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Cloning, Expression and Functional Characterization of the D-Amino Acid Oxidase from Rhodosporidium Diobovatum

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Abstract: *D-Amino Acid Oxidases(DAAO) Catalyze The Enantioselective Oxidation Of A Broad Variety Of D-Amino Acids To Their Corresponding A-Amino Acids, Which Spontaneously Hydrolyze To A-Keto Acids And Ammonium. The Enzyme Of Some Species Has Been Reported. But, In This Study, The Complete Nucleotide Sequence Of The DAAO In The Basidiomycetes Rhodosporidium Diobovatum Has Been Determined. The DAAO Gene Of R. Diobovatum Was Isolated Using Methods Of RACE And RT-PCR. The Results Showed That The Encoded Polypeptide Holds A Sequence Of 357 Amino Acid Residues With Homology To Those Of DAAO From Other Yeasts. The Deduced Protein Molecular Weight Is 38.33 Kda, Theoretical Isoelectric Point Is 7.60. The Expression Vectors Pet-DAAO Was Constructed And Expressed In Escherichia Coli. D-Amino Acid Oxidase Activity On Cephalosporin C Showed A Maximum Around 24 ℃ And The Optimum Reaction Ph Range From 7.5-8.5.*

Keywords: *Rhodosporidium Diobovatum, D-Amino Acid Oxidase, RACE, RT-PCR*

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I. Introduction

Rhodosporidium Diobovatum Was Described From 1970 [1]. It's A Kind Of Basidiomycete Yeast. Salient Features Are The Production Of Carotenoid Pigments, Which Originate Pink To Orange Or Red Cultures; Presence Of A Budding Yeast Stage That Is Unable To Form Ballistoconidia; Development Of A Mycelia Stage, Normally With Clamp Connections; Production Of Teliospores; And Formation Of Transversally Septate Basidia That Originate Multiple Basidiospores (Sporidia) From Each Basidial Cell [2]. The Generally Teliospores Of R. Diobovatum Are Quite Distinct From Those Of Other Rhodosporidium Species. Self-Sporulating Strains (Capable Of Producing Teliospores Without Prior Conjugation) Have Not Been Found For R. Diobovatum. It's Teliospores Generally Cleft, Spheroidal To Obovate [1]. Due To Live In The Marine Environment, The Physiological Mechanism And Regulatory Genes Of R. Diobovatum Has Been Changed To Adapt To Special Living Environment, Leading To Higher Osmosis Tolerance, High Pressure Resistance, Cold Resistance And Oligotrophic. This Kind Of Extreme Environment Endowed Many Special Properties For Yeasts, Which Might Possess Greater Value In Economic Exploitation Than Those On The Terrestrial Organism. Marine Yeasts Isolated From Marine Environments Are Able To Grow Better On A Medium Prepared Using Seawater Rather Than Freshwater [3]. Using Marine Yeasts In Industry Production Shows Distinctive Advantage On The Osmosis Tolerance And The Possibility Of Utilization Of Seawater Instead Of Fresh Water [4]. These Indicate That R. Diobovatum Have Great Potential To Research And To Be Applied In Various Industries.

DAAO Has Many Sources In Nature, From Animal Organs To Microorganisms And Is Also Present In Yeasts. Such As The Yeasts Rhodotorula Gracilis (Rgdaao) [5], Trigonopsis Variabilis (Tvdaao) [6], Rhodotorula Glutinis [7], Candida Boidinii [8], Fusarium Solani [9] And So On. In This Study, We Found The Important Enzyme D-Amino Acid Oxidase (DAAO) From R. Diobovatum Which Have Not Been Reported Before. The DAAO Was One Of The First Flavoproteins To Be Discovered In The Mid-30s Of The Last Century [10]. Biochemically, D-Amino Acid Oxidase (DAAO, EC 1.4.3.3) Is An FAD Flavoenzyme That Catalyzes Oxidative Deamination Of D-Amino Acids To Yield Corresponding Keto Acids, With Molecular Oxygen (O_2) Used As An Electron Acceptor And, Thus, Formation Of H_2O_2 [11]. Since 1935, DAAO Has Been The Object Of A Vast Mass Of Investigations, Becoming A Model For This Class Of Flavoproteins. Each DAAO Monomer Is Clearly Divided Into Two Domains [12], The FAD-Binding Domain With The Typical Rossman Fold And The Interface Domain That Forms The Contact Area With A Second Monomer In The Crystallographic Dimer. The FAD Cofactor Is Buried Inside The Protein, Adopts An Elongated Conformation, And Is Involved In A Number Of Interactions. In All Structures, The C-Terminal Tripeptide, Corresponding To The Peroxisomal Targeting Signal 1 Required For Targeting To Peroxisomes, Is Not Visible In The Electron-Density Map: The Flexibility Might

