# SYNERGISTIC INTERACTION OF PGPB ACDS AND ACDR GENES: THE PLANT STRATEGY TO COPE UP WITH BIOTIC AND ABIOTIC STRESSORS

## Garg Gunjan<sup>\*</sup> and Bhati Sharmista

School of Biotechnology, Gautam Buddha University, Greater Noida (U.P) India \*Author for Correspondence: gunjangarg@gbu.ac.in

### ABSTRACT

ACC deaminase is an inducible enzyme whose synthesis is induced in the presence of its substrate ACC. This enzyme encoded by gene AcdS is under tight regulation and regulated differentially under different environmental conditions. Regulatory elements of gene AcdS are comprised of the regulatory gene encoding LRP protein and other regulatory elements which are activated differentially under aerobic and anaerobic conditions. The role of some additional regulatory genes such as AcdB or LysR may also be required for expression of AcdS. Phylogenetic analysis of AcdS has revealed that distribution of this gene among different bacteria might have resulted from vertical gene transfer with occasional horizontal gene transfer (HGT). Application of bacterial AcdS gene has been extended by developing transgenic plants with ACCD gene which showed increased tolerance to biotic and abiotic stresses in plants. Moreover, distribution of ACCD gene or its homolog's in a wide range of species belonging to all three domains indicate an alternative role of ACCD in the physiology of an organism. Therefore, this review is an attempt to explore current knowledge of bacterial ACC deaminase, Enzymology and biochemical properties, genetics, mode of enzyme action, distribution among different species, future research avenues to develop transgenic plants expressing foreign AcdS gene to cope with biotic and abiotic stressors. Systemic identification of regulatory circuits would be highly valuable to express the gene under diverse environmental condition.

Keywords: ACC deaminase, acdS gene, IAA, LRP protein

### INTRODUCTION

### ACC Deaminase (ACCD): Prevalence, Enzymology and Biochemical properties

To feed all of the world's people, it is necessary to sustainably increase agricultural productivity. One way to do this is through the increased use of plant growth promoting bacteria (PGPB). Scientists have developed a synergistic interaction mechanisms between ACC deaminase and indole-3-acetic acid (IAA) synthesized by both plant and bacteria. This bacterial synergistic interaction mechanism (bac-SIM) not only directly promote plant growth, they also protect plants against different types of biotic and abiotic (specifically drought and salinity) stress. Under the bac-SIM, microorganisms colonize the rhizosphere of plants and extend drought tolerances by producing exopolysaccharides (EPS)-phytohormones, 1aminocyclopropane- 1-carboxylate (ACC) deaminase, Up-regulation or down regulation of stress responsive genes and do alteration in root morphology in acquirement of drought tolerance. ACCD enzyme is responsible for the cleavage of the plant ethylene precursor (i.e. Substrate of ACCD), ACC into ammonia and ά-ketobutyrate (Honma, 1978). By decreasing ACC levels in plants, ACC deaminaseproducing organisms decrease plant ethylene levels (Glick and Penrose et.al. 1998). which when present in high concentrations can lead to plant growth inhibition or even death (fig. 1). The enzyme ACC deaminase initially identified in the yeast Hansenula saturnus (now re-classified as Cyberlindnera saturnus) and was first purified to homogeneity from Pseudomonas sp. strain ACP ( Honma and Shimomura, 1978). It was subsequently partially purified from *Pseudomonas chloroaphis* 6G5 (Klee,

et.al.1991) and Pseudomonas putida GR12-2 (Jacobson, et.al. 1994) and then purified to homogeneity from P. putida UW4 (Hontzeas, et.al. 2004).

Pervasiveness of ACC Deaminase has been confirmed at the molecular level by amplification and sequence analysis of *AcdS* (a structural gene encoding ACCD). For this integrated microbial genomes database (IMG) is applied in the research. It has been noticed that the *AcdS* gene is commonly found in Actinobacteria, *Deinococcus-thermus*, three classes of Proteobacteria ( $\alpha$ ,  $\beta$ , and  $\gamma$ ), various fungi belonging to ascomycetes, basidiomycetes, and in some Stramenopiles. Altogether, 485 strains belonging to different genera showed presence of *AcdS* gene (Table: 1).

