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Comparative Analysis of 1-aminocyclopropane-1-carboxylate (ACC) Deaminase in Selected Plant Growth Promoting Rhizobacteria (PGPR)

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1-aminocyclopropane-1-carboxylate (ACC) deaminase promotes plant growth by sequestering and cleaving the ethylene precursor ACC to α-ketobutyrate and ammonium. Many plant growth promoting rhizobacteria producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase as a source of nitrogen has an eminent role in plant nutrition. In this work to perform comparative proteomics analysis of ACCD producing plant growth-promoting rhizobacteria (PGPR) i.e., *Azospirillum lipoferum, Phyllobacterium brassicacearum, Pseudomonas fluorescens, Francisella tularensis subsp. holarctica OSU18* and *Bacillus cereus.* The sequence and phylogenetic analysis of ACCD producing PGPR species represents the common conserved domain belonging to the tryptophan synthase beta subunit-like PLP-dependent enzymes superfamily and closely related to each other. The predicted homology models of ACCD of PGPR have similar protein structure with similar folds often share similar function. This analysis represents the evolutionary conservation and same biochemical function of ACCD producing plant growth-promoting rhizobacteria.

Key words: ACCD, PGPR, α-ketobutyrate, Ammonium, Proteome, Evolutionary conservation.

Soil microorganisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promote plant growth by sequestering and cleaving plant-produced ACC, and thereby lowering the level of ethylene in the plant. Many plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and this enzyme can cleave the ethylene precursor ACC to α-ketobutyrate and ammonium and thereby lower the level of ethylene in developing or stressed plants (Glick, 1995; Glick, *et al.*, 1998; Jacobson, *et al.*, 1994). Rhizosphere bacteria that favourably affect the plant growth and yield of commercially important crops are now denominated as "Plant growth promoting rhizobacteria" (PGPR) (Kloepper, *et al.*, 1980).The well known PGPR, include, bacteria belonging to the genera, namely, *Azospirillum, Azotobacter, Pseudomonas, Bacillus, Azoarcus, Klebsiella, Arthrobacter, Enterobacter, Serratia and Rhizobium* on non-legumes (Burdman, *et al.*, 2000). Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield (Wu, *et al.*, 2005). Certain strains of these plant-associated bacteria stimulate plant growth in multiple ways: (1) they may fix atmospheric nitrogen; (2) reduce toxic compounds; (3) synthesize phytohormones and siderophores; or (4) suppress pathogenic

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organisms (Bloemberg and Lugtenberg., 2001). Many plant growth-promoting bacteria contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and this enzyme can cleave the ethylene precursor ACC to α-ketobutyrate and ammonium and thereby lower the level of ethylene in developing or stressed plants. ACC deaminase activity has been found to be associated with a large number of different soil microorganisms (Klee *et al.*, 1991; Jacobson *et al*., 1994; Glick *et al*., 1995 and 1998; Campbell and Thomson, 1996). Interestingly, within a particular genus and species of microorganism, some strains have ACC deaminase activity, and others often do not. Thus, for example, while some strains of *Azospirillum* have recently been reported to contain an acdS gene (Blaha *et al*., 2006). In this study analysed the physiochemical properties and performed multiple sequence comparison of ACCD producing plant growth promoting rhizobacteria i.e., *Azospirillum lipoferum, Phyllobacterium brassicacearum, Pseudomonas fluorescens, Francisella tularensis subsp. holarctica OSU18* and *Bacillus cereus*. Analysed the protein family and conserved domain and find out sequence motifs . To predict the secondary structure and homology modelling to recognize the functional