Be Important For The Interaction With Peroxisomal Membrane Receptors [13]. However, DAAO From Microorganisms Show Properties That Render Them More Suitable For The Biotechnological Applications. Some Important DAAO-Producing Microorganisms Include Trigonopsis Variabilis, Rhodotorula Gracilis [14]. Tvdaao Exhibits The Highest Activity With Cephalosporin C And The Best Thermal Stability Among All Known D-Amino Acid Oxidases, And Together With Myeloperoxidase, May Play An Important Role In Antibacterial Systems In Mammals[15] . The Enzymatic Activity Of DAAO Has Been Correlated With The Incidence Of Schizophrenia [16]. Yeast Daaos Are Optimal Biocatalysts For These Enzyme-Based Technological Applications, As They Possess A High Activity, A Tight Binding Of The Coenzyme FAD, And A Broad Substrate Specificity [17]. So DAAO From Eucaryotic Cells Or Tissues Play An Important Role In Industry. As An Industrial Enzyme, DAAO Has Several Other Current And Potential Biotechnological Applications, Such As The Production Of A-Ketoacids, Chiral Intermediates, And Antibiotics[18] , Synthetic D-Aas Especially As The Catalyst In The Synthesis Of 7-Aminocepha-Losporanic Acid (7-ACA) From Cephalosporin C (CPC) In Conjunction With 7-Aminocephalosporanic Acid Acylase (GLA) [19]. This Compound Is The Starting Material For Producing Several Cephem Antibiotics. It Emerged Clearly That The Main Target Of DAAO Activity In Microorganisms Is To Make D-Amino Acids Available For Cell Metabolism [12]. A Number Of Daaos From Fungi Have Been Cloned With The Aim Of Obtaining Efficient Expression Systems. For Instance, DAAO From F. Solani Was Cloned And Expressed In Escherichia Coli, Reaching ≈8% Of The Whole Soluble Proteins [9]. T. Variabilis DAAO Gene Was Cloned And Expressed On K. Lactis And S. Cerevisiae Up To 150 U/G Cell Dry Weight [20]. R. Gracilis DAAO Cdna Was Also Cloned And Overexpressed In E. Coli , Leading To The Production Of About 2300 U/L Of Fermentation Broth [21].

In The Present Paper, A Gene Of D-Amino Acid Oxidase Was Obtained By Reverse Transcription-Polymerase Chain Reaction (RT-PCR) From R. Diobovatum, And The DAAO Gene Was Expressed In Recombinant E. Coli BL21(DE3)/Pet-DAAO. The Purified Enzyme Has Been Characterized In Its Physical And Chemical Characteristics And Also For Its Activity On Cephalosporin C.

II. Materials And Methods

2.1 Bacterial Strains, Plasmids And Media

R. Diobovatum MCCC 2A00023 Was Grown In A Basal Medium Supplemented With 30 Mm D-Alanine For 24 H At 26℃ With Sharking [22]. E. Coli Strains Were DH5α And BL21(DE3) And Cultured In LB Medium. Plasmids Pmd19-T Vector (Takara) And Expression Vector Pet-28a^{+} (Novagen) Were Used For Cloning And The Heterologous Expression.

2.2 Extraction Of Genomic DNA And RNA, And Synthesis Of Cdna

For DNA Extraction, Cell Culture (2 Ml) At Exponential Stage Was Collected By Centrifugation (12000 Rpm, 1 Min), Washed With Sterile Deionized Water, And Suspended In A Lysing Buffer (0.1% SDS, 100 Mm Nacl, 10 Mm Tris-Hcl (Ph 7.5), And 1 Mm EDTA). After Adding Equal Volumes Of Phenol/Chloroform (25:24, V/V) And Sand (20-35 Mesh, Promega.), Vigorous Vortexing For 10 Min, And Then, Total DNA Was Precipitated By Ethanol.

