In Silico Molecular Insights on the Structure-Function Aspects of ACC Deaminase of a Non-Pathogenic Klebsiella Pneumoniae

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***Abstract*—**Bacterial 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) is known to involve in breaking down the 1-aminocyclopropane-1-carboxylic acid (ACC), immediate precursor of ethylene, into a-ketobutyrate and ammonia. This is required when stress (biotic or abiotic) induced ethylene content is accelerated in plant cells resulting in reduction in plant biomass and yield. *Klebsiellapneumoniae*, although previously considered only as a pathogenic bacteria, there are some strains reported till date to prove it as a plant growth promoting bacteria (PGPB). ACCD activity has reported from the said strain but the present study is emphasized on its molecular proteomic structures and functions studied in silico. The present work revealed that the ACCD of *K. pneumoniae* is a 36.5 kDatetrameric stable protein found in intracellular condition. The phylogenetic analysis clearly depicts its similarity with several other ACCD reported from different bacterial genera. The structure-function insight would definitely help future researchers in designing wet lab as well as dry lab experiments.

Keywords-*Klebsiella pneumoniae; non-pathogenic; PGPR, in silico; protein modeling.*

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I. INTRODUCTION

ACC deaminase(EC3.5.99.7)is a microbial hydrolase family enzyme found both in fungi and bacteria [1]. On the other hand, ACC is the precursor of plant hormone ethylene found in plant cells, isolated from *Pseudomonas* sp. strain ACP [2]. Ethylene is a plant hormone essential for plant growth, fruit ripening; however excess ethylene has deleterious effect of root and soot development. The ACC deaminase producing rhizobacteria split the ACC into alpha ketobutyrate and ammonia using as carbon or nitrogen source, eventually reduce the level of ACC and ethylene [3]. Thus rhizobacteria are bound to root or seed and act as a sink for ACC and reduced the levels of ethylene. So ACC deaminase producing PGPR promote plant growth particularly under stress conditions by the regulation of accelerated ethylene production in response to various stress.

Thus ACC deaminase enzyme or protein (ACCD) has importance in agriculture particularly in stress condition. There are very few report of the in silico characterization of this protein found in rhizobacteria [4]. The present study describes in silico analysis of molecular proteomic structure of the ACCD protein isolated from *Klebsiellapneumoniae*. Attempts were also made the computational analysis of this protein to know the structural and functional insight essential for both dry and wet laboratory experiments.

II. MATERIAL AND METHODS

a. Retrieval and selection of reference sequence

Protein and corresponding gene sequence of *Klebsiellapneumoniae* was retrieved from NCBI (http://www.ncbi.nlm.nih.gov/) database in FASTA format. This sequence was used for further computational investigation including the molecular modeling.

b. Construction of phylogenetic tree of similar sequences

BLAST search was performed by using NCBI-BLAST to find the similar protein sequences with selected protein and phylogenetic tree was constructed using MEGA7 [5] software to find the evolutionary distances among the proteins. Whole genome sequences were omitted and partial sequences were selected from the search result.

c. Primary sequence analysis

Primary sequence analysis included the physicochemical characteristics of the *K. pneumoniae* protein (AEQ29825.1). ExpasyProtParam tool [6] was used to determine the molecular weight and amino acid composition.

d. Secondary structure prediction

Prediction of secondary structure (which involved the estimation of number of helices, sheets, turns, and coils in the amino acid sequence) was done from CFSSP: Chou and

Fasman secondary structure prediction server (www.biogem.org/tool/choufasman) [7-8].

e. Tertiary homology protein modeling and evaluation

SWISS-MODEL (ProMod3 version 1.0.2) workspace [9] was used for determining the homology protein model of AEQ29825.1. Evaluation of built model was done in SAVES server (http://services.mbi.ucla.edu/SAVES/). Ramachandran plot was constructed in RAMPAGE (http://morded.bioc.cam.ac.uk/~rapper/rampage.php) using the pdb file. The energetically allowed regions for backbone dihedral angles ϕ against ψ amino acid residues was visualized in the Ramachandran plot.

f. Functional analysis

Functionally interacting proteins of AEQ29825.1 were predicted by STRING server (http://string.db.org). The motif search tool (http://www.genome.jp/tools/motif/) was used to identify functional motifs in the AEQ29825.1 protein sequence.

