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Volume 2462

The Second International Conference on Genetic Resources and Biotechnology

Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Bogor, Indonesia • 24–25 May 2021

Editors • I Made Tasma, Dwinita Winkan Utami, Ika Roostika, Yadi Suryadi, Chaerani, Eny Ida Riyanti, Puji Lestari, Toto Hadiarto, Reflinur, Joko Prasetyono, Fatimah, Surya Diantina, Tri Puji Priyatno, Kusumawaty Kusumanegara, Wening Enggarini, Rerenstradika Tizar Terryana and Dani Satyawan

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

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In Silico Functional Prediction of CAS2, a Protein Specifically Expressed in Appressorium and Required for Pathogenicity of *Colletotrichum gloeosporioides*

Tri Puji Priyatno[1, a)], Farah Diba Abu Bakar[2], Rohaiza Ahmad Redzuan[2], Abdul Munir Abdul Murad[2] and Ifa Manzila[1]

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Abstract. Colletotrichum appressoria specific gene (*CAS2*) expressed during appressorium formation of *Colletotrichum gloeosporioides* play important role as virulence factors. Determination of the CAS2 protein structure could help deduction of its function and interaction with other factors. The current study aimed to assess in the functional and structural properties of the CAS2 by *in silico* analyses. CAS2 sequence submitted to the online tool Gene Ontology Functional Enrichment Annotation Tool (GO FEAT) in order to search for homology. The biological importance of the annotated products were characterized based on the analyses of physicochemical properties, subcellular localization, molecular function, protein-protein interaction networks, and 3D structural protein modelling. The results showed that CAS2 is a hydrophobic protein with an average molecular weight of around 46.2 kDa. The N-terminal amino acid (aa) sequence of CAS2 was composed of 22 amino acid residues of signal peptide. The high score probability (1.840) of subcellular location prediction showed that this protein located in plasma membrane. Based on putative transmembrane prediction software, the CAS2 protein is composed of three alpha-helical transmembrane domains with the longest of C-terminal of amino acid residues in cytoplasm. CELLO2GO server predicted that the CAS2 is a structural protein functioning as enzyme and ion binding. Protein interaction network resolved by STRING web server revealed 10 potential interacting protein associates with CAS2. These *in silico* analysis offered excellent and reliable information for functional characterization of CAS2 protein by using the advanced tools and techniques of computational biology.

INTRODUCTION

Colletotrichum gloeosporioides, the causal agent of anthracnose, infect an extensive range of tropical and subtropical crops, such as papaya (*Carica papaya*), mango (*Mangifera indica*), avocado (*Persea americana*), and chili peppers (*Capsicum* sp.). The infection process initialled with the adhesion of conidia onto the plant's surface followed by germination and formation of appressorium infection structure at the terminal end of germ tube. Subsequently, appressorium penetrates the plant epidermis, leading to the formation of primary and secondary hyphae in the development of the biotrophic and necrotrophic phases [1]. Due to the pivotal role of appresorium, including both of the previously mentioned stages in pathogenicity of the fungus, it is of great interest to determine the functional genes involved in appressorium morphogenesis and pathogenicity process of *C. gloeosporioides*.

In the previous studies we isolated and identified *CgCAS2* gene specifically expressed in appressorium morphogenesis of *C. gloeosporioides* [2, 3]. Priyatno *et al.* [3] reported that mutants with deleted *CAS2* were not defective in appressoria production compared to that of wild type, however, the *cas2* mutant produced multipolar germination and is non-virulent to mango fruits. This indicated that *CAS2* may encode a novel virulence factor in fungal pathogens (3). Recent studies found a link between dimorphism and virulence, becoming a new and

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promising target to develop compounds against fungal diseases [4]. The hallmark of pathogenic germination is unilateral formation of a single germ tube following the first cell division [5]. The positive correlation between unidirectional germination and virulence have also been reported in *Beauveria bassiana* against diamondback moth (*Plutella xylostella*) and the Colorado potato beetle (*Leptinotarsa decemlineata*) [6]. Bidirectional germination of *C. gloeosporioides* conidia occurred in rich medium and in the absence of plant signal causing fail formation of appressoria and reduced virulence [7].

All types of conidia germination are associated with cell polarization regulated by septin proteins at the growth sites , which serve as positional landmarks for recruitment and activation of the polarity-establishing machinery [5, 7]. Subsequently, the Spitzenkörper vesicles act as a vesicle supply center for the growing tip in hyphal polarity, then actin and the microtubule cytoskeleton play important role to continuous flow of secreted vesicles to the growing germ tube [9]. Regulation of these processes involves Rho-type GTPases acting as binary molecular switches [5]. Rho-type GTPases subfamily, such as RhoA, Rac1, and Cdc42, are involved in various aspects of cellular development, particularly morphogenesis, cell cycle, and vesicular trafficking in eukaryotic cells [10, 11].

According to Takemoto *et al*. [12], polarity proteins Rac1 and Cdc24 are components of membrane bound fungal NADPH oxidase (Nox) complex catalyzing reactive oxygen species (ROS) production. ROS are small molecules with high oxidative that plays a key role in fungal polarized growth, pathogenicity, sporulation, and spore germination [13]. Deletion mutant of *rac1* in *C. gloeosporioides* caused bipolar germination, difficulties in establishing and maintaining cell polarity, reduced conidial and hyphal adhesion, formation of immature appressoria, and non-pathogenic [5]. ROS producing systems via Nox usually localized at plasma membrane or endoplasmic reticulum membrane, and transport NADPH electrons through membranes to reduce oxygen molecule to superoxide anion ($O_2^{\bullet-}$) [14].