Table 1. Physicochemical analysis of protein sequence of ACCD from PGPR

Properties	*Azospirillum lipoferum*	*Phyllobacterium brassicacearum*	*Pseudomonas* fluorescens	*Francisella tularensis* subsp.holarctica OSU18.	*Bacillus cereus*
Number of amino	338	337	241	290	335
Molecular weight	36572.6	36750.7	26215.7	33504.4	36453.4
Theoretical PI	5.67	5.17	4.96	8.91	5.43
Negatively charged residues (Asp+Glu)	43	48	33	27	44
Positively charged residues(Arg+Lys)	38	36	24	33	36
Alanine	9.8%	10.1%	9.5%	4.8%	8.7%
Arginine	6.5%	5.9%	6.2%	2.8%	6.3%
Asparagine	2.1%	3.0%	2.9%	7.9%	3.3%
Aspartic acid	6.5%	5.6%	5.0%	5.2%	4.5%
Cysteine	0.9%	1.2%	2.1%	1.4%	1.8%
Glutamine	2.7%	1.8%	5.0%	5.2%	3.0%
Glutamic acid	6.2%	8.6%	8.7%	4.1%	8.7%
Glycine	11.8%	11.3%	10.8%	5.2%	11.6%
Histidine	1.5%	2.1%	1.2%	2.4%	1.8%
Isoleucine	6.8%	6.2%	6.6%	7.9%	5.1%
Leucine	6.2%	7.4%	6.6%	12.4%	8.4%
Lysine	4.7%	4.7%	3.7%	8.6%	4.5%
Methionine	2.7%	2.4%	2.1%	1.7%	2.4%
Phenylalanine	2.4%	3.9%	3.7%	5.5%	3.9%
Proline	4.4%	4.2%	3.7%	3.8%	4.8%
Serine	4.7%	4.2%	5.0%	7.2%	5.4%
Threoniine	5.3%	4.5%	4.1%	4.1%	3.9%
Tryptophan	0.9%	0.6%	0.8%	1.0%	0.6%
Tyrosine	4.7%	4.5%	2.9%	6.2%	3.6%
Valine	9.2%	8.0%	9.1%	2.4%	8.1%
Formula	$C_{1620}H_{2565}N_{449}O_{491}S_{12}$	$C_{1636}H_{2559}N_{445}O_{494}S_{12}$	$C_{1153}H_{1826}N_{322}O_{356}S_{10}$	$C_{1530}H_{2358}N_{394}O_{434}S_{9}$	$C_{1612}H_{2540}N_{448}O_{488}S_{14}$
Ext. Half life	30 hours	30 hours	4.4 hours	30 hours	1.4 hours
Instability index	26.10	31.68	41.23	26.76	40.03
Aliphatic Index	87.13	86.56	87.80	91.17	84.42
Gravy	-0.157	-0.159	-0.136	-0.342	-0.199

Fig. 1. Multiple sequence alignment results of ACCD of PGPR species

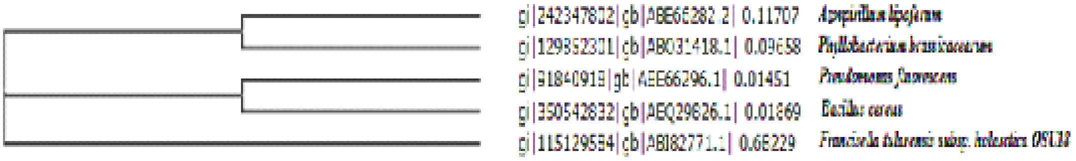

Fig. 2. Cladogram representation of phylogenetic tree of ACCD of PGPR species

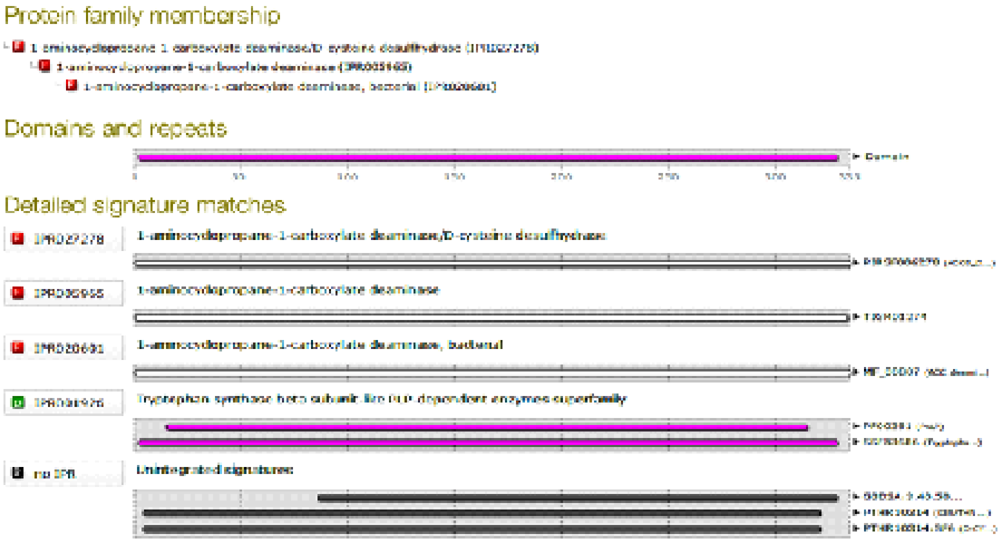

Fig. 3. Shows conserved domain of ACCD of PGPR with Detailed signature matches

Table 2. MOTIF Search results for conserved sequence motifs of ACCD of PGPR species

