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Active Sites Determination of Enzyme 1-aminocyclopropane-1-carboxylic acid synthase 2 (ACS2) of *Capsicum chinense* using Modeling and In Silico Docking

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Abstract
The growth of Chili pepper (*Capsicum chinense* Jacquin.) is affected by biotic and abiotic stresses. Abiotic stress such as waterlogging increases the expression of ACS (*1-aminocyclopropane-1-carboxylic acid synthase*) enzyme. This enzyme plays a great role in the process of ethylene biosynthesis and encoded by multiple genes. Waterlogging causes hypoxia condition. One of ACS enzymes that responds to hypoxia condition is ACS2. The different respond to hypoxia stress among plant was assumed to be caused by a different structure of the ACS2 enzyme. This study aimed to identify and confirm the active site of *Capsicum chinense* Jacquin ACS2 using modeling and in silico docking. The result of the three-dimensional (3D) structure modeling showed 91% similarity of *Capsicum chinense* Jacquin ACS2 with the structure of the tomato (*Solanum lycopersicum* L.). The *Capsicum chinense* ACS2 confirmed five active sites that bind to the substrates asparagin396B, valine397B, thyrosine152B, threonine128B, and thyrosine92A.

1. Introduction

Chili, an important spice and vegetable which is very much sensitive to waterlogging condition (Molla *et al*., 2022). During waterlogging, the diffusion of gases between plant cells and the outside environment is restricted, causing hypoxic conditions that affect physiological processes such as photosynthesis and respiration (Khan *et al*., 2024). In complete submergence, ethylene synthesis increases and is entrapped in plant tissues (Khan *et al*., 2020). The waterlogging condition makes the lack of oxygen or hypoxia (Ou *et al*., 2011). The chili pepper responses to waterlogging such as ethylene biosynthesis have been reported. Biosynthesis ethylene is a process to produce ethylene hormone. Ethylene is a plant hormone that plays a great role in the growth and development of plants (Lincoln Taiz and Eduardo Zeiger, 2002).

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Ethylene is an essential plant hormone, critical in various physiological processes. These processes include seed germination, leaf senescence, fruit ripening, and the plants response to environmental stressors (Khan *et al*., 2024). Ethylene biosynthesis is performed through the activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACO (ACC oxidase) in plants, the spermidine and spermine biosynthesis through the activity of the SAM decarboxylase, the synthesis of nicotinamine through the activity of nicotinamine synthase and the biotin biosynthesis through the activity of 7,8-diaminopelargonic acid aminotransferase (Roeder *et al*., 2009). Ethylene biosynthesis is tightly regulated by two key enzymes, namely 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO) (Khan *et al*., 2024). Wang *et al*. (2023) reported the regulation of ethylene biosynthesis through WRKY29, which transactivates the expression of ACS and ACO and brings about a pleiotropic effect on plant growth and development.

ACS enzyme plays a key role not only responds to biotic stress but also abiotic stress such as injury, drought, waterlogging & flooding (hypoxia) (Argueso *et al*., 2007). Under stress conditions, elevated levels of 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS) stimulate the production of increased amounts of the substrate 1-aminocyclopropane-1-carboxylate (ACC), consequently leading to higher ethylene synthesis within plant tissues (Pattyn *et al*., 2020). In response to biotic and abiotic stresses, the enzyme of ACS will be expressed in the high level immediately after the stress happened. The expression of ethylene related genes (such as ACO1, ACO2, ACS1, and ACS2) were found to be up-regulated in WT line soybean cultivar under waterlogging stress conditions (Sharmin *et al*., 2024). Previous research has indicated that the upregulation of ACS genes increases the synthesis of defensive proteins, paving the way for ACC production followed by ethylene (Eun *et al*., 2019). This enzyme of ACS encoded not only by a single gene but encoded by multiple genes as reported Peng *et al*. (2005) on *Arabidopsis* plant, this enzyme was encoded by 12 genes. Out of ACS genes in Arabidopsis, ACS3 is a pseudogene, whereas ACS10 and ACS12 are Asp, Phe, and Tyr aminotransferases, not ACSs, forming a putative aminotransferase clade (Yamagami *et al*., 2003). ACS is a multiple-gene-encoding polypeptide that varies from species to species (Khan *et al*., 2024).