III. RESULTS AND DISCUSSION

a. Retrieval and selection of reference sequence

In silico study of ACC deaminase protein of *Klebsiellapneumoniae* has not been previously studied unless in very preliminary form, that's why the present work was undertaken. But, similar computational investigation was done in other bacterial genera. Pramanik et al [4] worked on Mesorhizobium ACC deaminase giving a detailed structural and functional insight on the protein.

b. Construction of phylogenetic tree of similar sequences

Phylogenetic studies (Fig. 1) with similar protein sequences revealed that ACCD of *K. pneumoniae* (AEQ29825.1) clustered with ACCD of different bacterial species which includes *Serratiarubidaea* (AEQ29824.1), *Bacillus cereus* (AEQ29826.1), *Klebsiellaoxytoca* (ACJ12921.1), *Pseudomonas entomophila* (ACQ55296.1), *Pseudomonas putida* (ABJ91236.1) and *Pseudomonas fluorescens* (ACJ69586.1). Among this, closest clustering was observed with AEQ29825.1, AEQ29826.1 and ACJ12921.1. Similar phylogenetic assessment with protein sequences was observed in the works of Verma et al [10], Pramanik et al [4, 11-13].

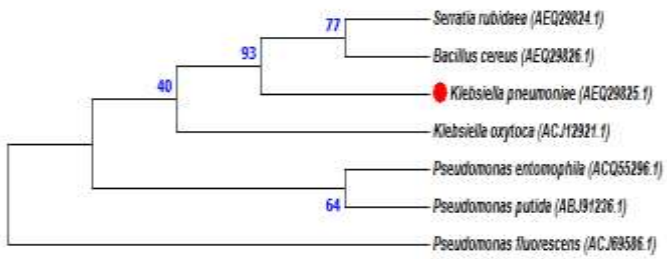

Figure 1. Phylogenetic tree of different ACCD of different species.

c. Primary sequence analysis

Primary sequence analysis revealed a set of physicochemical characteristics of the protein of interest. The protein consisted of 335 amino acid residues having molecular weight ~36.51 kDa. The Glycine was found highest in content in terms of percentage of the total amino acids (Fig. 2). Physicochemical characterization is important in terms of knowing the nature of protein [4].

Amino acid composition:

Ala (A)	28	8.4%
Arg (R)	21	6.3%
Asn (N)	11	3.3%
Asp (D)	15	4.5%
Cys (C)	6	1.8%
Gln (Q)	10	3.0%
Glu (E)	29	8.7%
Gly (G)	39	11.6%
His (H)	6	1.8%
Ile (I)	17	5.1%
Leu (L)	27	8.1%
Lys (K)	15	4.5%
Met (M)	8	2.4%
Phe (F)	14	4.2%
Pro (P)	16	4.8%
Ser (S)	18	5.4%
Thr (T)	13	3.9%
Trp (W)	2	0.6%
Tyr (Y)	12	3.6%
Val (V)	28	8.4%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Figure 2. Amino acid composition of selected protein AEQ29825.1.

d. Secondary structure prediction

Prediction of secondary structure (Fig. 3) showed that the proteins has secondary elements of alpha helix 62.7%, sheets 34.3% and turns 13.4%. The high content of alpha helices indicated the stable nature of the protein [4, 10].

e. Tertiary homology protein modeling and evaluation

After target-template alignment (Fig. 4), homology protein model was built (Fig. 5) using the best match template (1tzm.1.A) obtained from SWISS sever. The built model clearly showed that the protein actually consisted of four polypeptide chains. Ramchandran plot (Fig. 6, 7) revealed that 96.6% residues resided in favored region, 2.9% in allowed region and rests (0.5%) were in outlier region. A good quality model is expected to have more than 90% in the favored region [4]. Moreover, the overall quality factor for the pdb model was

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97.007% for each chain as found from SAVES server. It implied the characteristics of a good quality as well as a high resolution model.