Total RNA Was Isolated Using Trizol Reagent (Invitrogen, USA) Following The Manufacturer's Instructions. Briefly, Pellet Cells Were Mixed With Trizol And Chloroform. The RNA Present In The Aqueous Phase Was Precipitated By Adding Isopropyl Alcohol. The Resulting RNA Pellet Was Washed With 70% Ethanol And Suspended In Rnase-Free Water. For 5' And 3' Rapid Amplification Of Cdna Ends, Mrna Was Purified From Total RNA Using The Oligotex^{tm}-Dt30<Super> Mrna Purification Kit (Takara, Japan). Reverse Transcriptional Polymerase Chain Reaction (RT-PCR) Analysis Was Performed Using The Amplification Programme Described By The BD SMART^{TM} RACE Cdna Amplification Kit (Clontech, USA), And The Sequences Of The Oligonucleotide Primes Used Are Listed In Table 1. The Obtained Cdna Was Directly Used As The Template For Degenerate PCR And Amplification Of The Full Length Cdna.

2.3 Screening Of DAAO Gene

Obtaining The Sequences Of DAAO From The Genbanks As Follows: Rhodotorula Glutinis ATCC 204091 (Genbanks Accession: EGU13479); Rhodotorula Gracilis (Genbanks Accession: P80324); Trigonopsis Variabilis 0864 (Genbanks Accession: ACM44785); Fusarium Oxysporum Sp. (Genbanks Accession: EXM28874); Neurospora Crassa OR74A (Genbanks Accession: XP_962265); Aspergillus Niger (Genbanks Accession:CBS 513); Candida Boidinii (Genbanks Accession: BAB12222). Amino Acids Sequences Were Analyzed With The Clustal X, Two Fragment Of Amino Acids Sequences WAGANW And VGLRPA Were Found Which Are Conserved In DAAO. Degenerate Primers Rd-N1/ Rd-C1 Were Designed And Synthesized For Screening DAAO Genes.

5′-Full RACE Core Set And 3′-Full RACE Core Set (Takara) Were Used To Obtain The 5′ And 3′ Ends Of Cdna Sequence. PCR Amplification Was Carried Out Using Primers Rd-DAOF And Rd-DAOR With Cdna And DNA As A Template Respectively, And Under The Following Temperature Program: 94℃ For 5 Min, Followed By 30 Cycles Of 94℃ For 30s, 55℃ For 30s, And 72℃ For 2min And A Final Extension At 72℃ For

10 Min. Further Details And Information About The Primers Used Are Summarized In Table 1. The PCR Producer Were Checked By Agarose Gel Electrophoresis, And Purified Using The DNA Gel Extraction Kit (Takara). The Purified Fragment Were Subcloned Into The Pmd19-T Vector, Then Transformed Into E. Coli DH5α. Subsequently, Sequenced Reactions Were Carried Out By Invitrogen (China).

2.4 Sequence Analysis

Using The Online Software Of Genbank, Blastn And Blastp Find The Corresponding Homologous Amino Acid Sequences With Rd DAAO Nucleotide And Then Compared Nucleotide Sequences With DNAMAN. Analysis The Nucleotide Sequences And Predicted Domains Of Rd DAAO Protein With Using The Online Software Predictprotein (Http://Www Predictprotein Org/), Scanprosite (Http://Www. Expasy. Ch/Tools/Scanprosite/) And Pfam (Http://Pfam.Sanger.Ac.Uk/Search). Phylogenetic Tree Was Built Using Bioedit And MEGA5.

2.5 DAAO Cdna Expression In E. Coli

For Expression Studies, The Amplified Cdna Fragment Of DAAO Was Subcloned Into Pmd19-T And Then Digested By Ecor ⅠAnd Hind Ⅲ. Expression Vector Pet-28a^{+} Was Digested Using The Same Restriction Enzymes. The Above Mentioned Products Were Treated With T_4 DNA Ligase To Construct The Expression Vectors Pet-Rd DAAO. Then The Pet-Rd DAAO Was Transformed Into E. Coli BL21 (DE3) And Grown In LB Medium Containing 100 Mg/Ml Ampicillin At 37℃ With Shaking Until The OD_{600} Reached 0.4-0.6. The Culture Then Was Induced With 1 Mm Isopropyl B-D-Thiogalactopyranoside(IPTG). Rd DAAO Produced In E. Coli Was Extracted As Follows: Cells Were Harvested By Centrifugation, Washed With STE Buffer (10 Mm Tris-Hcl, Ph8.0, Containing 0.1 M Nacl And 1 Mm EDTA), And Suspended In Lysis Buffer (50 Mm Potassium Pyrophosphate Buffer, Ph 8.0, 2 Mm EDTA, 5mm 2-Mercaptoethanol And 1 Mm FAD). Cell Rupture Was Achieved By Sonication (5×1min) And The Cell Debris Was Removed By Centrifugation.

