

D-Amino Acid Oxidase and Metagenomics

Gao Chen, Yulong Xie, Jie Deng, Yu Liu, Peihong Shen, Bo Wu and Chengjian Jiang*

The Key Laboratory of Ministry of Education for Microbial and Plant Genetic Engineering, and College of Life Science and Technology, Guangxi University, 100 Daxue East Road, Nanning, Guangxi 530004, China

Abstract

D-amino acid oxidase (DAAO, EC 1. 4. 3. 3) converts D-amino acid to a corresponding α -keto acid via deamination. D-amino acid oxidase is one of the most important enzymes responsible for maintenance proper level of D-amino acids, which play a key role in regulation of many processes in living cells. This paper summary the applications of D-amino acid oxidase in agricultural and industry practices, as the target in human disease treatments, etc. Most important of all, we demonstrate the importance of metagenomic library in exploring the novel D-amino acid oxidase from the environmental microbiology to optimize their applications.

Keywords: D-amino acid oxidase; α -keto acid; Metagenomic library; Environmental microbiology

Introduction

D-amino acid oxidase (DAAO, EC 1. 4. 3. 3) can convert D-amino acid to a corresponding α -keto acid via deamination (Figure 1) [1]. However, many research results showed that D-amino acid oxidase could catalyze the most important substrate of application in industry of cephalosporin C to an important antibiotic intermediate of 7-aminocephalosporanic acid [2]. From a relatively simple structure in prokaryotes to a relatively complex structure in eukaryotes, various D-amino acid oxidases are present and function in biological evolution and physiological cellular activities. D-amino acid oxidase is a single peptide chain with approximately 400 amino acids. The primary sequence of D-amino acid oxidase contains conserved regions I to IV, in which region I is an extremely conserved region containing GXGXXG sequence with an α/β Rossmann structure at the starting position in the DNA sequence of the 5' end; this primary sequence is a conserved site that combines with flavin adenine dinucleotide [3]. D-amino acid oxidase has broad substrate specificity. The amino acids in conserved regions II to V usually contain two tyrosine residues and one arginine residue that exhibit different binding and catalytic functions. D-amino acid oxidase has a typical stereoisomer that exhibits a specific catalytic activity, that is, D-amino acid oxidase only catalyzes D-amino acids; a wide range of substrate specificity of this enzyme catalyzes all D-amino acids except D-glutamic acid and D-aspartate [4]. The activity center of D-amino acid oxidase is mainly composed of two parts [5]. The first part contains the main active center of the isoalloxazine ring of flavin adenine dinucleotide with two β -chains bent around the ring; the second part is composed of a β -substrate binding region of two short chains (Figure 2).

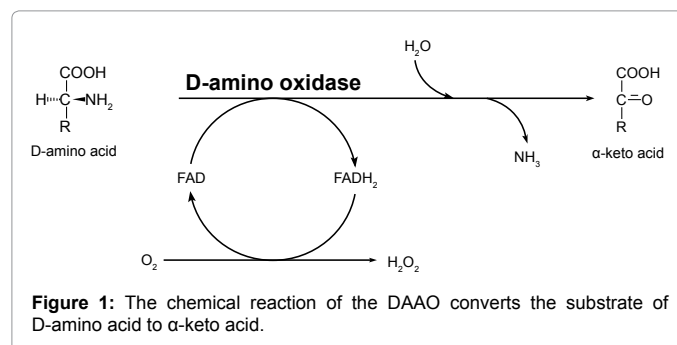
The Importance of D-Amino Acid Oxidase as the Target in Human Disease Treatments

D-amino acid oxidase is the target of human disease treatments, in which the mechanisms of these diseases highly produce D-amino acid oxidase [6]. For instance, neuropathic pain occurs as a result of damages in the central or peripheral nervous system and/or diseases caused by pain syndrome; however, the mechanism remains unclear. Sodium benzoate, an inhibitor of D-amino acid oxidase, elicits potent analgesic effect on chronic neuropathic pain. Benzene ring of the benzoate interaction with the isoalloxazine ring in the active center is competitive binding. This combination will cause the absorption spectrum disorder. Chronic spinal pain is mediated mainly by hydrogen peroxide free radicals, but the mechanism involves suppressing hydrogen peroxide production in the spinal cord [7,8]. In patients

with schizophrenia studied using D-amino acid oxidase and D-serine metabolic models, the pLG protein expression decreases, but human D-amino acid oxidase activity abnormally increases, thereby causing D-serine concentrations to decrease; as a result, human schizophrenia occurs [9,10]. Therefore, D-amino acid oxidase, as one of the important recognition sites and susceptible genes in schizophrenia and chronic neuropathic pain, can be used to understand the metabolic processes of these diseases and identify potential drug targets and drug usage [11,12].