Table 1		
Genera	Species	References
Gram-negative	Achromobacter xylosoxidans	Hontzeas, et al., 2004).Glick et
bacteria	Rhizobium leguminosarum	al., 1998, Duan, et al., 2013.
	Pseudomonas putida	
	Burkholderia phytofirmans	
Gram-positive bacteria	Brevibacterium iodinum	Glick and Penrose et al., 1998,
	Brevibacterium casei	Hontzeas, et al., 2005, Nie, et
	Bacillus licheniformis	al., 2002, Sergeeva, et al.,
	Zhihengliuela alba	2006.
	Micrococcus sp.	
	Brachybacterium saurashtrense	
Rhizobia species	Mesorhizobium loti	Duan, et al., 2009, Li and
	Rhizobium leguminosarum	Glick, 2001, Prigent, et al.,
	R. phaseoli	2008, Ma, et al., 2003.
Fungi	Penicillium citrinum	Jacobson, et al., 1994, Penrose,
	Trichoderma asperellum	et al., 2001, Nie, et al., 2002,
	Phytophthora sojae	Kaushal and Wani, 2015.
	Issatchenkia occidentalis	
Plants	Arabidopsis thaliana, poplar, and	Glick, 2007, Ma, et al., 2003.
	tomato	

Altogether, 485 strains belonging to different genera including Acidovorax, Bordetella, Brenneria, Collimonas, Cupriavidus, Curvibacter, Dickeya, Herbaspirillum, Halomonas, Lonsdalea, Methylibium, Pantoea, Polaromonas, Pseudomonas, Ralstonia, Serratia, Tatumella, Variovorax, and Xenophilus, showed presence of AcdS gene.

ACC deaminase (ACCD) is not a secreted enzyme. It is localized within the cytoplasm of the microorganism that produces it. It is a multimeric enzyme, carried 2-3 subunits. Native and subunit molecular mass of ACCD is approximately 69-105 kDa, and 35–42 kDa respectively. It is a sulfhydryl enzyme that utilizes pyridoxal 5-phosphate (PLP) as an essential co-factor. Two key amino acid residues: (i) a reactive thiol group at cysteine 162, and (ii) the pyridoxal phosphate binding site at lysine 51 have been located in the enzyme. Pyridoxal phosphate has the tendency to bind tightly with ACCD. D-amino acids like D-serine and D-cysteine act as substrates for ACC deaminase whereas, L-serine and L-alanine are act as effectual competitive inhibitors. Based on its protein fold, ACC deaminase belongs to the tryptophan synthase  $\beta$  super-family of PLP binding proteins (Glick, 2007). Maximal activity of this enzyme typically reported at 30°C (at pH 8-8.5). The affinity of an enzyme for a particular substrate (i.e., the Km value) reflects more than the tightness of substrate binding, rather it has a profound effect on the kinetics of conversion of substrate into product. When the Km values for the binding of ACC by ACC

deaminase were determined for enzyme extracts of several different microorganisms at pH 8.5, the values ranged from 1.5 to 17.4 mM, indicating that the enzyme does not have a particularly high affinity for ACC. There are two significant consequences of the low affinity of ACC deaminase for ACC. First, because the enzyme ACC oxidase (which catalyzes ethylene formation from ACC) has a much greater affinity for ACC than does ACC deaminase, the only way that ACC deaminase can effectively compete with ACC oxidase for ACC and thereby lower plant ethylene levels is for the amount of ACC deaminase to be much greater (100 to 1000 fold) than the amount of ACC oxidase. This is likely to often be the case since ACC oxidase is an induced enzyme that is normally present in non-senescent and non-stressed tissues in only very low levels. Second, since plant ACC levels are typically in the micromolar range and the Km is in the millimolar range, Michaelis–Menton kinetics indicate that every increase in ACC concentration will be accompanied by a parallel increase in the rate of ACC cleavage independent of the level of enzyme present. Thus, the enzyme will immediately respond to a 2 to 3fold increase in ACC levels as followed via environmental stress.



Figure 1: Bacterial synergistic interaction mechanism (bac-SIM): Directly promote plant growth, and also protect the plants against different types of biotic and abiotic stress. bac-SIM produce exopolysaccharides (EPS)-phytohormones, 1-aminocyclopropane- 1-carboxylate (ACC) deaminase. ACCD enzyme is responsible for the cleavage of the plant ethylene precursor, ACC into ammonia and  $\dot{\alpha}$ -ketobutyrate. (Elisa Gamalero *et al.*, 2015)