2.6 DAAO Activity Assay.

Enzyme Activity Was Assayed Polarographically At 25℃ In A Thermostatted Hansatech Oxygen Electrode, Measuring The Oxygen Consumption In The Presence Of 28 Mm D-Alanine Substrate [23]. Determination Of Kinetic Parameters, Ph And Temperature Optima Of The DAAO Reaction With Cephalosporin C And D-Alanine Were Carried Out In 75 Mm Sodium Pyrophosphate Buffer, Ph 8.5, At 25℃ [24]. DAAO Concentration Was Determined As Flavin Content Assuming E455=12600 /Mcm [25].

III. Results

3.1 Cloning Of R. Diobovatum DAAO Cdna And Genomic DNA

Total Mrna Was Isolated From R. Diobovatum Grown Under DAAO Induction Conditions In The Presence Of D-Alanine As Nitrogen Source. Under These Conditions DAAO Represents 0.3% Of The Total Soluble Protein In The Yeast Extract And A Comparatively High Amount Of Its Specific Mrna Is Supposed To Be Present [26]. To Clone The DAAO Gene Of R. Diobovatum, Amino Acid Sequences Of D-Amino Acid Oxidase From Several Other Species Were Aligned. Degenerate Primers Rd-N1 And Rd-C1 Were Designed Based On Two Conserved Sequence Fragments WAGANW And VGLRPA To Amplify The Core Sequence Of Encoding Gene. An Approximate 720 Bp Fragment Was Obtained. Sequencing Of The Cloned Product Gave A Cdna Fragment That Could Encode A Polypeptide With High Homology To DAAO Protein From Other Yeast Species. These Data Suggested That The Degenerate PCR Product Was Indeed A Part Of The DAAO Gene.

To Obtain The Full-Length DAAO Gene Nucleotide Sequence, The Regions Adjacent To The Sequenced Fragment Were Cloned Using The 5'RACE And 3'RACE. The Nucleotide Sequence Obtained By 5'RACE Had The Starting Codon ATG And By 3'RACE Had A TGA Translation Stop Codon. Based On These Sequence, Two Gene Specific Primers, GSP1 And GSP2, Were Designed And Applied For The Full-Length Gene Amplification. We Obtained A Full-Length Cdna And DNA Clones Of The Gene DAAO, 1071bp And 1361bp, Respectively (Fig. 2). The High Similarity Of The Deduced Amino Acid Sequence Encoded By This Fragment With That Of Other Daaos Strongly Suggested That It Corresponded To The DAO Gene Of R. Diobovatum. Comparison Of DNA With Cdna Sequences Revealed That DAAO DNA Contains Four Introns (Fig. 2). The Coding Region Consists Of 1071 Bp Encoding A Protein Of 357 Amino Acids. A Partial Sequence Comparison Between Yeasts, Fungus DAAO Proteins Of The Regions Containing The Highly Conserved Residues Which Play A Key Role Is Reported In Figure 3.

3.2 Analysis Of DAAO Gene Sequence

The Corresponding Full-Length Cdna Cloned Contains A Complete Open Reading Frame (ORF) Which Can Encode A Polypeptide That Have The Number Of 357 Amino Acid. The Deduced Protein Molecular Weight Is 38.34 Kda, Theoretical Pi Is 7.60. The Number Of Negatively Charged Residues (Asp And Glu) Is 36 And Positively Charged Residues (Arg And Lys) Content Is 37. In Yeast, The Estimated Half-Life Is 20 Hours, In Vivo. The Instability Index Could Provide An Estimate Of The Stability Of DAAO. It Is Computed To Be 41.51 And Is Classifies The Protein As Unstable. The Aliphatic Index Of A Protein Is Defined As The Relative Volume Occupied By Aliphatic Side Chains (Alanine, Valine, Isoleucine, And Leucine). It May Be Regarded As

A Positive Factor For The Increase Of Thermostability Of Globular Proteins [13]. The Aliphatic Index Is 87.44, Which Reveals That The Protein Is Lipophilic. There Are Three Glycosylation Sites, Which Play An Important Role In Modification Of Protein And Regulating Protein Function, At The Position 114, 295 And 304, Respectively. In This Protein Sequence , There Are 3 Transmembrane Helices From 73 To 93, 172 To 193, 316 To 332. The Amino Acid Sequence Was Blasted In The NCBI Database, Revealing That The DAAO Has 68% Identities With Rhodosporidium Toruloides (Genbank Accession: P80324), 69% Identities With Rhodotorula Glutinis ATCC 204091 (Genbank Accession: EGU13479). The Phylogenetic Tree For The DAAO Is Presented In Fig. 4. The Phylogenetic Analysis Proves The Evolution Diversity And The Evolution Pathway For This Enzyme. The First Step Of Evolution Divided The DAAO Precursor Into Two Groups: Fungi Including Basidiomycota And Ascomycota, And Mammal And Prokaryotic Bacteria. Strangely, In Presence Of Ascomycota Of Neurospora Crassa, Ascomycetes And Basidiomycetes Are Much More Close Than Basidiomycetes And Mammalian DAAO. Without The Neurospora Crassa, Basidiomycetes DAAO Are Distant Relative Of Ascomycota. Which Is Different With V. I. Tishkov And S. V. Khoronenkova [27].