Rhizobacteria	No. of found motifs	Found motifs	Position	Pfam ID
Azospirillum lipoferum	1	FGPTPIEHLPRLTAALGGKVEIYAKRDDCNSGLAMGGNKLRKLEYIVPDAIASGTDTLVSIGGVQSNHTRMVAATAAKIGMKCVVVQESWVPHEDAVYDRVGNILLTRLMGADSRIVPDGFDIGIRKSWEEAIQSVKDAGGKPYGIPAGASVHKYGGLGYVGFAEEVRKQEADLGFKFDYIVVCVVTGSTQAGMIVGFAADGRGDRVIGIDASGTPEQTRTQVRQIVDSTAELVELGRKVRDDEIVILEDYAYPAYGVPSAETNEAIRLAARTEAMITDPVYEGKSMQGMIDLVRKGWFPEGSKVL	13..318	PALP
Phyllobacterium brassicacearum	1	FGPTPIETLDRLSEHLGGKVHLYAKREDCNSGLAFGGNKLRKLEYIIPDAIASGADTLVSIGGVQSNHTRMVAAVAAKIGFKCRLVQESWVPHEDAAYDRVGNILLSRIMGADVEMVDEGFDIGIRESWENAIEDVKANGGKPYPIPAGASVHKYGGLGYVGFAEEVRAQEAELGFKFDYIVVCTVTGSTHAGMLVGFAKDGRERNVIGIDASATPEKTRAQVFEIARRTADLVELERELTEDDVVLNEDYAYPIYGVPSQETKAAIRLCARLEGMITDPVYEGKSMQGMIDLVGKGFFPEGSRVL	12..317	PALP
Pseudomonas fluorescens	2	FGGNKTRKLEYLIPEAIEQGCDTLVSIGGIQSNQTRQVAAVAAHLGMKCVLVQENWVNYSDAVYDRVGNIEMSRIMGADVRLDAAGFDIGIRPSWEKAMSDVVEQGGKPFPIPAGCSEHPYGGLGFVGFAEEVRQQEKELGFKFDYIVVCSVTGSTQAGMVVGFAADGRSRNVIGIDASAKPE	2..184	PALP
		EQGCDTLVSIGGIQSNQTRQVAAVAAHLGMKCVLVQEN	19..56	LYTB
Francisella tularensis subsp.holarctica OSU18	1	FIVMRDDLSHPIFSGNKARKLAYLLNNPEKYSHIQTIISFGGNQSNFMLALSQLAELKGWNFHYWIKPLPKFLRQTKNGNLKLALDNGMQLFETLSSLNLEKIKANYHTDSSLYFFDQGGRNTLAEQGIAECAKEIKKYCKQNNIDDYSVIVASGTGTTALYLEKYLPYKVYTIPCVG	15..192	PALP
Bacillus cereus	2	TPLKRLSEHLGGKVELYAKREDCNSGLAFGGNKTRKLEYLIPEAIEQGCDTLVSIGGIQSNQTRQVAAVAAHLGMKCVLVQENWVNYSGAVYDRVGNIEMSRIMGADVRLDAAGFDIGIRPSWEKAMSDAVERGGKPLPIPAGCSEHPYGGLGFVGFAEEVRQQEKELGFKFDYIVVCSVTGSTQAGMVVGFAADGRSKNVIGVDASAKPEQTKAQILRIARHTAELVELGREITEEDVVLDTRFAYPEYGLPNEGTLEAIRLCGSLEGVLTDPVYEGKSMHGMIEMVRRGEFPDGSKVL	16..315	PALP
		EQGCDTLVSIGGIQSNQTRQVAAVAAHLGMKCVLVQE	61..97	LYTB

Table 3. Predicted secondary structure results of ACCD of PGPR pecies using GOR4

Species	αHelix (%)	Extended Strand (%)	Random Coil(%)
Azospirillum lipoferum	33.43	18.64	47.93
Phyllobacterium brassicacearum	34.12	16.62	49.26
Pseudomonas fluorescens	36.10	14.94	48.96
Francisella tularensis subsp. holarctica OSU18	36.90	15.52	47.59
Bacillus cereus	29.85	19.40	50.75

Table 4. Z-score results for *Azospirillum lipoferum*, *Pseudomonas fluorescens*, *Phyllobacterium brassicacearum*, *Francisella tularensis subsp. holarctica OSU18* and *Bacillus cereus* using swiss model