Practices of D-Amino Acid Oxidase in Agriculture

D-amino acid oxidase is applied in agricultural practices to improve crop growth. This oxidase can replace antibiotic resistance and herbicide genes. Plants easily absorb D-amino acids from the environment, but plant cells cannot generate endogenous D-amino acid oxidase; thus, the metabolism of D-amino acid is limited, and the accumulation of D-amino acid in plant cells possibly inhibits growth [13]. Lin et al. verified that *Trigonopsis* D-amino acid oxidase is successfully expressed in rice and indicated that heterologous plant D-amino acid oxidase causes partial abnormality in plant growth

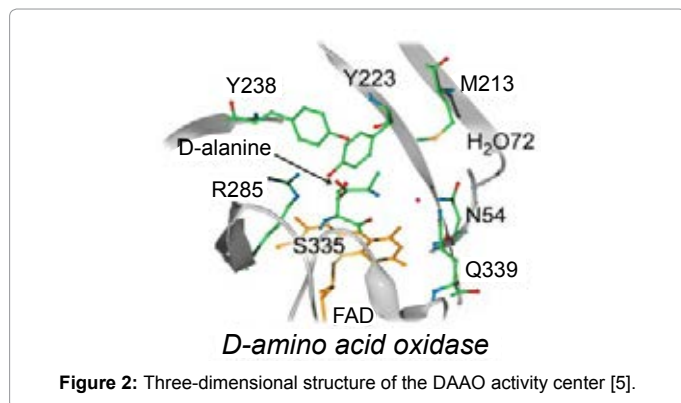


*Corresponding author: Chengjian Jiang, The Key Laboratory of Ministry of Education for Microbial and Plant Genetic Engineering, and College of Life Science and Technology, Guangxi University, 100 Daxue East Road, Nanning, Guangxi 530004, China, Tel: 0086-771-3239403; Fax: 0086-771-3237873; E-mail: jiangcj0520@gmail.com

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[14]. This characteristic has been used as an effective biotechnological development tool; a reverse selection of plants can be achieved by growing plants on a medium containing D-alanine and by integrating the target gene in D-amino acid oxidase [15]. D-amino acid oxidase in *Arabidopsis*, apple, and rice crops has been successfully expressed. Studies have also shown that these transgenic plants can efficiently convert D-amino acid to harmless α -keto carboxylic acids [16].

Practices of D-Amino Acid Oxidase in Industry

D-amino acid oxidase is widely used in industrial applications (Table 1). D-amino acid oxidase extracted from pig kidney can also convert D-amino acids to α -keto acids by deamination [19]. D-amino acid oxidase can act on D-amino acid, thereby producing ketonic acid based on specific stereoisomers of D-amino acid oxidase, in which L-amino acid remains uncatalyzed to separate D-amino acid and thus obtain L-amino acids. D-amino acids constitute the peptidoglycan and muramic acid of cell walls, and microorganisms containing D-amino acid oxidase can be grown using D-amino acids. Therefore, D-amino acid assays in food are indicators of food bacterial contamination. Wcislo et al. reported about D-amino acid oxidase immobilized on an amp biosensor of carbon nanotubes modified by Prussian blue as a vector [20]. D-amino acid oxidase is also immobilized on the surface to detect D-amino acids in food; the sensitivity of this detection method can be achieved at 5 nmol/L to 200 nmol/L.