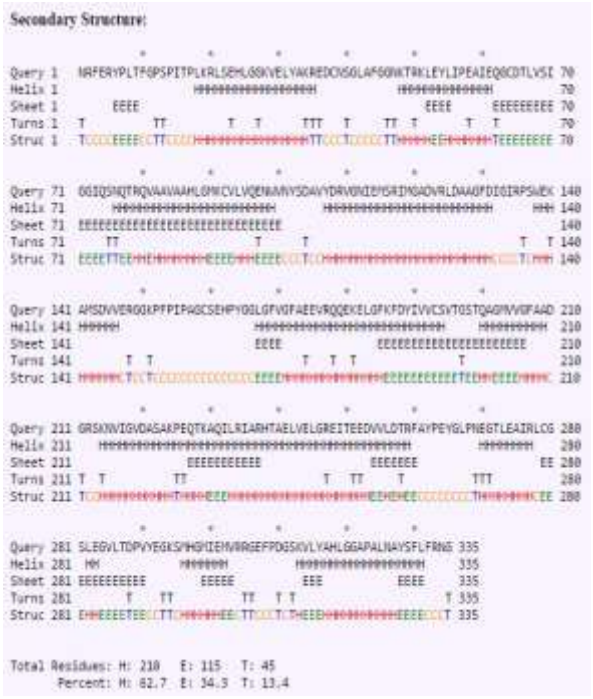

Figure 3. Secondary structure of AEQ29825.1 protein.

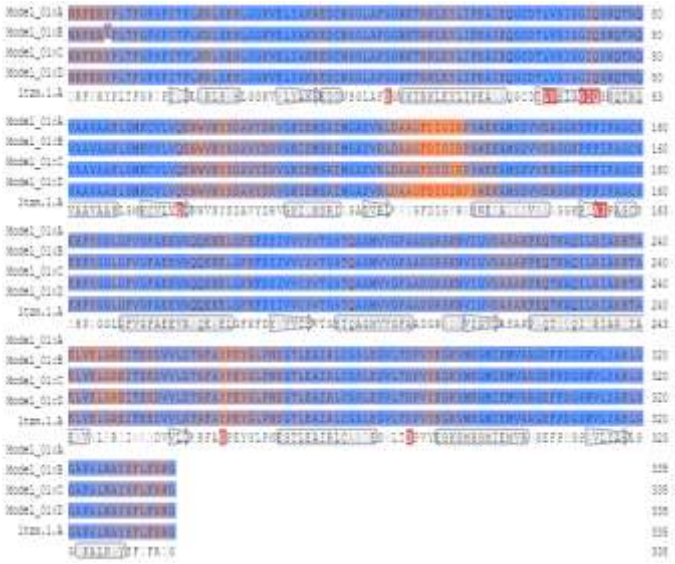

Figure 4. Target-template alignment of AEQ29825.1 with 1tzm.1.A proteins.

f. Functional analysis

STRING analysis (Fig. 8, 9) detected a list of ten functionally interacting protein partners with AEQ29825.1 (written as "dcyD" in the center of the STRING network). These proteins were yecS, yecC, sseA, metC, KPN_02418, ynjE, malY, KPN_04233, KPN_03410 and yfbQ. Besides, two functional motifs were found from motif search (Fig. 10) which includes PALP-pyridoxal-phosphate dependant enzyme (PF00291) and Sin_N- Sin-like protein conserved region (PF4801).

IV. CONCLUSION

The present study encompasses the in silico detailed investigation on the important ACC deaminase enzyme of *Klebsiellapneumoniae*, a PGPR. From this study it was revealed that ACC deaminase of *K. pneumoniae* is a 36.5 kDatetrameric stable protein found intracellularly within the said bacterial species. The constructed phylogenetic tree exposed its similarity with some of the other ACCD of different bacterial species. Morever, this study is first to work out the "in detailed", in silico approach of *Klebsiellapneumoniae* ACC deaminase. For future laboratory experiments and to design primers specific for the amplification of the cDNA of the particular protein, this study might be very helpful to the researchers.