Regardless of its important role, CAS2 protein is yet not well characterized structurally and functionally. Considering the importance of this protein in pathogenicity of *C. gloeosporioides*, the present study is carried out to systematically analyze CAS2 protein with the prediction of physiochemical properties, subcellular localizations, domain/motif predictions, secondary and tertiary structure prediction, and function annotation using established bioinformatics databases and tools.

MATERIALS AND METHODS

CAS2 nucleotide sequence was retrieved from previous studies [2, 3]. This sequence was used as an input for Expert Protein Analysis System (ExPASy), a proteomic server of Swiss Institute of Bioinformatics (SIB) (https://www.expasy.org/) [15].

Primary Protein Structure Analysis

The coding sequence of CAS2 gene was translated to protein sequence by using ExPASy translated tool (http://web.expasy.org/translate/). Primary structural analysis of CAS2 protein was determined using ProtParam from ExPASy. The biophysical and biochemical properties include molecular weight (Mw), isoelectric point (pI), extinction coefficients (EC-quantitative study of protein-protein and protein ligand interactions), instability index (II-stability of proteins), aliphatic index (AI-relative volume of protein occupied by aliphatic side chains), grand average hydropathicity (GRAVY-sum of all hydropathicity values of all amino acids divided by number of residues in a sequence), half-life, and number of positive and negative residues.

Secondary Protein Structure Characterization

The amino acid sequence was subjected to secondary protein structure prediction by using Psipred (http://bioinf.cs.ucl.ac.uk/psipred/).

Tertiary Protein Structure Prediction

The tertiary structure prediction of CAS2 was modeled through ab initio approach using online available tool LOMETS (https://zhanglab.ccmb.med.umich.edu/LOMETS/) and visualized using EzMol, a molecular display wizard (http://www.sbg.bio.ic.ac.uk/ezmol/cgi-bin/loadingPage.cgi). The model, thus obtained, was further

validated by Ramachandran plot using the RAMPAGE online tool (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Subcellular Localization and Protein Classification

The subcellular localization of the target protein was predicted using CELLO2GO server with gene ontology annotation (http://cello.life.nctu.edu.tw/cello2go/) [16].

Functional Analysis

The MyHits web server (http://myhits.isb-sib.ch) is used to predict the annotation of protein sequences and to analysis of their domains and signatures of CAS2. Potential phosphorylation sites of the protein were studied using NetPhos2.0. Location of signal peptide cleavage sites was predicted using PrediSi (http://www.predisi.de). Transmembrane topology of CAS2 was predicted using Consensus Constrained TOPology prediction (CCTOP; http://cctop.enzim.ttk.mta.hu) and was visualized to use Protter, a web service (http://wlab.ethz.ch/protter).

Protein-Protein Interaction Analysis

STRINGv10.0 web server (http://string-db.org) was used to predict the interaction of CAS2 protein with other closely allied proteins.

RESULTS AND DISCUSSIONS

Primary Structure and Physicochemical Properties of CAS2 Protein

ExPASy ProtParam server was used to analyze the amino acid composition (Table 1) and physicochemical properties (Table 2) of CAS2 protein that consisted of 369 amino acids. Leucine (L) and alanine (A) are the most abundant amino acid present; isoleucine (I), glycine (G), and threonine (T) are the next abundant amino acids predominantly presence. Leucine (L), alanine (A), isoleucine (I), and glycine (G) are hydrophobic amino acids which normally locate inside the protein core and participate in van der Waals interactions [17]. In particular, hydrophobic amino acids can be involved in binding/recognition of hydrophobic ligands such as lipids [18]. The alanine is rarely directly involved in protein function, but it can play a role in substrate recognition or specificity, particularly in interactions with other non-reactive atoms such as carbon [18]. The presence of glycine can play a distinct functional role, such as using its backbone (without a side chain) to bind to phosphates [19]. If a conserved glycine changing to any other amino acid, the change could have a drastic impact on function, such as in protein kinases [18].

The deduced CAS2 protein has a calculated molecular mass of 40,229 Da and content identical amount of positive and negative charges of amino acid residues. Because of the isoelectric point (pI) of 8.84, CAS2 was classified as alkaline protein. In isoelectric focusing method, this computed pI will be supportive for developing buffer system for purification [20]. The instability index is computed to 31.08 which classify the CAS2 protein as stable. Guruprasad *et al*. [21] suggested that the instability index can be used as a measure of *in vivo* half-life of a protein. Proteins with an instability index of more than 40 have shown an *in vivo* half-life of less than 5 h, whereas those that have an instability index of less than 40 have *in vivo* half-life of more than 16 h [22]. Correlate with low instability index, the expected half-life of CAS2 was about 30 h.

The extinction coefficients of CAS2 were 55015 and the aliphatic index was 104.44. Relative volume of a protein occupied by it aliphatic side chains (alanine, isoleucine, leucine, and valine) is denoted by aliphatic index [20]. The higher the aliphatic index, the higher will be the stability of the protein [23]. CAS2 has 0.104 of the grand average of hydropathy (GRAVY) value. The positive GRAVY indicates that the protein was hydrophobic, and, hence, cannot interact with the aqueous environment or the amide water [24]. A positive value >-0.4 suggests increased probability for membrane association [25].

TABLE 1. Amino acid composition of CgCAS2 protein.

