
CONIFERYL ALDEHYDE DEHYDROGENASE GENE FROM *PSEUDOMONAS NITRORELECTUS* JIN1

Praveen P.Balgir*, Dinesh Kalra

Department of Biotechnology, Punjabi University, Patiala, Punjab. INDIA.

E-mail : balgirbt@live.com

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Abstract

Coniferyl aldehyde dehydrogenase gene (calB) oxidises coniferyl aldehyde into ferulic acid, the last step in the metabolic pathway of eugenol to ferulic acid. It occurs in various bacterial strains like *Pseudomonas nitrorelectus* Jin1, *Ralstonia eutropha*, *Escherichia coli*, *Pseudomonassp.*. HR199, *Rhodococcus opacus*. Coniferyl aldehyde produced as the intermediate product by the biotransformation of eugenol, is oxidised to ferulic acid, NADPH and H⁺. Aldehyde dehydrogenase-related bacterial sequences were aligned and using *in silico* methodology analysed to determine functionally important conserved residues in this protein. A total of 16 residues have been identified to be conserved in this protein. A total of 16 residues have been identified to be conserved in this protein. *calB* gene from *Pnitrorelectus* Jin1 located in region 107290-108732 (1442bp) was found to contain all the functionally important residues and hence was selected for isolation.

INTRODUCTION

Coniferyl aldehyde dehydrogenase enzyme is member of the family oxidoreductase, which acts on the aldehyde or oxo group of donor with NAD⁺ or NADP⁺ as acceptor. The systematic name is aldehyde:NAD⁺ dehydrogenase. NAD⁺-dependent aldehyde dehydrogenase, NAD⁺-linked aldehyde dehydrogenase, propionaldehyde dehydrogenase and aldehydedehydrogenase (NAD⁺)[1]. Aldehyde dehydrogenase belongs to class of NADPH dependent enzymes which catalyze reduction of various phenylpropenyl aldehyde derivatives. These enzymes mainly catalyse the oxidation (dehydrogenation) of aldehydes to carboxylic acid [2]. Aldehyde dehydrogenase gene is found in many organisms from prokaryotes such as bacteria, fungus to eukaryotes such as mammals. They belong to aldehyde dehydrogenase domain (Aldehyde dehydrogenase domain (IPR015590).

Aldehyde dehydrogenase family which consists of enzymes involved in conversion of a broad variety of aliphatic and aromatic aldehydes to their respective carboxylic acids [2-4]. Dehydrogenase enzymes may be dimers or tetramers of identical subunits [3]. It is clear from crystal structures of enzymes that subunits of different ALDHs contain very similar domains like each has catalytic, nucleotide binding and oligomerization domains [5-11]. Aldehyde dehydrogenases participate in different metabolic pathways [12] such as glycolysis/gluconeogenesis[13], ascorbate and aldurate metabolism[14], bile acid synthesis[15].

**Aldehyde + NAD⁺ + H₂O ⇌ an acid + NADH + H⁺**

It is an enzyme that catalyses the oxidation of aldehyde to ferulic acid. Coniferyl aldehyde, NAD⁺ and H₂O are the 3 substrates of this reaction giving rise to 3 products ferulic acid, NADH and H⁺. Structurally the enzyme consists of three domains which are: a NAD(P)+ cofactor-binding domain, a catalytic domain and a bridging domain. The catalytic mechanism of enzyme involve cofactor (NAD(P)+) binding, which results in a conformational change and activation of an invariant catalytic cysteine nucleophile. It has been found that ALDH reaction is completed in two steps, acetylation and deacetylation [6,8,16,17].

In first step invariant active site cysteine makes a nucleophilic attack on the carbonyl carbon of the aldehyde molecule (Fig.1) and thus thio-hemiacetal intermediate is formed. From this intermediate hydride ion is transferred from aldehyde to the C4 atom of the nicotinamide ring of the NAD(P)⁺. This causes a collapse of the thio-hemiacetal to thioester intermediately. In second step an activated water, hydrolyses the thioester intermediate which releases the product. Deprotonation of catalytic cysteine before nucleophilic attack on the substrate, is an essential step in this mechanism. It may be caused by either glutamate 268 or 399[6,8,18,19,20]. The resulting thiolate ion is likely stabilized by the positively charged nicotinamide ring of the coenzyme and/or adjacent main chain amide groups. The proton abstracted by Glu-268 may then go to bulk water [11, 21]. In addition, Glu-268 may be most likely residue which activates water molecule in the second step of the reaction [6, 8, 11, 17, 22]. Beside cysteine and glutamate many other residues interact with the NAD(P) to hold it in place. In reaction, the aldehyde enters the active site through a channel located on the outside of the enzyme. The active site contains a Rossman fold. Interactions between the cofactor and the fold allow the isomerization of the enzyme while keeping the active site functional [5]. In aldehyde dehydrogenase enzyme active site is largely conserved throughout the different classes of the enzyme and although the number of amino acids present in a subunit can change but there is little change in overall function of the site [17,22].

Different organisms have several distinct ALDH genes. In *E. Coli* in one species the largest number of sequences of ALDH present are 13. In human eleven sequences of ALDH are present, excluding the ALDH8 protein [23]. In *Bacillus subtilis* 10 sequences and 14 from various species of *Pseudomonas* are known. Enzyme is used in the bacteria for degradation of toxic aromatic compounds. During degradation, the enzyme's crystal structure shows that intermediates are shuttled directly between
Domain Analysis and Isolation of Coniferyl Alcohol Dehydrogenase Gene from *Pseudomonas nitroreducens* Jin1

Praveen P. Balgir¹ and Dinesh Kalra¹*

¹Department of Biotechnology, Punjabi University, Patiala - 147002, India.

**Authors’ contributions**

This work was carried out in collaboration between both authors. Author PPB designed the study. Author DK analyzed the data. Both authors contributed equally to the study. Both authors read and approved the final manuscript.

**ABSTRACT**

**Aim:** The Aim of present study is to analyse conserved functional Short Dehydrogenase Reductase (SDR) domain from bacteria. Based on the domain analysis selection of coniferyl alcohol dehydrogenase gene for isolation from *Pseudomonas nitroreducens* Jin1.

**Place and Duration of Study:** Department of Biotechnology, Punjabi University, Patiala. From July, 2012 to November, 2012.

**Methodology:** Bioinformatics tools were used to analyse various calA genes from bacteria based on the presence of conserved domain in members of SDR family. Based on insilico analysis, *Pseudomonas nitroreducens* Jin1 calA was selected. PCR was used for amplification of the gene from the genome of *Pseudomonas nitroreducens* Jin1.

**Result:** Multiple sequence alignment results for conserved domains amongst members of SDR family identified presence of all domains of Short Dehydrogenase Reductase members in *Pseudomonas nitroreducens* Jin1 calA gene. Amongst the various sequences compared the *P. nitroreducens* Jin1, calA was found to be the smallest in size. The locus of calA in the genome resides at 103513-104280 bases. It was amplified from the genome of *Pseudomonas nitroreducens* Jin1. The calA gene that was amplified is of size 768bp.

**Conclusion:** calA gene isolated from *Pseudomonas nitroreducens* Jin1 is a small gene with all the functional domains and can be used for biotransformation of coniferyl alcohol to coniferyl aldehyde.

*Corresponding author: Email: dinkalra@gmail.com;