3.3 DAAO Characterization And Its Activity

The Recombinant Enzyme Oxidized Efficiently Cephalosporin C (V_{max}=103.5 U (Mg Protein)$^{-1}$ And K_m≈5 Mm, At 25℃) As Well As D-Alanine, The Substrate Usually Employed For This Enzyme (V_{max}=120.0 U (Mg Protein)$^{-1}$ And K_m≈1 Mm, At 25℃) (Tab. 2). D-Amino Acid Oxidase Activity On Cephalosporin C Over The 15-40℃Temperature Range Showed A Maximum Around 24℃(Fig.5). Moreover, The Reaction Reached An Activity Maximum In The 7.5-8.5 Ph Range (Fig. 6) .

IV. Discussion

D-Amino Acid Oxidase (DAAO, EC 1.4.3.3) Is A Flavoprotein That Catalyses The Oxidation Of D-Amino Acids To The Corresponding 2-Imino Acids And Hydrogen Peroxide. Each Amino Acid Is Non-Enzymatically Hydrolysed To A-Keto Acids And Ammonium [28]. This Enzyme Is Useful In Several Areas Of Biochemistry And Biotechnology. Most Important Applications Are In Qualitative And Quantitative Analyses In Either A Soluble Or Immobilized Manner, The Oxidation Of Cephalosporin C [29]. In This Research, We Cloned And Characterized The Gene Encoding DAAO In R. Diobovatum. The Cloned Rd DAAO Cdna Was Shown To Encode A Functional DAAO Enzyme Expressed In Relatively High Yield In E. Coli. The Level Of Expression Obtained In E. Coli Was Considerably Higher Than That Obtained From R. Gracilis Under Optimal Conditions: A Development Which Will Facilitate Both Structural Studies And Biotechnological Applications. By Using The Primary Sequence Of R. Diobovatum DAAO As A Query, A Number Of Sequences Coding For Putative Daaos Were Identified In The Fungi Kingdom (The Identity Degree Ranges From 30 To 70%).

DAAO Activity Is Ubiquitous: It Is Found In Numerous Eukaryotic Organisms, Including Yeasts, Fungi, Insects, Amphibians, Reptiles, Birds And Mammals [30]. Concerning The In Vivo Function Of The Enzyme, Several Findings On The Physiological Role Of D-Amino Acid Oxidase Point To A Detoxifying Function Of The Enzyme In Metabolizing Exogenous D-Amino Acids In Animals [31]. In Yeast Cells, The Flavoprotein DAAO Is Involved In Primary Metabolism Where The Efficiency Of D-Amino Acid Utilization Is Fundamental For Cell Growth [32]. According To Figure 3, Comparison Of The Primary Structure Of R.D DAAO With Related Sequence, R.D DAAO Is Relative Conserved With Several Other Yeast DAAO Sequences. D-Amino Acids Have Long Been Known To Play A Prominent Role In Bacterial Physiology As Constituents Of Bacterial Cell Walls (As Components Of Peptidoglycans And Teichoic Acids); Thus, Microorganisms And Some Of Their Products Are Likely To Provide An Important Source Of Dietary D-Amino Acids [31]. DAAO In Vitro Displays Broad Substrate Specificity [33]. In Our Experiment, R.D DAAO Induced Under The Condition Of The Presence Of D-Alanine As Nitrogen Source And Also Have Enzyme Activity To Utilize Other D-Amino Acids, And R.D DAAO Have High Catalytic Efficiency. In This Study, We Confirmed That The Putative DAO Gene In The R. Diobovatum Encodes A Functional DAAO Protein, And Showed That DAAO Is Induced By D-Amino Acids. These Findings, With The Full Genome Sequence And Molecular Genetic Tools Availability Of The Yeast, Indicated That DAAO Of The Yeast R. Diobovatum Could Be Play An Important Role In Industry.

In Conclusion, We Have Successfully Cloned And Functionally Characterized The DAAO Gene Of The Red Yeast R. Diobovatum MCCC 2A00023. However, The Induction Mechanism Remains To Be Elucidated. This Work Will Be Valuable For Further Study Of DAAO.