No.	Amino acid	Number of amino acid	Composition (%)
1	Ala (A)	36	9.8
2	Arg (R)	17	4.6
3	Asn (N)	18	4.9
4	Asp (D)	13	3.5
5	Cys (C)	2	0.5
6	Gln (Q)	6	1.6
7	Glu (E)	16	4.3
8	Gly (G)	29	7.9
9	His (H)	9	2.4
10	Ile (I)	33	8.9
11	Leu (L)	38	10.3
12	Lys (K)	20	5.4
13	Met (M)	7	1.9
14	Phe (F)	11	3.0
15	Pro (P)	23	6.2
16	Ser (S)	20	5.4
17	Thr (T)	28	7.6
18	Trp (W)	7	1.9
19	Tyr (Y)	11	3.0
20	Val (V)	25	6.8
21	Pyl (O)	0	0.0
22	Sec (U)	0	0.0

TABLE 2. Physicochemical properties of CgCAS2 protein.

No.	Biophysical and biochemical properties	Values
1	No. of amino acids	369
2	Molecular weight	40,229.77
3	Isoelectric point	9.34
4	Negatively charged residues (Asp + Glu)	29
5	Positively charged residues (Arg + Lys)	37
6	Extinction coefficients	55,015
7	Abs 0.1%	1.368
8	Instability index	31.08
9	Aliphatic index	104.44
10	Grand average of hydropathicity (GRAVY)	0.104
11	Half-life	30 h

Secondary Structure of CAS2 Protein

The secondary protein structure of CAS2 was determined using the PSIPRED online tool. Total amino acids contributed to helix is 45.8%, 43.9% to coils, and 10.3% to strands (Fig. 1). This shows that helix and coils have identical amount in the secondary structure elements of CAS2 followed by strands. Secondary structure is defined by the pattern of hydrogen bonds between the amino hydrogen and carboxyl oxygen atoms in the peptide backbone [26]. The various secondary structures were influenced by amino acid composition [27]. Amino acids alanine, leucine, and glutamine are classified as strong helix admire, whereas glycine, proline, and asparagine are known as strong coils formers [26]. The dominant helix and coil structure content of CAS2 is due to the rich content of alanine, leucine, glycine, and proline. Proline has a special property of creating kinks in polypeptide chains and disrupting ordered secondary structure [27].

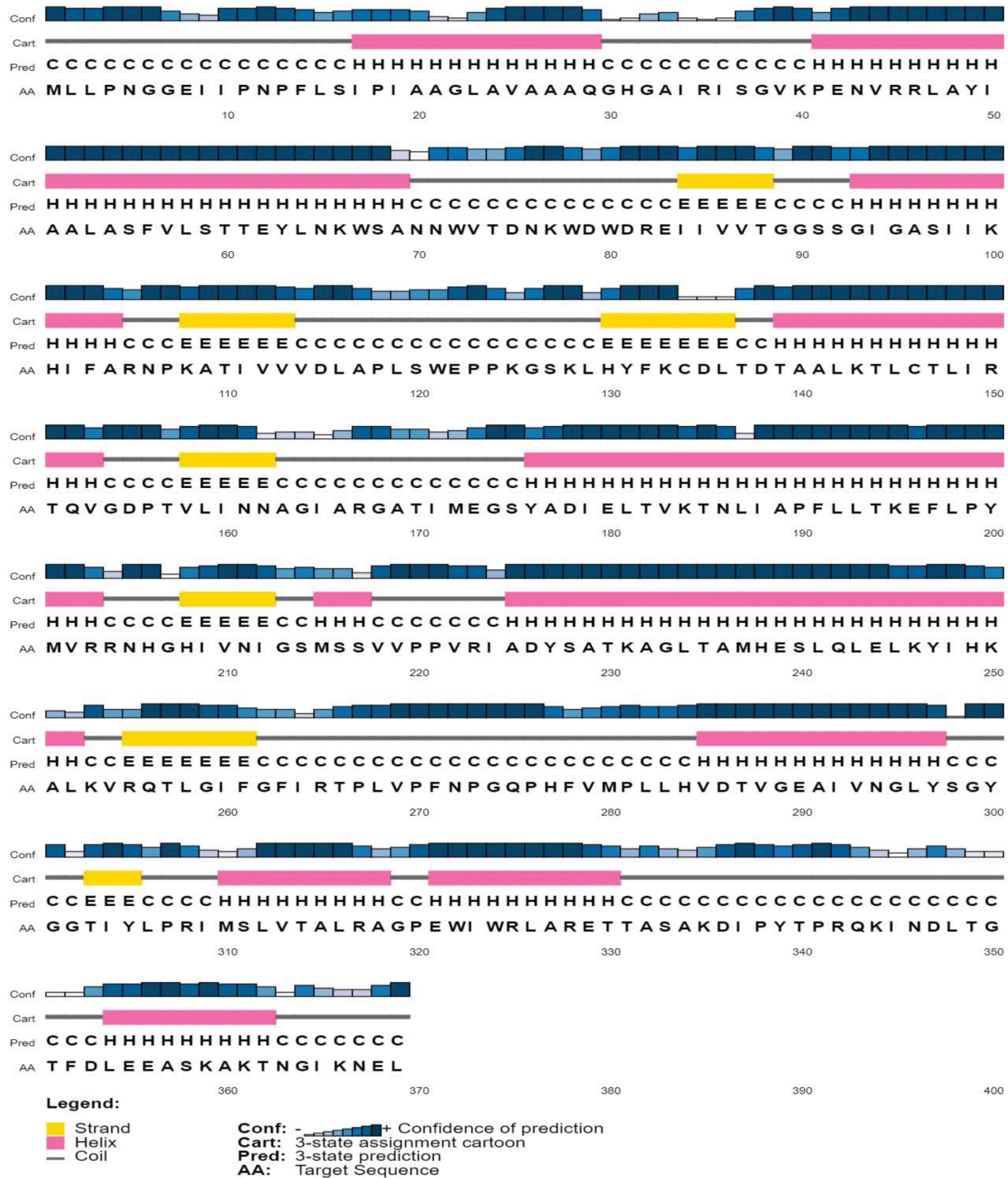


FIGURE 1. CAS2 secondary structure prediction using PSIPRED. Helix and oil identically dominates the secondary structure elements of CAS2 protein.