Scoring function term	*Azospirillum lipoferum*		*Phyllobacterium brassicacearum*		*Pseudomonas fluorescens*		*Bacillus cereus*		*Francisella tularensis subsp. holarctica OSU18*	
	RAW SCORE	Z-SCORE	RAW SCORE	Z-SCORE	RAW SCORE	Z-SCORE	RAW SCORE	Z-SCORE	RAW SCORE	Z-SCORE
C_beta interaction energy	-120.00	-1.21	-85.81	-1.49	-86.10	-0.53	-141.33	-1.07	-64.55	-1.54
All-atom pairwise energy	-13520.09	-1.27	-12616.27	-1.54	-5063.08	-0.77	-14271.23	-1.27	-5245.35	-1.81
Solvation energy	-53.86	-0.83	-46.47	-1.32	-9.55	-1.79	-57.19	-0.75	-0.85	-2.90
Torsion angle energy	-69.78	-2.55	-65.15	-2.72	-49.86	-1.08	-99.44	-1.94	-26.15	-2.89
QMEAN6score	0.730	-0.36	0.708	-0.64	0.677	-1.00	0.707	-0.65	0.624	-1.59
Template	1f2d.1.A	1f2d.1.A	1tzm.1.A	1j0d.1.A	4d92.1.A					
Sequence Identity	61.04%	71.73%	81.33%	64.74%	20.57%					
GMQE	0.79	0.85	0.91	0.81	0.60					
Energy	-921.40 KJ/mol		-950.02 KJ/mol		-284.29 KJ/mol		-949.79 KJ/mol		-368.38 KJ/mol	

Table 5. Given a list of protein structure validation using SAVES server

Stereochemical parameters	*A. lipoferum*	*Phyllobacterium* brassicacearum	*Pseudomonas fluorescens*	*Francisella tularensis* subsp. holarctica OSU18	*Bacillus cereus*
Residues in most favoured regions [A,B,L]	455 (83.6%)	457 (82.2%)	179 (87.7%)	223 (87.8%)	447 (81.3%)
Residues in additional allowed regions [a,b,l,p]	81 (14.9%)	89 (16.0%)	23 (11.3%)	27 (10.6%)	91 (16.5%)
Residues in generously allowed regions [~a,~b,~l,~p]	7 (1.3%)	9 (1.6%)	2 (1.0%)	2 (0.8%)	9 (1.6%)
Residues in disallowed regions	1 (0.2%)	1 (0.2%)	0 (0.0%)	2 (0.8%)	3 (0.5%)
Number of non-glycine and non-proline residues	544 (100.0%)	556 (100.0%)	204 (100.0%)	254 (100.0%)	550 (100.0%)
Number of end-residues (excl. Gly and Pro)	4	4	3	2	4
Number of glycine residues (shown as triangles)	78	74	26	15	76
Number of proline residues	30	28	9	11	32
Total number of residues	656	662	242	282	662
G-factor	0.07	0.04	0.11	-0.57	0.06
Verify-3D	99.09% of the residues had an averaged 3D-1D score > 0.2	97.29% of the residues had an averaged 3D-1D score > 0.2	82.64% of the residues had an averaged 3D-1D score > 0.2	67.14% of the residues had an averaged 3D-1D score > 0.2	94.58% of the residues had an averaged 3D-score > 0.2

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MRLDRFERYPLTFGPTPIEHLPRLTAALGGKVEIYAKRDDCNSGLAMGGNKLRKLEYIVPDAIASGTDTL
ccccccccccccccccccchhhhhhhhhccceeeeeccccccccee cccchhhhheeeccccccccccee
VSIGGVQSNHTRMVAATAAKIGMKCVVVQESWVPHEDAVYDRVGNILLTRLMGADSRIVPDGFDIGIRKS
eeeccccccchhhhhhhhhhhcceeeeeeccccccccchhhhhhchhhhhhhcccccccccccceechhhh
WEEAIQSVKDAGGKPYGIPAGASVHKYGGLGYVGFAEEVRKQEADLGFKFDYIVVCVVTGSTQAGMIVGF
hhhhhhhhhccccccccccccceecccccceeecchhhhhhhhhhhccccceeeeeeeecccccceeeeee
AADGRGDRVIGIDASGTPEQTRTQVRQIVDSTAELVELGRKVRDDEIVILEDYAYPAYGVPSAETNEAIR
eccccccceeeeeccccccccchhhhhhhhhchhhhhhhhhhccccchhhhhhhccccccccccchhhhhhh
LAARTEAMITDPVYEGKSMQGMIDLVRKGWFPEGSKVLYAHLGGAPAINGYSYTYRNG
hhhhhhhhhccceecccchhhhhhhhhcccccccceeeeeeccccccccceeeeeeec
```

Alpha helix (Hh): 113**,** Extended strand (Ee): 63, Beta turn (Tt): 0, Random coil (Cc): 162.

Fig. 4. Secondary structure prediction of ACCD of *Azospirillum lipoferum*

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cccccccccccccccccchhhhhhhhcccceeeeeccccccccccccccccceeeeeccccccccccceee
SIGGVQSNHTRMVAAVAAKIGFKCRLVQESWVPHEDAAYDRVGNILLSRIMGADVEMVDEGFDIGIRESW
eecccccccchhhhhhhhhhhcceeeeeecccccccchhhhhhhhhhhhhhhccchhhhhcccccchhhhh
ENAIEDVKANGGKPYPIPAGASVHKYGGLGYVGFAEEVRAQEAELGFKFDYIVVCTVTGSTHAGMLVGFA
hhhhhhhhcccccccccccccceecccccceeechhhhhhhhhhhhhcccceeeeeeeecccccchhhhhh
KDGRERNVIGIDASATPEKTRAQVFEIARRTADLVELERELTEDDVVLNEDYAYPIYGVPSQETKAAIRL
ccccccceeeecccccchhhhhhhhhhhhhhhhhhhhhhhcccchhhhccccccceecccccchhhhhhh
CARLEGMITDPVYEGKSMQGMIDLVGKGFFPEGSRVLYAHLGGAPAINGYAYTFRNG
hhhhccccccceecccccceeeeeeecccccccccchhhhhhccccccccceeeeeeec
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Alpha helix (Hh): 115**,** Extended strand (Ee): 56, Beta turn (Tt): 0, Random coil (Cc): 166

Fig. 5. Secondary structure prediction of ACCD of *Phyllobacterium brassicacearum*