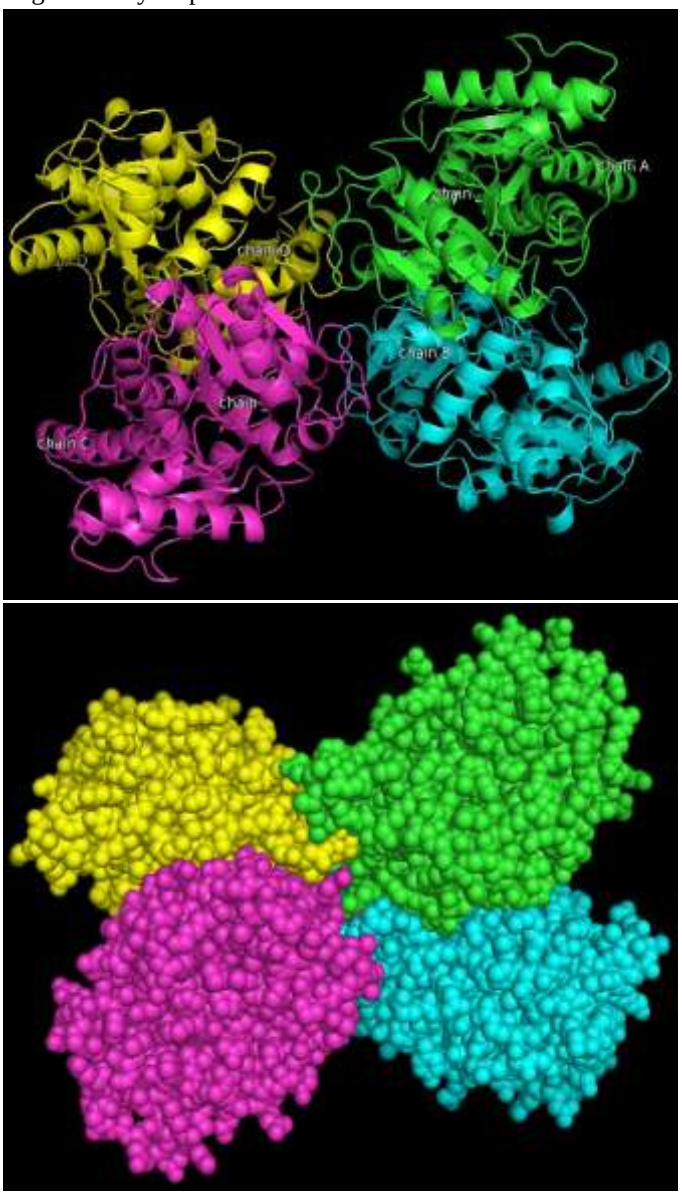

Figure 5. Built tertiary protein model for the selected protein AEQ29825.1.

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Acknowledgements

The authors are gratefully acknowledged to Department of Science and Technology, New Delhi, Government of India for financial supports to K. Pramanik (1st author) as DST-INSPIRE Research fellow (Reg. No. IF150197) and P.K.Ghosh (2nd author) as National Post-Doctoral Fellow (DST, SERB) (Reg. No. NPDF/2016/00323 dt.05.07.16).