The feature-based function prediction for all Gene Ontology domains was presented in Table 3. GO prediction categorize CAS2 protein within three classes: biological process, molecular function, and cellular component. For biological processes, CAS2 referred to oxidation-reduction process, metabolic process for some compounds (such as small molecule, carboxylic acid, phosphate-containing compound, lipid, monocarboxylic acid, nucleotide metabolic, and alcohol), and transport ions. The molecular function of CAS2 was predicted involved in enzymes activity and molecular binding, while the cellular component category was involved in membrane function of mitochondria and endoplasmic reticulum. The importance of fungal membrane proteins in the morphogenesis and pathogenesis of

fungal pathogen is well-established [28]. However, several membrane proteins are not yet well characterized due to difficult in the preparation of stable membrane proteins with the preservation of their native structure for further studies. The cell membrane is the most external structure of the fungal cell in the interaction between the cell and the environment in a highly dynamic interplay [29]. Therefore, the identification of CAS2 as membrane proteins may be the key of understanding the pivotal role of CAS2 in morphogenesis and pathogenesis mechanisms of *C. gloeosporiodes*.

TABLE 3. Feature-based function prediction for all Gene Ontology domains.

GO term	Name	Probability	SVM Reliability
Biological process predictions			
GO:0055114	Oxidation-reduction process	0.909	H
GO:0044281	Small molecule metabolic process	0.900	H
GO:0019752	Carboxylic acid metabolic process	0.886	H
GO:0006796	Phosphate-containing compound metabolic process	0.795	H
GO:0006629	Lipid metabolic process	0.792	H
GO:0032787	Monocarboxylic acid metabolic process	0.755	H
GO:0009117	Nucleotide metabolic process	0.748	H
GO:0006810	Transport ions	0.744	H
GO:0006082	Organic acid metabolic process	0.726	H
GO:0006066	Alcohol metabolic process	0.700	H
Molecular function predictions			
GO:0003824	Catalytic activity	0.991	H
GO:0016491	Oxidoreductase activity	0.957	H
GO:0016746	Transferase activity, transferring acyl groups	0.850	H
GO:0001882	Nucleoside binding	0.850	H
GO:0016740	Transferase activity	0.839	H
GO:0000166	Nucleotide binding	0.823	H
GO:0032549	Ribonucleoside binding	0.822	H
GO:0030554	Adenyl nucleotide binding	0.806	H
GO:0020037	Heme binding	0.803	H
GO:0001883	Purine nucleoside binding	0.791	H
Cellular component predictions			
GO:0005743	Mitochondrial inner membrane	0.979	H
GO:0016020	Membrane	0.978	H
GO:0016021	Integral component of membrane	0.975	H
GO:0031224	Intrinsic component of membrane	0.963	H
GO:0005740	Mitochondrial envelope	0.949	H
GO:0031966	Mitochondrial membrane	0.940	H
GO:0031090	Organelle membrane	0.911	H
GO:0005739	Mitochondrion	0.892	H
GO:0005789	Endoplasmic reticulum membrane	0.836	H
GO:0098588	Bounding membrane of organelle	0.829	H

Subcellular Localization of CAS2 Protein

Localization study using CELLO2GO server predicted CAS2 protein located in plasma membrane (Fig. 2) suggesting that it might have an important regulatory role in perceive environmental signal into internal cell. The use of putative transmembrane domain prediction software (HMMTOP) predicted that the CAS2 protein is composed of three alpha-helical transmembrane domains (amino acids 15–34, 45–64, and 84–103). The HMMTOP analysis also revealed that the N-terminus and a second loop is outside of the membrane, the first loop and the longest C-terminus is inside of the membrane. Using SignalP software, the N-terminal CAS2 were composed of 24 amino acids as signal peptide. The presence of both signal peptide sequence and two transmembrane domains strongly suggested that CAS2 is a membrane protein. According to Kapp *et al.* [30], signal peptides were considered

as an additional resource of the function of secretory and membrane proteins. In eukaryotes, signal sequences direct the insertion of proteins into the membrane of the endoplasmic reticulum and are usually cleaved off by signal peptidase [30]. The transmembrane CAS2 protein was visualized using Protter software as presented in Fig. 3.

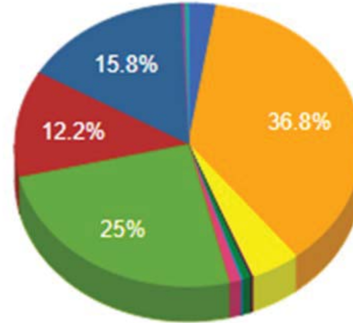


FIGURE 2. Subcellular localization prediction of CAS2 using CELLO2GO server.