V. Conclusions

In This Study, We Have Successfully Cloned And Functionally Characterized The DAAO Gene Of The Red Yeast R. Diobovatum MCCC 2A00023.We Confirmed That The Putative DAO Gene In The R. Diobovatum Encodes A Functional DAAO Protein, And Showed That DAAO Is Induced By D-Amino Acids. The Complete Nucleotide Sequence Of The DAAO In The Basidiomycetes Rhodosporidium Diobovatum Has Been Determined. These Findings Indicated That DAAO Of The Yeast R. Diobovatum Could Be Play An Important

Role In Industry.

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Table 1. Primer Sequences Used In This Study

Primers	Sequences	Purpose	Origin
Rd-N1	5′ - TGGGCNGGNGCNAAYTGG - 3′	Screening	This Study
Rd-C1	5′ - GCGGGNCGNAGCCCNAC- 3′	Screening	This Study
Rd-3F	5′ - GTCGCCCTGCTCGTTAACGCG - 3′	3′RACE 1st PCR	This Study
R-3F	5′ - TACCGTCGTTCCACTAGTGATTT- 3′	3′RACE 1st PCR	3′-Full RACE Direction
Rd-3S	5′ - ATCATTCCGCGCCCCGG- 3′	3′RACE 2st PCR	This Study
R-3S	5′ - CGCGGATCCTCCACTAGTGATTTCACTA TAGG- 3′	3′RACE 2st PCR	3′-Full RACE Direction
Rd-5F	5′ - CGCCCTCGGGGAGGAGGC - 3′	5′RACE 1st PCR	This Study
R-5F	5′ - CATGGCTACATGCTGACAGCCTA - 3′	5′RACE 1st PCR	5′-Full RACE Direction
Rd-5S	5′ - AGAGGGGACGAGCGAGACC- 3′	5′RACE 2st PCR	This Study
R-5S	5′ - CGCGGATCCACAGCCTACTGATGATCA GTCGATG -3′	5′RACE 2st PCR	5′-Full RACE Direction
Rd-DAOF	5′ - ATGGCCTCAAGCAACACGG - 3′	Prokaryotic Expression	This Study
Rd-DAOR	5′ - CTACAGCCTGGCCTTTGCGC - 3′	Prokaryotic Expression	This Study

Table 2. Kinetic Parameters Of The DAAO Reaction With Cephalosporin C And D-Alanine.

Substrate	V_{max} (U /Mg Protein)	K_m(±0.01) (Mm)
Cephalosporin C	103.5	5
D-Alanine	120.0	1

Reactions Were Carried Out In 75 Mm Sodium Pyrophosphate Buffer, Ph 8.5, At 25℃.

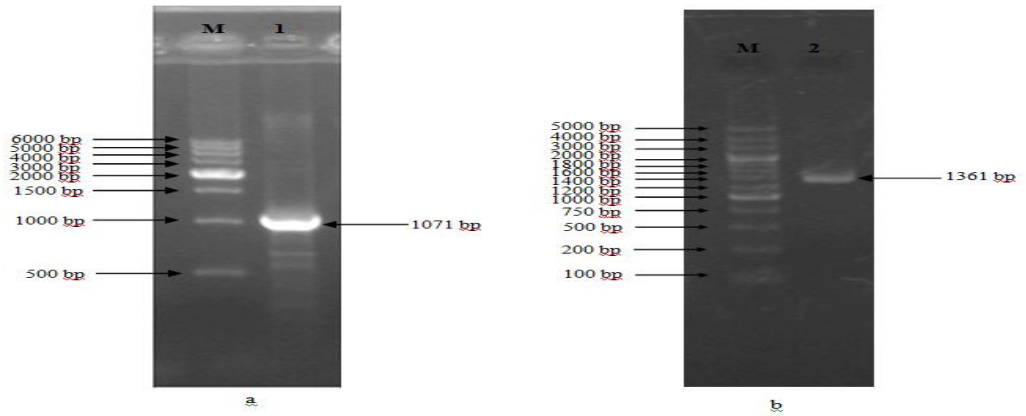

Figure 1. A: Agarose Gel Electrophoresis Of PCR Products Obtained From Cdna Of The R.D DAAO, 1071bp.
B: Agarose Gel Electrophoresis Of Obtained A Full-Length DNA Clones Of The Gene DAAO, 1361bp.