```
AFGGNKTRKLEYLIPEAIEQGCDTLVSIGGIQSNQTRQVAAVAAHLGMKCVLVQENWVNYSDAVYDRVGN
cccccccchhhhcchhhhhccccceeecccccccchhhhhhhhhhhhcceeeeeecccccccceeeecccc
IEMSRIMGADVRLDAAGFDIGIRPSWEKAMSDVVEQGGKPFFPIPAGCSEHPYGGLGFVGFAEEVRQQEKE
hhhhhhhchhhhhhhcccccccccchhhhhhhhhhccccccccccccccccccccceecchhhhhhhhhhh
LGFKFDYIVVCSVTGSTQAGMVVGFAADGRSRNVIGIDASAKPEQTKAQILRIARHTAELVELGREITEE
hcceeceeeeeeecccccccceeeeeecccccccceeeecccccchhhhhhhhhhhhhhhhhhhhhhhhcccc
DVVLDTRFAYPQYGLPNEGTLEAIRLCRSLE
Ccccccccccccccccccccchhhhhhhceec
```

Alpha helix (Hh): 87**,** Extended strand (Ee): 36, Beta turn (Tt): 0, Random coil (Cc): 118

Fig. 6. Secondary structure prediction of ACCD of *Pseudomonas fluorescens*

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MLSLFQKISFENKDFIVMRDDLSHPIFSGNKARKLAYLLNNPEKYSHIQTIISFGGNQSNFMLALSQLAE
cccccccccccccceeeeeccccccccccchhhhhhhhccccccccceeeeeecccccchhhhhhhhhhh
LKGWNFHYWIKPLPKFLRQTKNGNLKLALDNGMQLFETLSSLNLEKIKANYHTDSSLYFFDQGGRNTLAE
hcccceeecccccccccchhhhccchhhhhhccchhhhhhhhhhhhhhhhcccccccceeeecccccchhhh
QGIAECAKEIKKYCKQNNIDDYSVIVASGTGTTALYLEKYLPYKVYTIPCVGSSDYLKEQFNNIDSDLVH
hhhhhhhhhhhhhcccccccceeeeeecccccchhhhhhccccceeeeeeeecccccchhhhhccccccccc
PKIISPNFKNNFGQLDIANYNIYLKLLRETKIEFDLLYDPIAWRTLLSKYHQLPKPIIYIHCDGVSGNQT
cceecccccccccccchhhhhhhhhhhhhhhhhhhhhhcccchhhhhhhccccccccceeeeeeccccccch
MLARYQRFSQ
hhhheeeeec
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Alpha helix (Hh): 107**,** Extended strand (Ee): 45, Beta turn (Tt): 0, Random coil (Cc): 138

Fig. 7. Secondary structure prediction of ACCD of *Francisella tularensis subsp. holarctica OSU18*