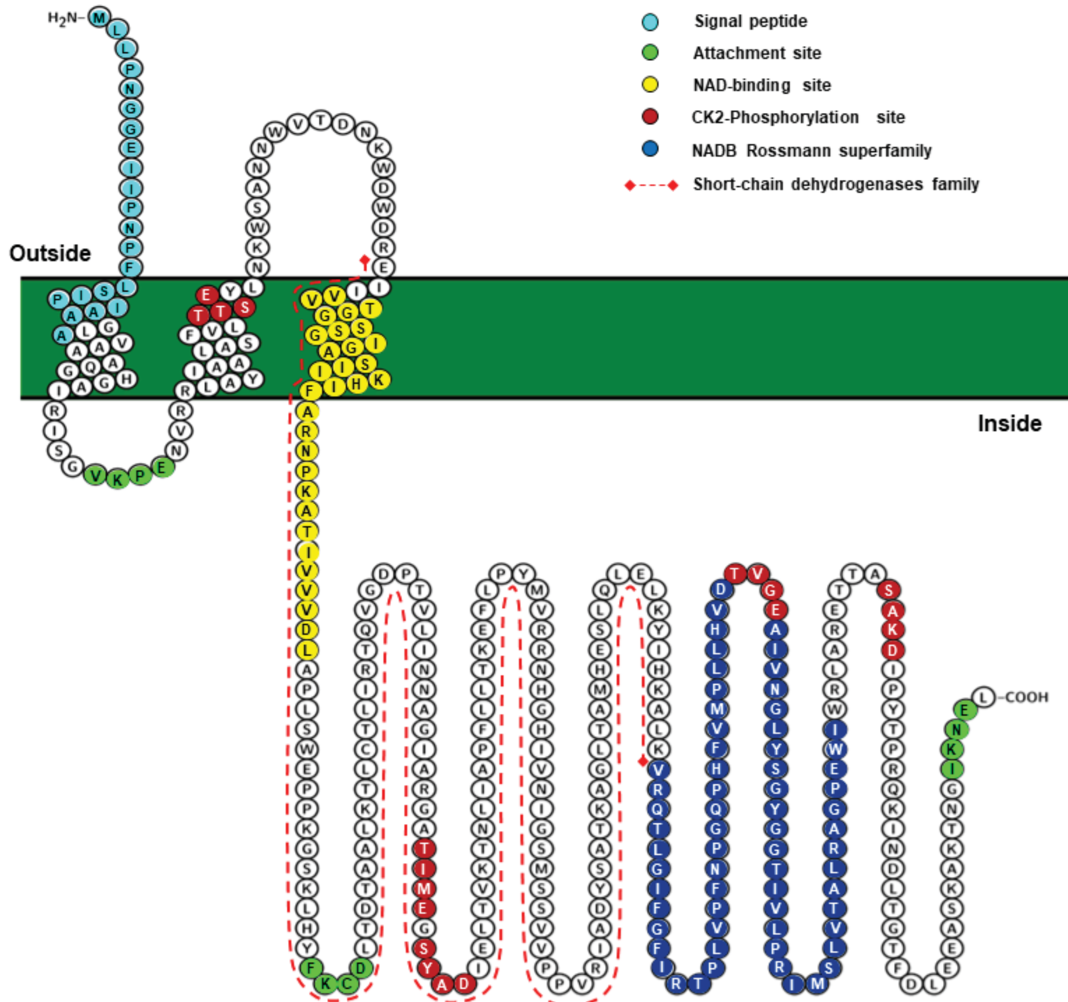


FIGURE 3. Visualization of transmembrane CAS 2 protein based on Protter software.

Biological Importance of CAS2 Protein

Correlate with molecular function predictions, based on motif scan using MyHits (https://myhits.sib.swiss/cgi-bin/motif_scan), CAS2 protein contained motif for enzymes activity such as short-chain dehydrogenases/reductases family signature, NAD binding site, 3-beta hydroxysteroid dehydrogenase/isomerase family, NAD-dependent epimerase/dehydratase family and Ketoreductase domain (Table 4). CAS2 protein also contain motif for casein kinase II phosphorylation site, N-myristoylation site, and protein kinase C phosphorylation. NAD-dependent epimerase/dehydratase family and short chain dehydrogenase are motif with longest amino acid sequences located inside of membrane. Amino acid sequence containing motif for NAD-binding site is partly located in the transmembrane and the other sequences in inside of membrane (Fig. 3). Short-chain dehydrogenases/reductases (SDRs) are very large family of enzymes and great functional diversity [31]. There are two common type families of this enzymes and are called 'classical' and 'extended' families. NAD-dependent epimerase/dehydratase is extended type of SDR characterized by conserved catalytic tetrad (N-S-Y-K) and cofactor binding site (GxxxGxG) [32] with having common α/β-folding pattern, and characterized by presence of a central β-sheet typical to Rossmann-fold with helices on either side [33]. Proteins in this family bind with NAD/NADP as cofactor using the conserved Rossmann-fold domain in the N-termini, while their C-terminal domains bind diverse substrates such as sugars, alcohols, steroids, aromatic compounds and xenobiotics [34].

TABLE 4. Motif amino acid sequence prediction of CAS2.