### Regulation of ACC deaminase structural genes (acdS) Gene

The plant hormone ethylene is commonly associated with the control of a wide variety of developmental and

stress regulated processes. The immediate precursor to ethylene in flowering plants, 1aminocyclopropane-1-carboxylic acid (ACC). ACC is produced from S-adenosyl methionine (SAM) by ACC synthase (ACS) which is then converted to ethylene by the action of ACC oxidase (ACO). It is generally believed that the level of ethylene is regulated by the activity of ACS and ACO. Another potential mechanism to down-regulate ethylene levels is through the conjugation of ACC into malonyl ACC (MACC) via ACC malonyl transferase or to 1-y -L-glutamyl-amino-cyclopropane-1-carboxylic acid (GACC) via  $\gamma$ -glutamyl-trans-peptidase. Through a mutualistic or symbiotic relationship with soil-borne microorganisms, another mechanism exists to regulate ethylene levels in plants. The enzyme ACC deaminase (ACCD), produced by bacteria, cleaves the cyclo propane ring of plant ACC to produce  $\alpha$ ketobutyrate and ammonia (Glick et al., 1998). This process is irreversible, reducing plant ethylene production and thereby affecting plant growth (Grichko and Glick 2001), (Mayak, et al., 2004), (Penrose, et al., 2001). To date, ACCD has only been found in microorganisms, not in plants (Hontzeas, et al., 2005), (Minami, et al., 1998). Using transgenic approaches, the action of bacterial ACCD on plant development has been studied in a wide variety of models. It has been shown to delay fruit ripening, increase tolerance to heavy metals and decrease susceptibility to a variety of environmental stressors such as flooding, drought and salinity (Grichko and Glick, 2000), (Grichko and Glick, 2001b), (Klee, et al., 1991),(Nie, et al., 2002), (Sergeeva, et al., 2006).



**Figure 2:** Regulation of bacterial ACC deaminase activity in *P. putida* UW4 (previously considered as an Enterobacter cloacae strain of PGPB) and the endosymbiont *Rhizobium leguminosarum* 128C53K: ACC deaminase gene consist of regulatory gene AcdR located 5' upstream of ACC deaminase structural gene (AcdS). Analysis of the acdS upstream region bared the presence of a acdR or lrpL gene, encoding a protein homologous to the Leucine Responsive regulatory Protein (Lrp). acdS is specifically regulated by acdR. Promoter regions for binding of regulatory proteins like Lrp-box for binding of Lrp protein, AcdB box for binding regulatory protein AcdB. In the presence of ACC, LRP forms an active octamer that binds to a complex of ACC; ACC deaminase gene negatively regulated by leucine which is synthesized from a-ketobutyrate (a breakdown product of ACCD catalyzed reaction). Higher concentration of leucine, favors the formation of inactive LRP dimer-form, leads to switching off the transcription of *AcdS gene* 

The regulation of bacterial ACC deaminase activity has been investigated in two plant-beneficial bacteria, i.e. the PGPR *P. putida* UW4 (*Enterobacter cloacae* strain) and the endosymbiont *Rhizobium leguminosarum* 128C53K strain (Fig. 2). In both strains, analysis of the acdS upstream region revealed

the presence of, (1) a gene (named acdR or lrpL) encoding a protein homologous to the Leucine Responsive regulatory Protein (Lrp), (2) a putative Lrp-like binding site in the vicinity of acdS, (3) a CRP (cyclic AMP receptor protein) binding site, and (4) an FNR (fumarate nitrate reduction regulatory protein) binding site (which is a known anaerobic transcriptional regulator). All of these features were shown to be involved in the transcriptional regulation of the ACC deaminase gene, with the *P. putida* UW4 ACC deaminase structural gene (acdS) promoter under the transcriptional control of the regulatory Lrp protein (encoded by acdR). AcdS is highly regulated and expresses differentially depending on presence or absence of oxygen, concentration of substrate, and accumulation of products. Gene consist of regulatory gene AcdR located 5' upstream of ACC deaminase structural gene (AcdS), promoter regions for binding of regulatory proteins like Lrp box for binding of Lrp protein, AcdB box for binding regulatory protein AcdB, FNR box for binding of fumarate and nitrate reductase protein and, CRP box for binding of cAMP receptor protein.

In *Pseudomonas putida* UW4, study of the regulatory path of AcdS gene expression of ACCD enzyme has been shown that, ACC-deaminase structural genes (acdS) have acdR gene, which is responsible for leucine responsive regulatory protein (LRP) synthesis. acdR gene is located around 50 to a few hundred base pairs upstream of the start of the acdS gene and transcribed in a direction opposite to acdS (Blaha, *et al.*, 2006), (Duan, *et al.*, 2009), (Prigent-Combaret, *et al.*, 2008).