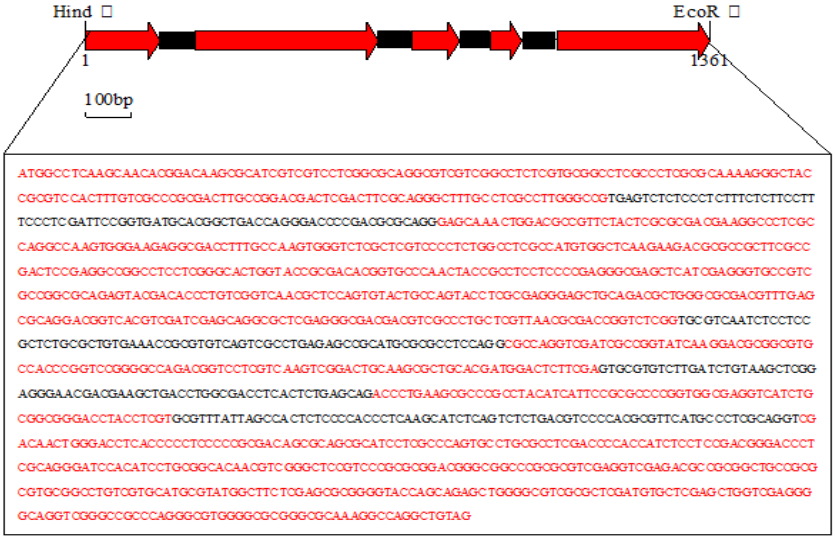

Figure 2. Bases Sequences Comparison Of R. Diobovatum MCCC 2A00023 DAAO Genomic DNA And Cdna.