Amino acid position	Motif information
59–62	Casein kinase II phosphorylation site
170–173	Casein kinase II phosphorylation site
175–178	Casein kinase II phosphorylation site
287–290	Casein kinase II phosphorylation site
333–336	Casein kinase II phosphorylation site
22–27	N-myristoylation site
89–94	N-myristoylation site
164–169	N-myristoylation site
213–218	N-myristoylation site
295–300	N-myristoylation site
182–184	Protein kinase C phosphorylation site
333–335	Protein kinase C phosphorylation site
340–342	Protein kinase C phosphorylation site
214–242	Short-chain dehydrogenases/reductases family signature
366–369	Endoplasmic reticulum targeting sequence
86–143	3-beta hydroxysteroid dehydrogenase/isomerase family
86–152	Ketoreductase domain
83–246	Short chain dehydrogenase
85–307	NAD-dependent epimerase/dehydratase family
209–226	Bacterial transferase hexapeptide (three repeats)
201–214	Phage tail fibre repeat

According to Islam *et al*. (2019), NAD-dependent epimerase/dehydratase plays vital rule in cell surface properties, exoenzyme production, and virulence in *Pectobacterium carotovorum*. The deletion mutant of *WcaG* gene encodes NAD-dependent epimerase/dehydratase, a homologue of GDP-fucose synthetase, caused *P. carotovorum* to have more hydrophobic cell surfaces, formed more biofilm on abiotic surfaces, and were ultimately impaired in host tissue maceration [35]. In *Mycobacterium tuberculosis*, *HadDMtb* gene annotated as encoding a NAD-dependent epimerase/dehydratase family protein has pivotal role for ketomycolic acid biosynthesis and virulence [36]. The role of NAD-dependent epimerase/dehydratase family did not well characterized yet in plant pathogenic fungi. The SDR family that has been deeply characterized in fungi is 1,3,6,8-tetrahydroxynaphthalene reductase mediating the naphthol reduction reactions in melanin biosynthetic pathway [37].

NAD binding sites of CAS2 locating on the transmembrane and within the membrane (Fig. 3) indicated that CAS2 may be involved in the transport of electrons (NAD^+) within oxidation-reduction reactions (redox) as well as being a highly important component of cellular signalling. NAD^+ plays a significant role in the reactions associated

with glycolysis, oxidative phosphorylation, fermentation, Ca_2^+ signaling, chromatin structure, DNA repair and life span [38]. The first NAD^+ transporter, ScNDT, was identified in the mitochondrial envelope of bakers' yeast exhibiting a high affinity for NAD^+ [39]. Then, Palmieri *et al.* [40] reported that the gene products of YIL006W and YEL006W, namely AtNdt1p and AtNdt2p, respectively, are two isoforms of the mitochondrial NAD transporter (ScNDT) in *S. cerevisiae*. So far, there are no reports of NAD^+ transporter in plant pathogenic fungi. CAS2 also have no homology to ScNDT and AtNDT.

Tertiary Structure of CAS2 Protein

The amino acid sequence of CAS2 was submitted in LOMETS for the template selection for homology modeling. The LOMETS gave 100% estimated precision for oxidoreductase (PDB ID: c6k8sA). The final model was selected and was visualized using EzMol software (Fig. 4a). The model was further validated by Ramachandran plot which concluded that 64.1% of amino acids were in favored and 35,9% were outliers suggesting moderate degree of the stability of the predicted protein structure (Fig. 5). The 'favorable region' is that region where the amino acid residues mostly reside [41]. The amino acid residues are sometimes found in the outside of the 'favorable region', that are also permitted for the stable protein structure, known as 'allowed region'; and rest regions in the Ramachandran plot is known as 'outlier region'. A model having more than 90% residues in favorable region is considered as good quality model [42].

Binding sites of protein in the 3D model of CAS2 was predicted by using Q-site finder. The residues identified in the burial cavity of protein are G89, S91, S92, G93, I94, V112, V113, V114, D115, C134, D135, L136, N162, A163, G164, I165, T185, G213, S214, Y227, K231, and R308 revealing a deep cleft as shown in Fig. 4b. Those amino acid residues indicated the hydrophobic nature of the binding site of the CAS2 protein. This emphasized that protein binding site geometry might influence the NAD-CAS2 interaction and may play important role in electron transfer.