```
NRFERYPLTFDPSPITPLKRLSEHLGGKVELYAKREDCNSGLAFGGNKTRKLEYLIPEAIEQGCDTLVSI
ccccccccccccccccchhhhhhhccceeeeeeccccccccee cccccccchhhhcchhhhhccccceeec
GGIQSNQTRQVAAVAAHLGMKCVLVQENWVNYSGAVYDRVGNIEMSRIMGADVRLDAAGFDIGIRPSWEK
cccccchhhhhhhhhhhhcceeeeeecccccccceeeecccchhhhhhhchhhhhhhcccccccccchhh
AMSDAVERGGKPLPIPAGCSEHPYGGLGFVGFAEEVRQQEKELGFKFDYIVVCSVTGSTQAGMVVGFAAD
hhhhhhhcccccccccccccccccccceecchhhhhhhhhhhhcceeceeeeeeeccccccceeeeeecc
GRSKNVIGVDASAKPEQTKAQILRIARHTAELVELGREITEEDVVLDTRFAYPEYGLPNEGTLEAIRLCG
ccccceeeecccccchhhhhhhhhhhhhhhhhhhhhhhhccccccccccccccccccccccchhhhhhcc
SLEGVLTDPVYEGKSMHGMIEMVRRGEFPDGSKVLYAHLGGAPALNAYSFLFRNG
Cccceeccceeccccchhhhhhheeecccccccceeeeeecccccceeeeeeeeeec
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Alpha helix (Hh): 100**,** Extended strand (Ee): 65, Beta turn (Tt): 0, Random coil (Cc): 17.

Fig. 8. Secondary structure prediction of ACCD of *Bacillus cereus*

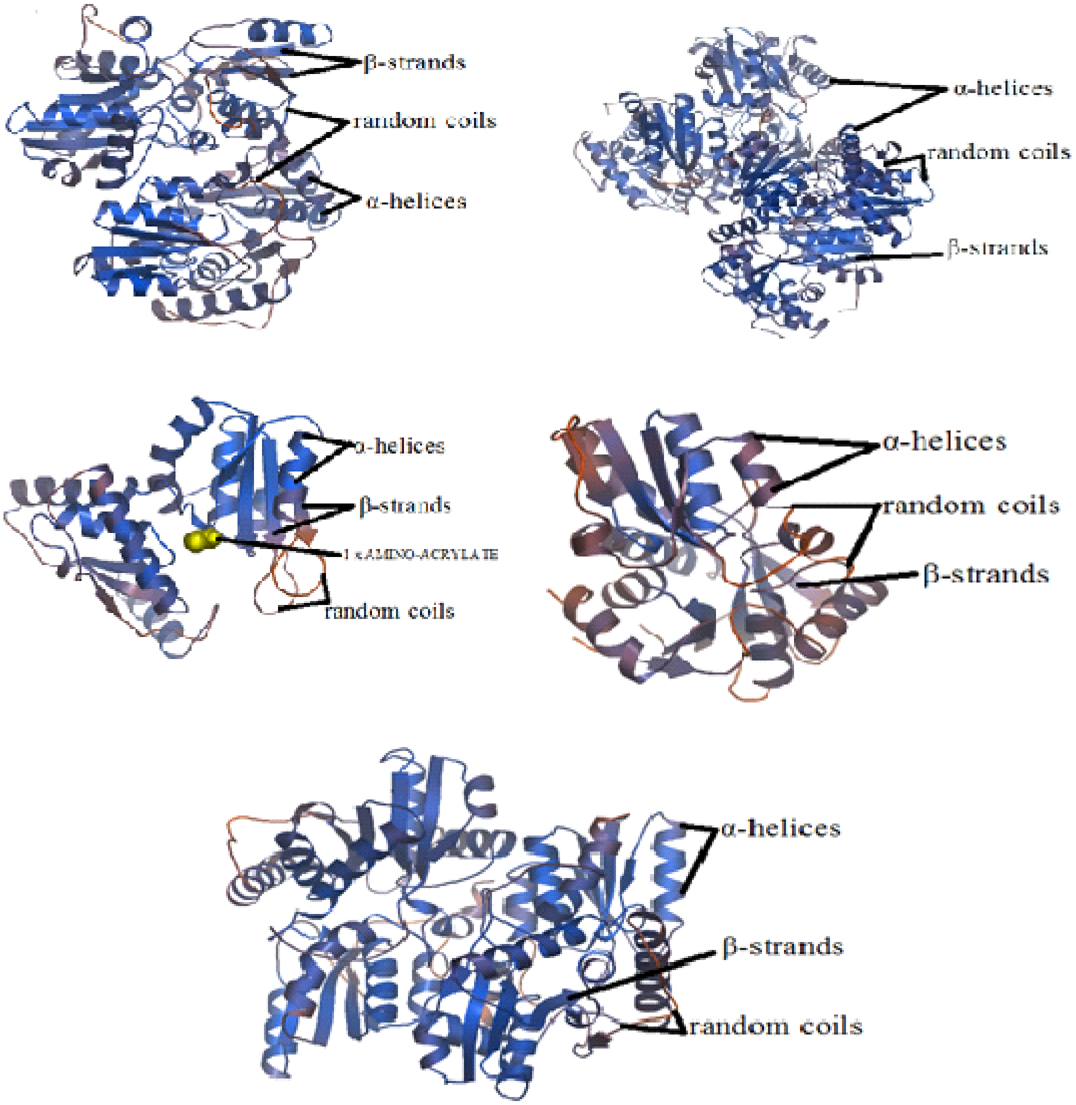

Fig. 9. Predicted homology model of ACCD of *Azospirillum lipoferum* (a), *Phyllobacterium brassicacearum* (b), *Pseudomonas fluorescens* (c), *Francisella tularensis subsp. holarctica OSU18* (d) *and Bacillus cereus* (e).