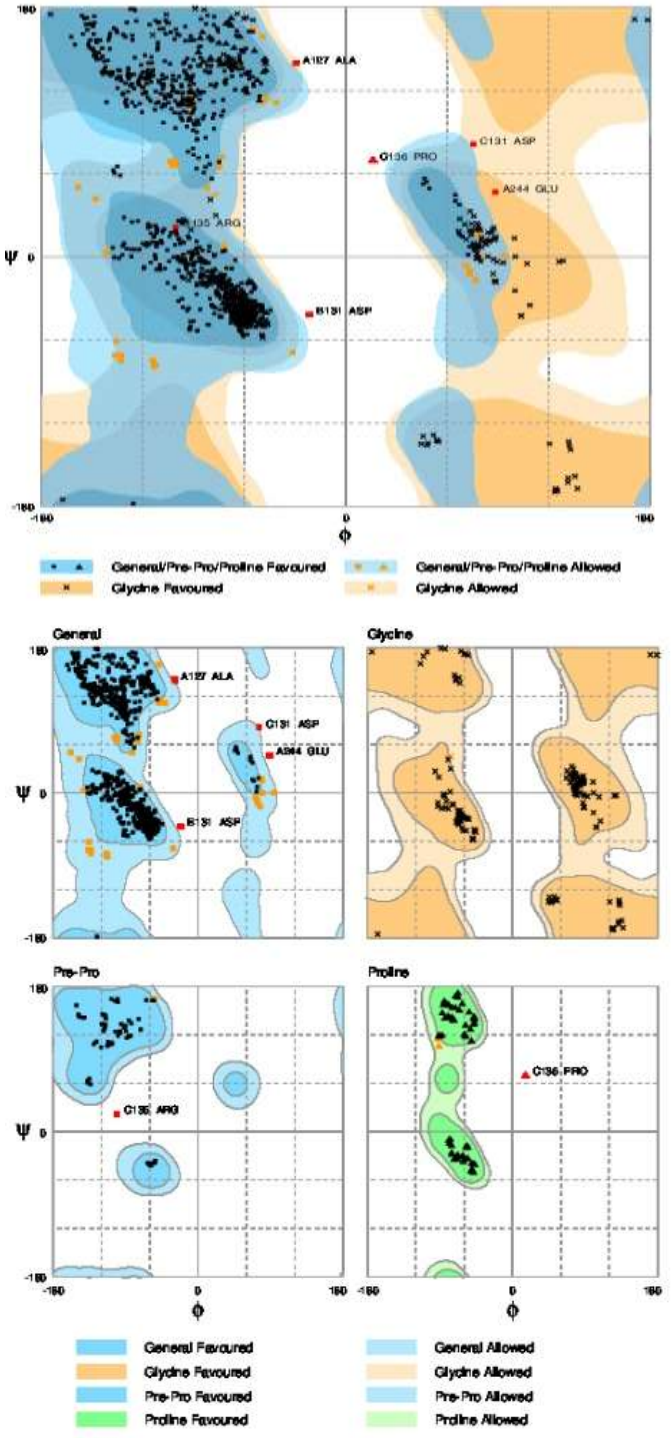

Figure 6.Ramachandran plot for the selected protein AEQ29825.1.

Evaluation of residues

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Residue [A  18 :LEU] (-103.55,  68.94) in Allowed region
Residue [A  44 :PHE] (  73.53, -12.22) in Allowed region
Residue [A 101 :ASN] ( -74.82,  64.61) in Allowed region
Residue [A 126 :ASP] ( -48.91, 159.04) in Allowed region
Residue [A 134 :ILE] ( -81.79,  49.93) in Allowed region
Residue [A 136 :PRO] ( -91.35, 106.85) in Allowed region
Residue [A 157 :ALA] ( -48.96, 111.31) in Allowed region
Residue [A 195 :VAL] (-132.84, -75.24) in Allowed region
Residue [A 291 :TYR] (-112.33, -78.48) in Allowed region
Residue [B  18 :LEU] (-102.26,  66.38) in Allowed region
Residue [B  44 :PHE] (  76.81, -16.82) in Allowed region
Residue [B 109 :ARG] (-141.12,   3.54) in Allowed region
Residue [B 128 :ALA] (  79.45,  -0.21) in Allowed region
Residue [B 154 :PRO] ( -91.61, 116.10) in Allowed region
Residue [B 195 :VAL] (-132.84, -70.87) in Allowed region
Residue [B 223 :ALA] (  79.15,  15.86) in Allowed region
Residue [B 262 :TYR] ( -54.64, 164.47) in Allowed region
Residue [B 291 :TYR] (-113.91, -74.95) in Allowed region
Residue [C  18 :LEU] (-100.12,  65.61) in Allowed region
Residue [C  44 :PHE] (  71.12,  -6.38) in Allowed region
Residue [C 103 :SER] (-158.12,  50.27) in Allowed region
Residue [C 157 :ALA] ( -48.77, 111.77) in Allowed region
Residue [C 195 :VAL] (-136.28, -71.09) in Allowed region
Residue [C 223 :ALA] (  77.56,  17.21) in Allowed region
Residue [C 246 :GLY] ( -76.88,  45.15) in Allowed region
Residue [C 291 :TYR] (-113.16, -78.94) in Allowed region
Residue [D  18 :LEU] (-100.95,  69.05) in Allowed region
Residue [D  44 :PHE] (  73.57,  -9.79) in Allowed region
Residue [D 101 :ASN] ( -73.16,  72.14) in Allowed region
Residue [D 103 :SER] (-147.78,  41.93) in Allowed region
Residue [D 109 :ARG] (-141.44,   3.54) in Allowed region
Residue [D 134 :ILE] (  93.83,   0.36) in Allowed region
Residue [D 137 :SER] ( -31.21, -69.11) in Allowed region
Residue [D 154 :PRO] ( -91.96, 116.80) in Allowed region
Residue [D 157 :ALA] ( -46.98, 114.63) in Allowed region
Residue [D 195 :VAL] (-135.46, -60.91) in Allowed region
Residue [D 214 :LYS] ( -72.10,   7.57) in Allowed region
Residue [D 223 :ALA] (  77.26,  17.22) in Allowed region
Residue [D 291 :TYR] (-113.19, -78.14) in Allowed region
Residue [A 127 :ALA] ( -29.49, 139.70) in Outlier region
Residue [A 244 :GLU] (  88.58,  46.75) in Outlier region
Residue [B 131 :ASP] ( -21.31, -41.50) in Outlier region
Residue [C 131 :ASP] (  75.57,  81.31) in Outlier region
Residue [C 135 :ARG] (-100.41,  21.64) in Outlier region
Residue [C 136 :PRO] (  16.27,  70.16) in Outlier region
Number of residues in favoured region   (~98.0% expected)  : 1287 ( 96.6%)
Number of residues in allowed region    ( ~2.0% expected)  :   39 (  2.9%)
Number of residues in outlier region                       :    6 (  0.5%)
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Figure 7. Amino acid residues of Ramachandran plot.