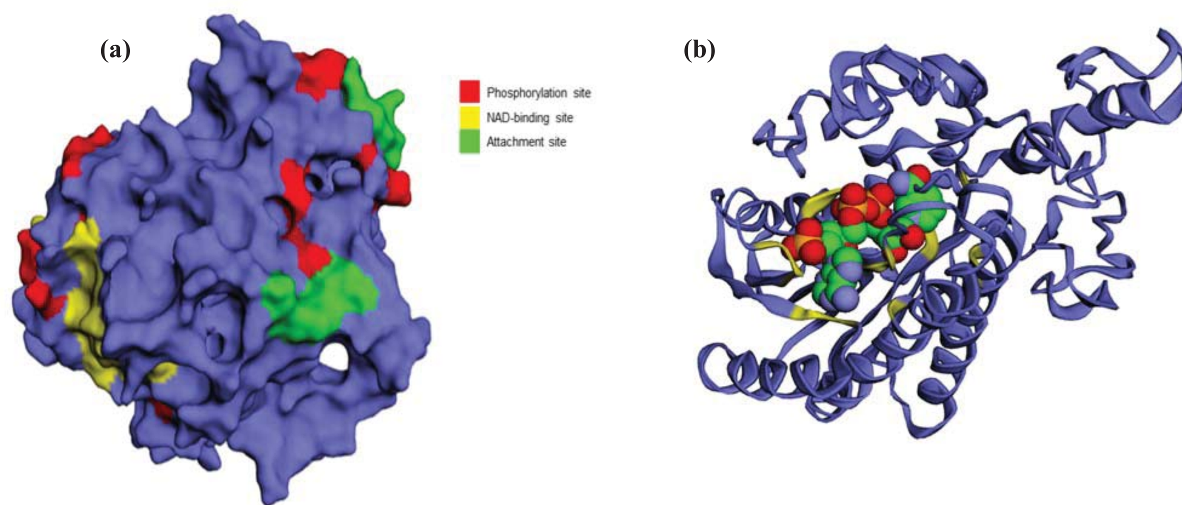
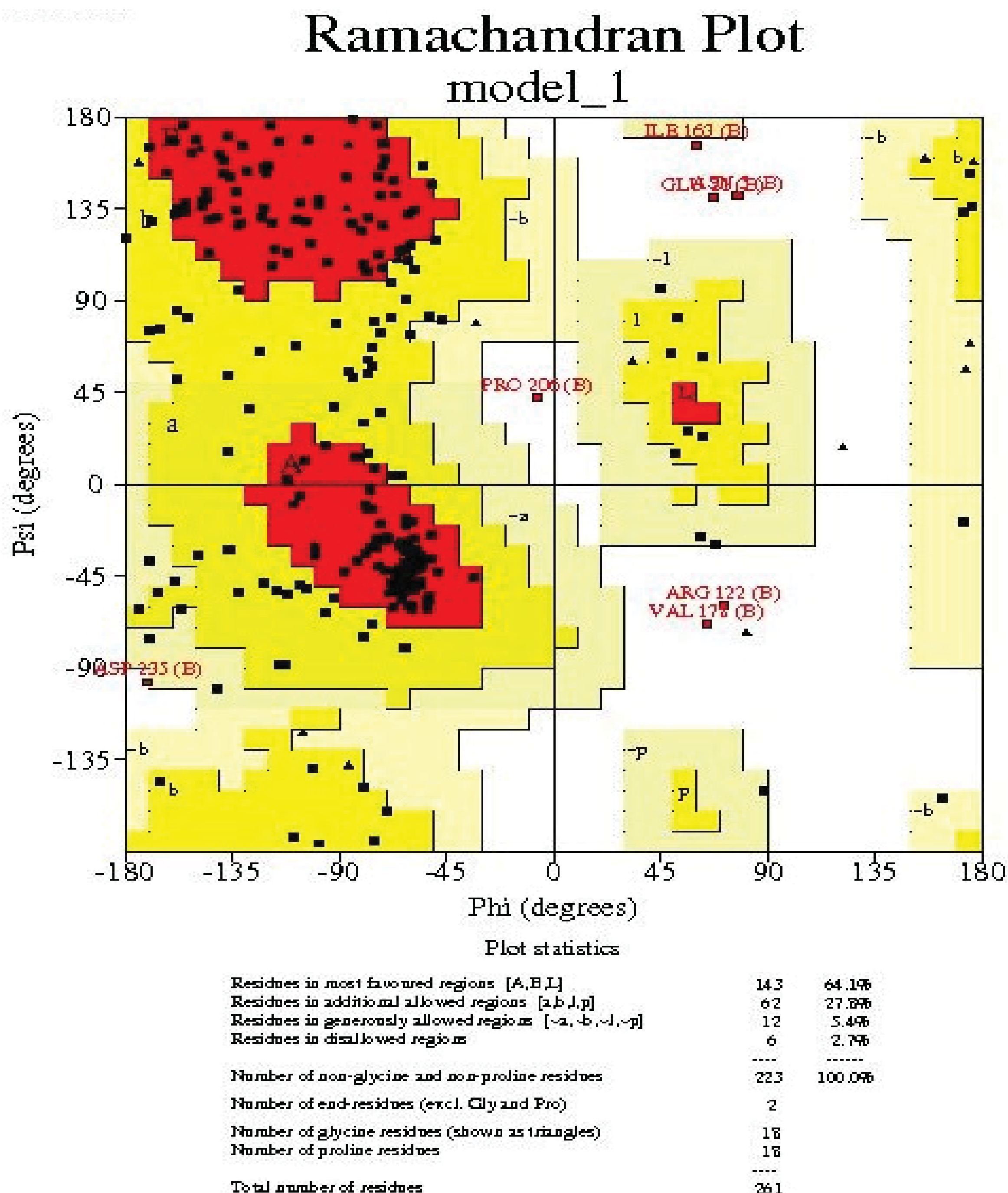


FIGURE 4. Homology model of CAS2 protein by LOMETS (a) and identified NAD^+ binding site was visualized by EzMol (b).

Protein-Protein Interaction Network of CAS2 Protein

Protein interaction network analyzed by STRING web server revealed 10 potential interacting protein associates (Fig. 6) based on various network parameters like text mining, gene fusion, co-occurrence, co-expression, neighborhood, and databases. A node indicates a protein while as a connecting edge represents their interaction. The closest interacting protein having the shortest node was found oxidoreductase YKL107W (putative short-chain dehydrogenase/reductase) while the distant interacting protein was oxidoreductase YGL039W (aldehyde reductase) and tRNA methyltransferase (methylates the N-1 position of guanine at position 9 in tRNAs). Potential interacting protein associates with CAS2 protein are listed in Fig. 7. CAS2 closely interacted with YKL107W encodes a novel aldehyde reductase for detoxification of acetaldehyde, glycolaldehyde, and furfural to prevent the yeast cells

(*S. cerevisiae*) from damage caused by furfural [43]. In *Magnaporthe oryzae*, four family of aldehyde dehydrogenases (MoMSDH, MoBADH1, MoKDCDH, and MoP5CDH) influence on the morphological and infectious development [44]. However, the contributions of CAS2 in interaction with aldehyde dehydrogenases are not yet understood.



