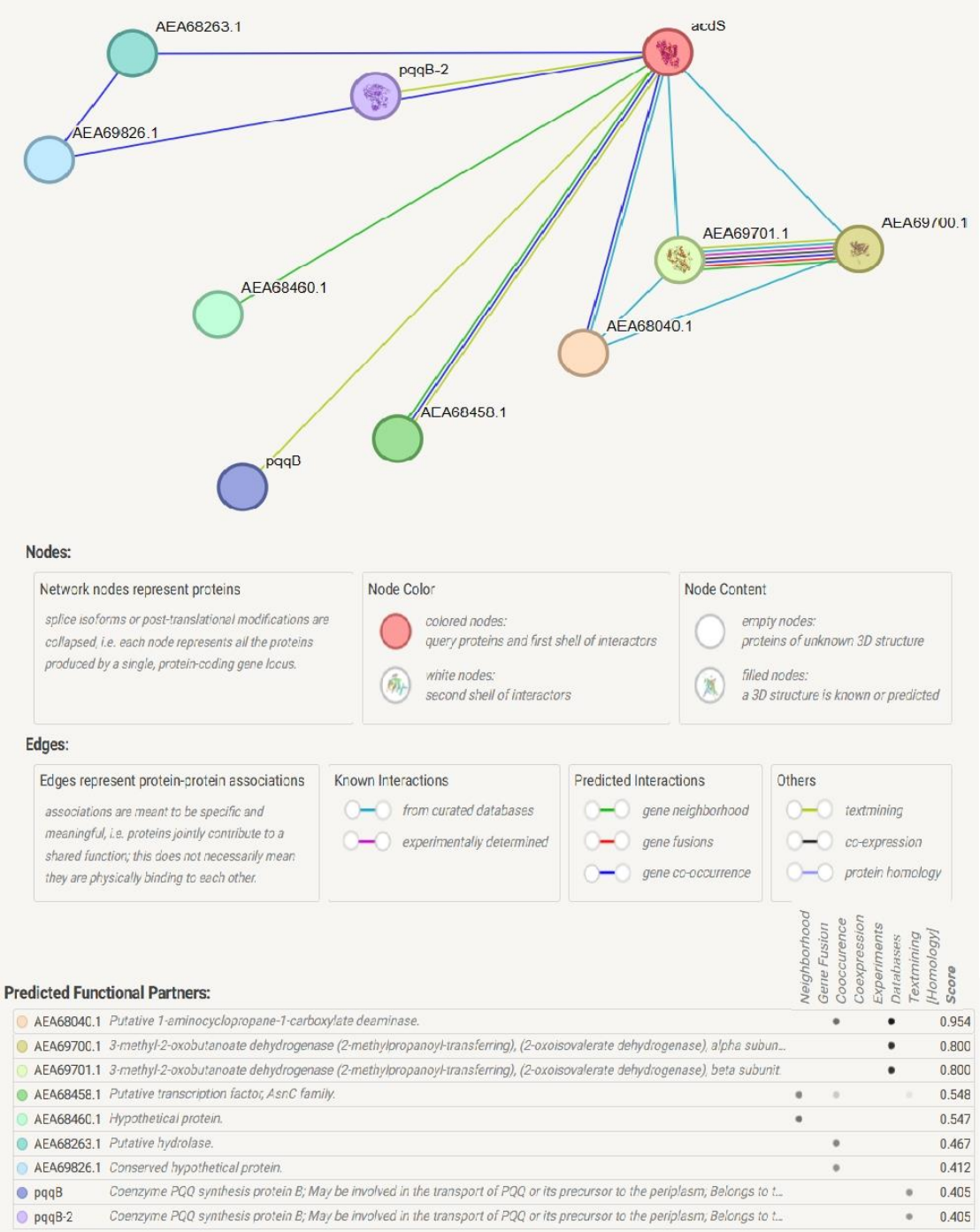


Figure 8: STRING analysis of acdS protein of *P. brassicacearum* TY1210

## Conclusion

ACC deaminase is an important and widely studied bacterial enzyme involved in reducing ethylene levels within plant cells during stress conditions. This enzyme plays a vital role in benefiting legume plants, enabling them in effectively fixing atmospheric nitrogen within their root

nodules. In this study, the *in silico* characterization showed that ACC deaminase is a thermostable, acidic protein with a molecular mass ranging between 25.30 to 37.01 kDa. Its secondary structure mainly consists of alpha helices (42.31%), coils (35.90%), followed by extended strands (15.38%) and beta turns (6.41%). Different servers were used to assess the quality of the 3D-modeled protein. Further, through phylogenetic comparison among the selected taxa, a strong correlation between the ACC deaminase enzyme and its corresponding genes was observed. However, this study this study offers a theoretical understanding of the functional and structural attributes of the ACC deaminase enzyme, shedding light on its significance in the symbiotic relationship between plants and microbes. Further *in vivo* research is needed to validate its potential.

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