annotation of genes recognized in an organism's genome (Gopal, *et al.*, 2001). This analysis may be helpful to understand the biological function of PGPR species.

Materials and methodology

Selection and protein sequence retrievals

Selected the five species of PGPR contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and retrieved ACCD protein sequences of PGPR using NCBI. >gi|242347802|gb|ABE66282.2|[*Azospirillum lipoferum*] (Blaha, *et al.*, 2006), >gi|129852301|gb|ABO31418.1|[*Phyllobacterium brassicacearum*] (Contesto, *et al.*, 2008), >gi|91840918|gb|ABE66296.1|[*Pseudomonas fluorescens*] (Blaha, *et al.*, 2006), >gi|115129584|gb| ABI82771.1|[*Francisella tularensis subsp. holarctica OSU18*] (Petrosino, *et al.*, 2006), and >gi|350542832|gb| AEQ29826.1| [*Bacillus cereus*] (Chen, *et al.*, 2013).

Primary sequence and Phylogenetic analysis of ACCD of PGPR species

Comparison of amino acid compositions of ACCD producing PGPR species using ProtParam tool (web.expasy.org/protparam/). The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY) of PGPR species in this analysis. ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/) is used for multiple sequence alignment of PGPR species. The phylogenetic tree of encoded amino acid sequence was constructed from the PGPR species with ClustalW2 by using the Percent identity matrix. Analysed a group of evolutionary protein, structural and functional unit in sequences by Pfam (pfam.sanger.ac.uk/), InterPro (www.ebi.ac.uk/interpro/) and MOTIF Search (www.genome.jp/tools/motif/).

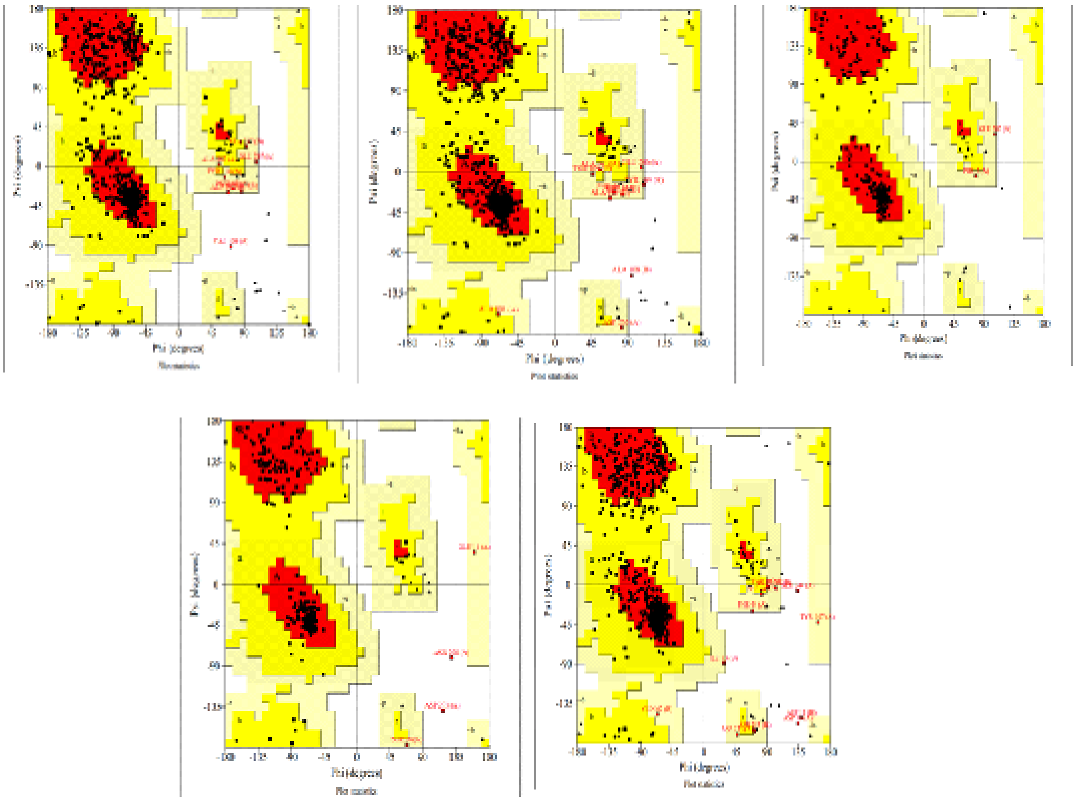

Fig. 10. Ramachandran plot of ACCD of *Azospirillum lipoferum* (a), *Phyllobacterium brassicacearum* (b), *Pseudomonas fluorescens* (c), *Francisella tularensis subsp. holarctica OSU18* (d) *and Bacillus cereus* (e)