Residues in most favoured regions [A,B,L]	143	64.1%
Residues in additional allowed regions [a,b,l,p]	62	27.8%
Residues in generously allowed regions [~a,~b,~l,~p]	12	5.4%
Residues in disallowed regions	6	2.7%
	----	------
Number of non-glycine and non-proline residues	223	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	18	
Number of proline residues	18	

Total number of residues	261	

FIGURE 5. Structure validation by Ramachandran plot depicts the general as well as specific distribution of amino acids.

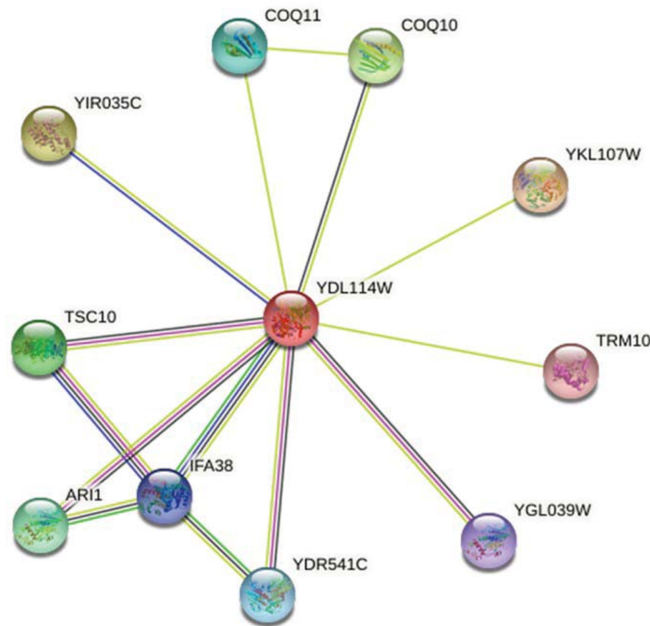


FIGURE 6. Protein-protein interaction map of CAS2 protein by STRING web server. The closest interacting protein having the shortest node was found oxidoreductase YKL107W (putative short-chain dehydrogenase/reductase), while the distant interacting protein was oxidoreductase YGL039W (aldehyde reductase) and tRNA methyltransferase (methylates the N-1 position of guanine at position 9 in tRNAs).

Nodes:

Network nodes represent proteins

splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Color

colored nodes: query proteins and first shell of interactors

white nodes: second shell of interactors

Node Content

empty nodes: proteins of unknown 3D structure

filled nodes: some 3D structure is known or predicted

Edges:

Edges represent protein-protein associations

associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

Known Interactions

from curated databases

experimentally determined

Predicted Interactions

gene neighborhood

gene fusions

gene co-occurence

Others

textmining

co-expression

protein homology

Your Input:

YDL114W *Uncharacterized oxidoreductase YDL114W; Putative short-chain dehydrogenase/reductase; YDL114W is not an essential gene; Belongs to the short-chain dehydrogenases/reductases (SDR) family (308 aa)*

Predicted Functional Partners:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	(Homology)	Score
YKL107W	*Uncharacterized oxidoreductase YKL107W; Putative short-chain dehydrogenase/reductase; proposed to be a palmitoylate...*							•		0.960
YIR035C	*Uncharacterized oxidoreductase YIR035C; Putative cytoplasmic short-chain dehydrogenase/reductase*			•				•		0.947
COQ10	*Coenzyme Q-binding protein COQ10, mitochondrial; Coenzyme Q (ubiquinone) binding protein; functions in the delivery of ...*							•		0.947
TSC10	*3-ketodihydrosphingosine reductase TSC10; 3-ketosphinganine reductase; catalyzes the second step in phytosphingosine ...*					•		•		0.934
ARI1	*NADPH-dependent aldehyde reductase; utilizes aromatic and alophatic aldehyde substrates; member of the short-chain d...*							•		0.925
COQ11	*MIOREX complex component 2; Putative oxidoreductase, subunit of Coenzyme Q biosynthetic complexes; required for syn...*							•		0.896
YDR541C	*Putative uncharacterized oxidoreductase YDR541C; Aldehyde reductase; substrates include both aromatic and aliphatic al...*							•		0.886
IFA38	*Very-long-chain 3-oxoacyl-CoA reductase; Microsomal beta-keto-reductase; contains oleate response element (ORE) sequ...*			•				•		0.885
YGL039W	*Putative uncharacterized oxidoreductase YGL039W; Aldehyde reductase; reduces aliphatic aldehyde substrates using NA...*							•		0.775
TRM10	*tRNA methyltransferase; methylates the N-1 position of guanine at position 9 in tRNAs; protein abundance increases in res...*							•		0.694

FIGURE 7. The screenshot from STRING server of predicting interacting proteins with the query sequence. Top 10 interacting partners have been displayed.

CONCLUSION

In conclusion, CAS2 is hydrophobic protein localizing in transmembrane of mitochondria and is involved in catalytic activity of oxidation-reduction process. This protein showed high homology with short-chain dehydrogenase RED2 (RNA-editing deaminase) containing motif for NAD binding site and NAD-dependent epimerase/dehydratase family. Genes encode a NAD-dependent epimerase/dehydratase family protein has pivotal role for ketomycolic acid biosynthesis and virulence in fungal pathogen. This result is fundamental understanding of *CAS2* gene for their crucial role in pathogenesis and the identification of novel classes of control targets.

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