In the presence of ACC, LRP forms an active octamer that binds to a complex of ACC and another protein, AcdB (Cheng, *et al.*, 2008). (This tripartite complex activates transcription of acdS by binding to its promoter region. Upon synthesis of ACC deaminase, ACC is cleaved to form ammonia and ketobutyrate (a precursor of branched chain amino acid ssuch as leucine), and when a cell accumulates a sufficiently high level of leucine, this amino acid binds to the LRP octamer causing it to dissociate into inactive dimers shutting down further transcription of acdS. This mode of regulation ensures that ACC deaminase is synthesized only when it is needed. cAMP receptor protein, FNR (under anaerobic conditions), fumarate–nitrate reduction regulatory protein are the some other examples, which act as acdS promoters and helps in the transcription process (Duan, *et al.*, 2013 and Li and Glick, 2001). Hence ACC deaminase gene activity is negatively regulated by leucine which is synthesized from  $\alpha$ -ketobutyrate, a breakdown product of ACCD catalyzed reaction. As the concentration of leucine increases, it favors formation of inactive LRP dimer form which leads to switching off the transcription of *AcdS* gene (Fig. 2). In the absence of ACC, the acdR gene is transcribed until an excess amount of LRP builds up and then binds to an LRP box, part of the DNA sequence immediately upstream of the acdR gene and overlapping with the promoter for this gene, preventing further transcription of acdR.

### Genetics of ACC Deaminase (AcdS gene):

ACC deaminase gene has been reported in several species of bacteria, including *R. leguminosarum Trifoli* (Itoh, 1996) and *Mesorhizobium loti* MAFF303099 (Kaneko, *et al.*, 2002). The phylogenetic analysis of *AcdS* and *AcdR* genes suggested that both of them were evolved in a similar manner. In some bacteria it has been additional identified that AcdS and *AcdR* genes are evolved through horizontal gene transfer (Hontzeas, *et al.*, 2005 and Prigent, *et al.*, 2008). Data recovered from IMG revealed that nucleotide sequences of *AcdS* gene is shown closeness to the other genes namely dcyD and yedO which encode for another PLP-dependent enzyme D-cysteine sulfhydralase.

Regulatory machinery for *AcdS* expression varies in different species if compared with regulatory elements of *P. putida* UW4. For example LRP like protein and  $\sigma$ 70 promoter are involved in regulation of *AcdS* gene in *Bradyrhizobium japonicum* USDA 110 and *Rhizobium leguminosarumbv. Viciae* 128 C53K (Kaneko, *et al.*, 2002). (Ma, *et al.*, 2003). In *Burkholderia* sp. CCGE 1002, two copies of *AcdS* gene are involved in expression regulatory mechanism. In *M. loti* (an important symbiotic nitrogen fixing bacteria), *AcdS* and *nif-H* genes (which contain *nifA1* and *nifA2* [regulatory N<sub>2</sub> fixing units] and  $\sigma$ <sup>54</sup> RNA polymerase [sigma recognition site]) are involved in the regulatory process. The *nifA2* encoded protein NifA2 interact with  $\sigma$ <sup>54</sup> RNA polymerase favoring *AcdS* transcription.

However role of *nifA1* in the expression of *AcdS* is not properly understood, but it has been confirmed that up to certain some extent it affect the transcription of *AcdS* gene (Nukui, *et al.*, 2006). In many *Actinobacteria* and *Meiothermus*, GntR protein coding gene is found next to *AcdS* gene. These evidence indicate a possibility that some downstream elements are also involved in regulation of ACC deaminase expression. In some members of these genera, the absence of promoter region strongly suggests that the interaction of *AcdS* gene and some downstream element next to *AcdS* gene is involved in the regulation of *AcdS* gene transcription. In certain species of *Actino-bacteria* and *Proteobacteria* like *Brenneria* sp. EniD312, *Pantoea sp.* At-9B, LysR family of transcription regulatory elements, are found in close vicinity of *AcdS* gene. However, the exact mechanism of regulation of ACC deaminase in many of the micro-organisms is still poorly known.

# CONCLUSION

Agriculture is considered as most vulnerable sectors to climate-change. Exploiting plant-microbe interaction is a relevant approach to increase food production for the growing population in the current scenario of climate change. The future research needs to be in developing efficient microbial formulation for boosting plant performance under salinity stress that substantially reduces the use of chemical fertilizers and pesticides. The research should focus on to isolate indigenous PGPR from the stress-affected soils that could be used as bioinoculants for crops grown in stressed ecosystems (Kaushal and Wani, 2015). Better understanding of what makes a beneficial PGPR interaction with plant would provide an important insight to better handle microbes. Despite the recent progress and perspectives highlighted on microbial mediated drought tolerance in plants, we are still at the beginning of our understanding of PGPR mechanisms imparting drought tolerance in plants. Nevertheless, current progress in the area conveys that future research has great potential to give new insights for sustainable food production.

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