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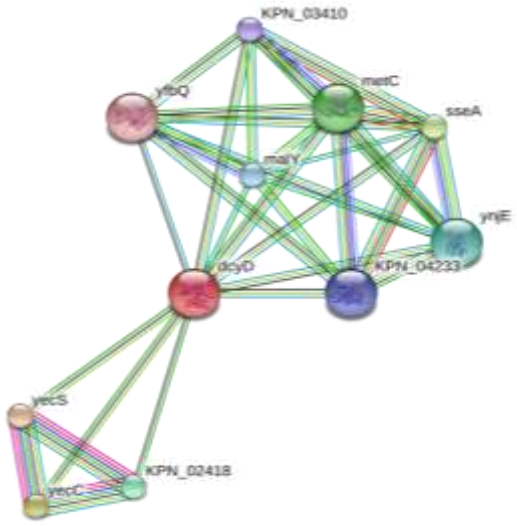


Figure 8.STRING network showing functional interacting protein partners with the selected proteinAEQ29825.1.

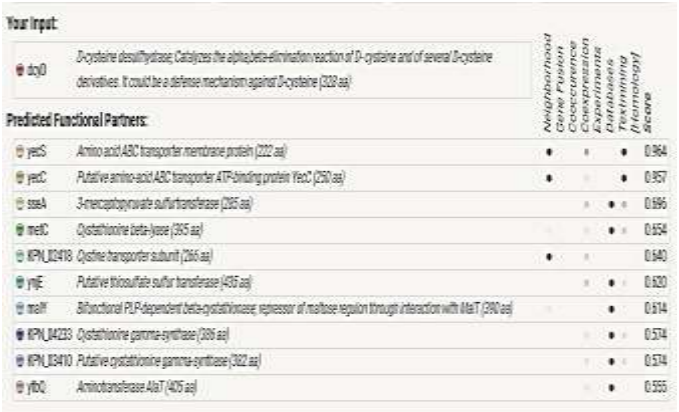
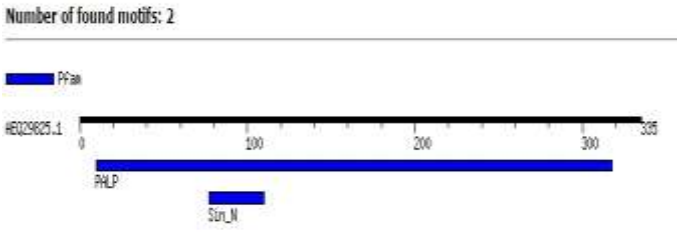


Figure 9. List of proteins interacting with the selected proteinAEQ29825.1.



Pfam (2 motifs)

Pfam	Position(Independent E-value)		Description
PALP	10..318(3.5e-41)	Detail	PF00291, Pyridoxal-phosphate dependent enzyme
Sin_N	77..110(0.13)	Detail	PF04801, Sin-like protein conserved region

Figure 70. Motif search result showing functional motifs for the selected protein AEQ29825.1.

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