Structure prediction and evalutation of ACCD of PGPR species

The GOR IV (npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html) method analyzed the sequences of PGPR species to predict alpha helix, beta sheet, turn, or random coil secondary structure. To perform comparative protein modeling of the 3-D structure of PGPR protein sequences by using SWISS-MODEL program in the automated mode (http://swissmodel.expasy.org/). The template used in this modeling was selected from template identification function in the SWISS-MODEL program. SAVES (http://nihserver.mbi.ucla.edu/SAVES/): **s**erver was used to analyze and visualize the predicted PGPR structure by using tools such as PROCHECK, WHAT_CHECK, ERRAT, VERIFY_3D, PROVE.

RESULTS

Primary sequence and Phylogenetic analysis of ACCD of PGPR species

Comparison of ACCD amino acid compositions from plant growth promoting rhizobacteria i.e., *Azospirillum lipoferum, Phyllobacterium brassicacearum, Pseudomonas fluorescens, Francisella tularensis subsp. holarctica OSU18* and *Bacillus cereus* are shown in table 4.1

DISCUSSION

Plant growth promoting rhizobacteria that produce the enzyme 1-aminocyclopropane 1- carboxylate (ACC) deaminase and this enzyme can cleave the ethylene precursor ACC to α-ketobutyrate and ammonium, there by lowers the level of ethylene under various biotic and abiotic stresses. Conceptually PGPR can have an impact on plant growth and development in two different ways: indirectly or directly. In this study selected the well known PGPR with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture, bacteria belonging to the genera, namely *Azospirillum lipoferum, Phyllobacterium brassicacearum , Pseudomonas fluorescens, Francisella tularensis subsp. holarctica OSU18 and Bacillus cereus.*

In this work to performed comparative proteomics analysis of ACCD producing PGPR species. The Physiochemical properties analysis of ACCD of PGPR species *Azospirillum lipoferum, Phyllobacterium brassicacearum* and *Francisella tularensis subsp. holarctica OSU18* are stable protein and *Pseudomonas fluorescens, Bacillus cereus* are unstable protein with instability index 26.10, 31.68, 41.23, 23.76 and 40.03. They were favor in charged, aliphatic and hydrophobic nature. An amino acid sequence alignment shown more conserved residues are GNKRKLYTSGGQSNAGMGGGAEVTGTVQIL in among sequences and phylogenetic tree described the closely related to each other via percent identity matrix 78.64%, 65.98%, 67.16% and 15.33%. using clustal w2 program. These species wee common conserved domain belonging to the tryptophan synthase beta subunit-like PLP-dependent enzymes superfamily. It consist of diverse proteins including threonine dehydratase, cysteie synthase, pyridoxal phosphate-dependent deaminase. The CD corresponds to 1- aminocyclopropane-1-carboxylate deaminase (ACCD)/ D-cysteine desulfhydrase family Proteins in this family included deaminase, D-cysteine desulfhydrase, phenylserine dehydratase and L-cysteate sulfo-lyase that catalyses a cyclopropane ring-opening reaction, the irreversible conversion of 1-aminocyclopropane-1-carboxylate (ACC) to ammonia and alpha-ketobutyrate [PMID: 21244019]. Prediction of secondary structure was mainly composed of ± Helix, random coil and extended strand. To perform comparative modelling of 1- aminocyclopropane-1-carboxylate deaminase (ACCD) producing rhizobacteria *Azospirillum lipoferum, Phyllobacterium brassicacearum , Pseudomonas fluorescens, Francisella tularensis subsp. holarctica OSU18 and Bacillus cereus* respectively using this template PDBID 1f2d.1.A, 1tzm.1.A, 1j0d.1.A, 4d92.1.A and 1j0d.1.A with sequence identity 61.04% , 71.73%, 81.33%, 20.57% and 64.74%. Predicted homology models were evaluated using SAVES server. This analysis will be helpful in knowing the evolutionary conservation and biochemical function of ACCD producing plant growth-promoting rhizobacteria (PGPR).

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