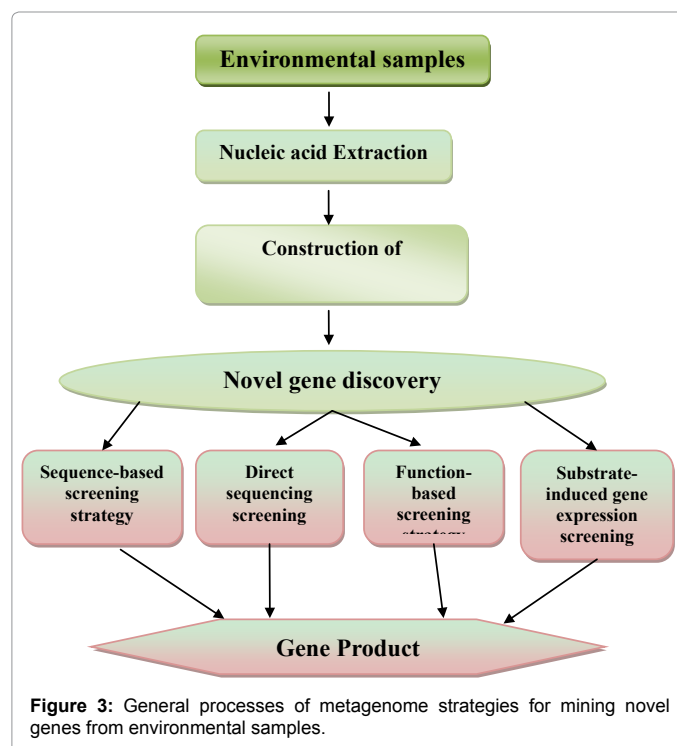
D-Amino Acid Oxidase and Metagenomics

Environmental microbes are large genetic resources. DNA-DNA hybridization and other independent culture techniques have been conducted using 100 g of soil sample containing microbial species ranging between types of 4,000 and 13,000 [21], at an average of 500 mg of the soil, can be extracted from 10 μ g of DNA, which contains numerous genes with unknown functions [22]. In contrast to pure culture techniques, metagenomic library technology can be applied to develop environmental uncultured microbial resources; with this technology, 99% of uncultured microbial flux can be utilized, thereby expanding the microbial gene space a hundredfold [23]. Metagenomic library has been a new strategy that involves the cloning of the total microbial genome, or "metagenome", directly isolated from natural environments into cultivable bacterium such as *Escherichia coli* [24]. This approach does not require the in vitro culturing of microorganisms from environmental samples and can avoid the enrichment of dominant strains under specific selective conditions. Scientists utilize *Escherichia coli* or *Streptomyces lividans* (variable lead *Streptomyces lividans*) as a cloning host to build various libraries such as plasmid libraries, cosmid libraries, bacterial artificial chromosome genomic library, and fosmid libraries (Figure 3) [25]. On the basis of gene expression and sequence-

specific functional homology screening, several new active enzymes and biologically active substances have become available to humans [26]. Therefore, metagenomic library technology is important to study uncultured microorganisms in extreme environmental systems and clone new genes as well as isolate and screen various D-amino acid oxidases. To our knowledge, few researches of cloning of D-amino acid oxidases from uncultured microorganisms have been reported. In our currently study, a novel D-amino acid oxidase gene had been isolated through sequence-based screening strategy successfully from a metagenomic library of contaminated agricultural soil sample from South China (22°51'N, 108°17'E). The possible novel gene was composed of 1074 base pairs, which encoded a 358 amino acids polypeptide about 40 kDa. Bioinformation analysis result showed that the possible novel gene shared no similarity with the other known genes in the Genbank

Items	Pig kidney DAAO	<i>Rhodotorula gracilis</i> DAAO	<i>Trigonopsis variabilis</i> DAAO	Human kidney DAAO
Base pairs	1041	1104	1068	1041
Amino acids	347	368	356	347
kDa	39.6	40	39.3	38.4
pKa of FAD	2.2×10^{-7}	2.0×10^{-8}	1.5×10^{-7}	$8 \pm 2.0 \times 10^{-6}$; $3 \pm 1.0 \times 10^{-7}$
pl	7.0, 7.2	7.2, 7.4, 7.8	5.1	-
Benzoate dissociation constant(mM)	0.002	0.245	18.8	0.007 ± 0.002
Activity state	Monomer+FAD	D-dimer+2FAD	D-dimer+2FAD	-
Active site residues	Y224	Y223	C108	Y224
	Y228	Y238		Y228
	R283	R285		R283
	G313	S335		G313
	Q53	Q339		Q53
		M213		

Table 1: Properties of DAAOs from different sources [2,17,18].



database at DNA level, and shared a similarity of 72% with a D-amino acid oxidase from *Flavobacterium psychrophilum* (YP_001296835.1) at the amino acid level. Multi-alignment sequence compared results also revealed a special I-V conserved domain existed in the amino acids (Detail data not shown). More detailed biochemical characterizations of the novel protein are currently in progress. The finding of a novel D-amino acid oxidase from contaminated agricultural soil sample points out the advantage of metagenomic library in cloning novel genes through sequence-based screening using the *E. coli* host. This study demonstrates the potential of metagenome bioprospecting for novel genes and biocatalysts, and broadens the diversity of D-amino acid oxidases, which should provide a rational basis for genetic modification and direct evolution to optimize their applications. In addition, our future research will focus on various substrates of the D-amino acid oxidase and also to explore its basic enzymatic properties through molecular structure alteration and modification method.

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