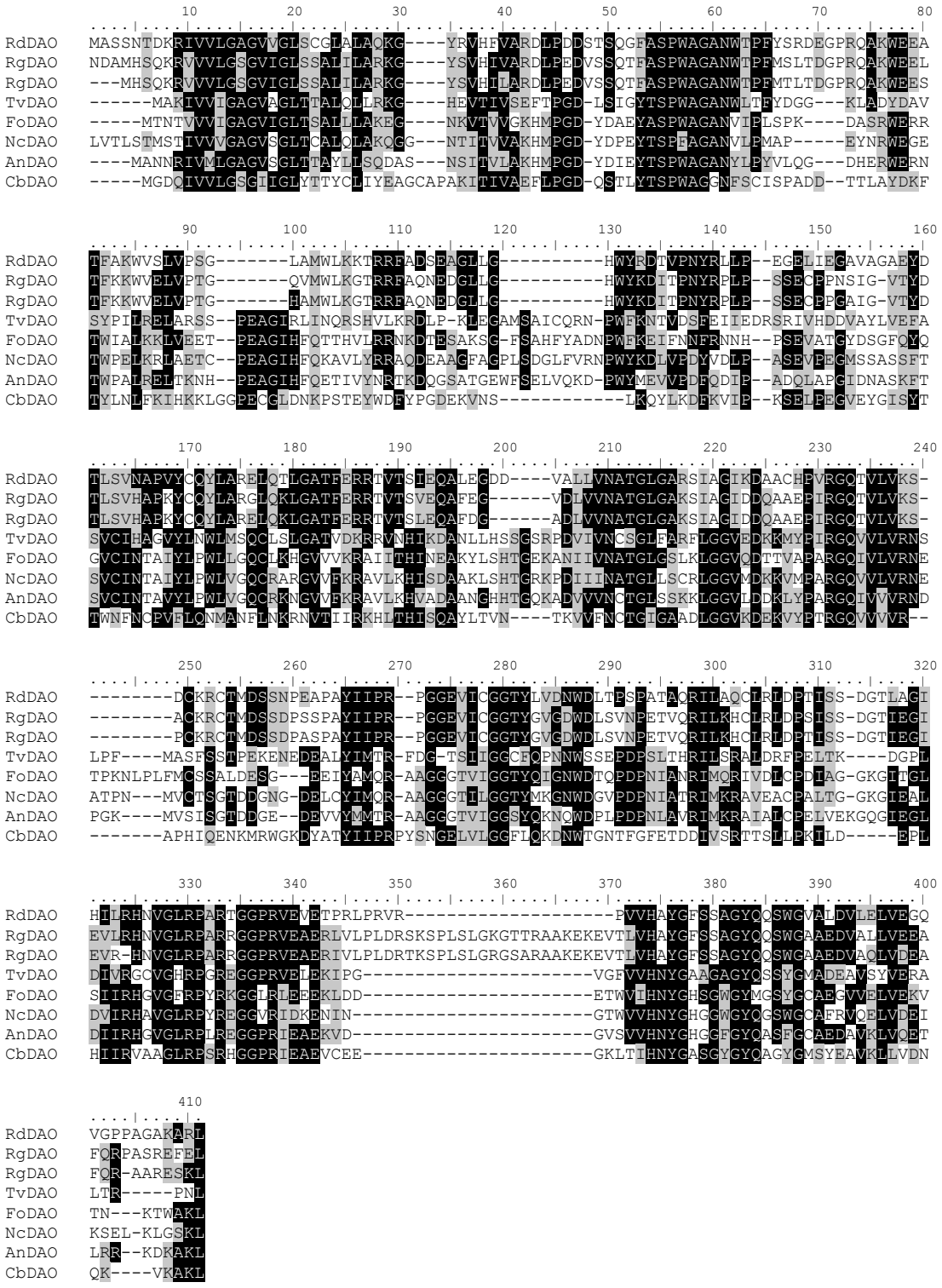

Figure 3. Comparison Of The Primary Structure Of Deduced Amino Acid Sequence Of DAAO From R. Diobovatum MCCC 2A00023 With Related Sequence. The Picture Was Generated By Clustalw Multiple Alignment Of Sequences From Eight Organisms Using The Bioedit Software. The Highly Conserved Region Are Shaded.

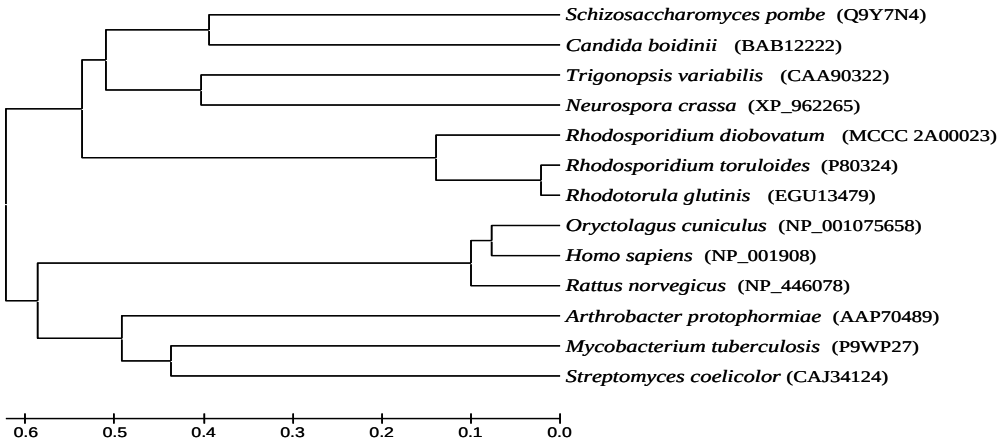

Figure 4. Unrooted Phylogenetic Tree (UPGMA) Of R. Diobovatum DAAO. The Tree Was Built Using Bioedit And MEGA5.

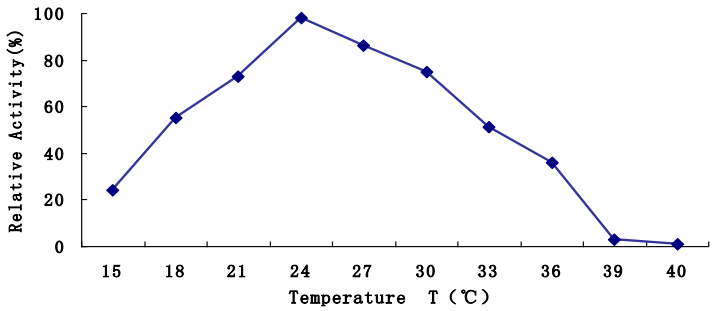

Figure 5. Effect Of Different Temperature On The DAAO Activity.

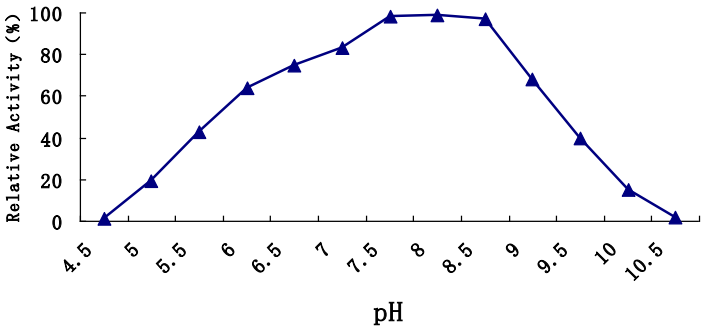

Figure 6. Effect Of Different Ph On The DAAO Activity.

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