In the activity to convert SAM become ACC, this enzyme requires PLP (*pyrodoxal-5-phosphate*) as a cofactor (Argueso *et al*., 2007). A monomeric ACS may also be catalytically active (Huai *et al*., 2001). The ACS protein is located in the cytosol. It is an enzyme that depends on PLP and is evolutionarily related to the aminotransferase superfamily. It also requires pyridoxal as a cofactor (Xu *et al*., 2021). The homo- and heterodimerization of the ACS isoform influence enzyme activity and stability. Studies have revealed that the eight functional proteins of Arabidopsis have the potential to form up to 45 different combinations of homodimers or heretodimers (Khan *et al*.,

2024). However, due to structural constraints, only 25 of these combinations are functional and capable of forming active sites (Park *et al*., 2021).

Active sites ACS enzyme that binding to SAM has been examined Yip *et al*. (1990) using C^{14}-*AdoMet*. Study of Capitani *et al*. (1999) reported that active site of an apple of this enzyme there are Arg281, Arg497, Asn202, Asp230, Lys273, Ser270, Thr121, Tyr233, and Tyr85 that binding to SAM. These amino acids serve as the enzymes homodimer interface and are crucial for PLP binding (Capitani *et al*., 1999). Many techniques have been used to determine active site, one of them is docking in silico. *Docking* is binding between the ligand with a specific cavity of protein (Ahmed *et al*., 2007). Objectives of this study to identify active sites of an ACS2 enzyme of chili pepper (*Capsicum chinense* Jacquin.) using modeling and docking in silico.

2. Method

2.1. Materials and Methods

Sequence of *acs2* gene of *Capsicum chinense* with accession GenBank: AB434927.1. was collected from NCBI *database* (https://www.ncbi.nlm.nih.gov/ nuccore/186200776). The sequence was used as a gene template. Protein from this gene later was confirmed from UniProt database (www.uniprot.org/*)* for the availability of the 3D structure of enzyme ACS2. If the 3D structure is not available in the database, then continued to conduct online modeling of the 3D structure using Swiss model (https://swissmodel. expasy.org/).

Substrate S-Adenosyl-L-methionine (SAM) was obtained from PubChem database (https://pubchem.ncbi.nlm.nih. gov/). Structure of 3-D of enzyme ACS2 and SAM then were analyzed using docking technique approach. The docking was performed using Pyrx (autodox vina) (Trott and Olson, 2010) and Phymol software to bind between the substrate and the enzyme.

3. Result and Discussion

Acs2 gene sequence of Capsicum *chinense* from NCBI (Genbank AB434927.1) encoded a protein that 100% similar to the protein sequence of *Capsicum chinense* from Uniprot database (B2NIX2). Modeling of the 3D structure using online Swiss model program obtained a sequence with similarity about 91,59% and resolution 2.7 Å. The 3D structure is similar to ACS2 complex-ligand PLP of tomato (*Solanum lycopersicum) with* PDB identification: 1iay.1 (Huai *et al*., 2001). Sequence similarity of the amino acid model between *Capsicum chinense* and *Solanum lycopersicum* start at position 11-439 of amino acid (Figure 1). According to Claverie and Notredame (2006), modeling in silico based on highest similarity (%), highest score and low of *E-value*. Sanchez and Šali (1997) reported that good sequence similarity for modeling in the study of docking in silico more than 30%. In this study, sequence similarity was 91,59% that proper 3D structure that can be used to advanced step.

Figure 1. Modeling of amino acid sequence between Capsicum *chinense* and *Solanum lycopersicum* with sequence similarity about 91%.

The structure of 3D consists of the conserved region and a variable region, the conserved region is the part that consists of helix and sheet at protein. The presence of outlier at conserved region results in alteration of structure and function of the protein (Lukitaningsih *et al*., 2015).

Three-Dimensional structure of ACS2 was docked with S-*adenosylmethionine* (*Adomet*) obtained *binding affinity* energy -8,3 kcal/mol and resulted in active sites between ligand SAM and ACS2 with 5 positions (Table 1 and Figure 2).

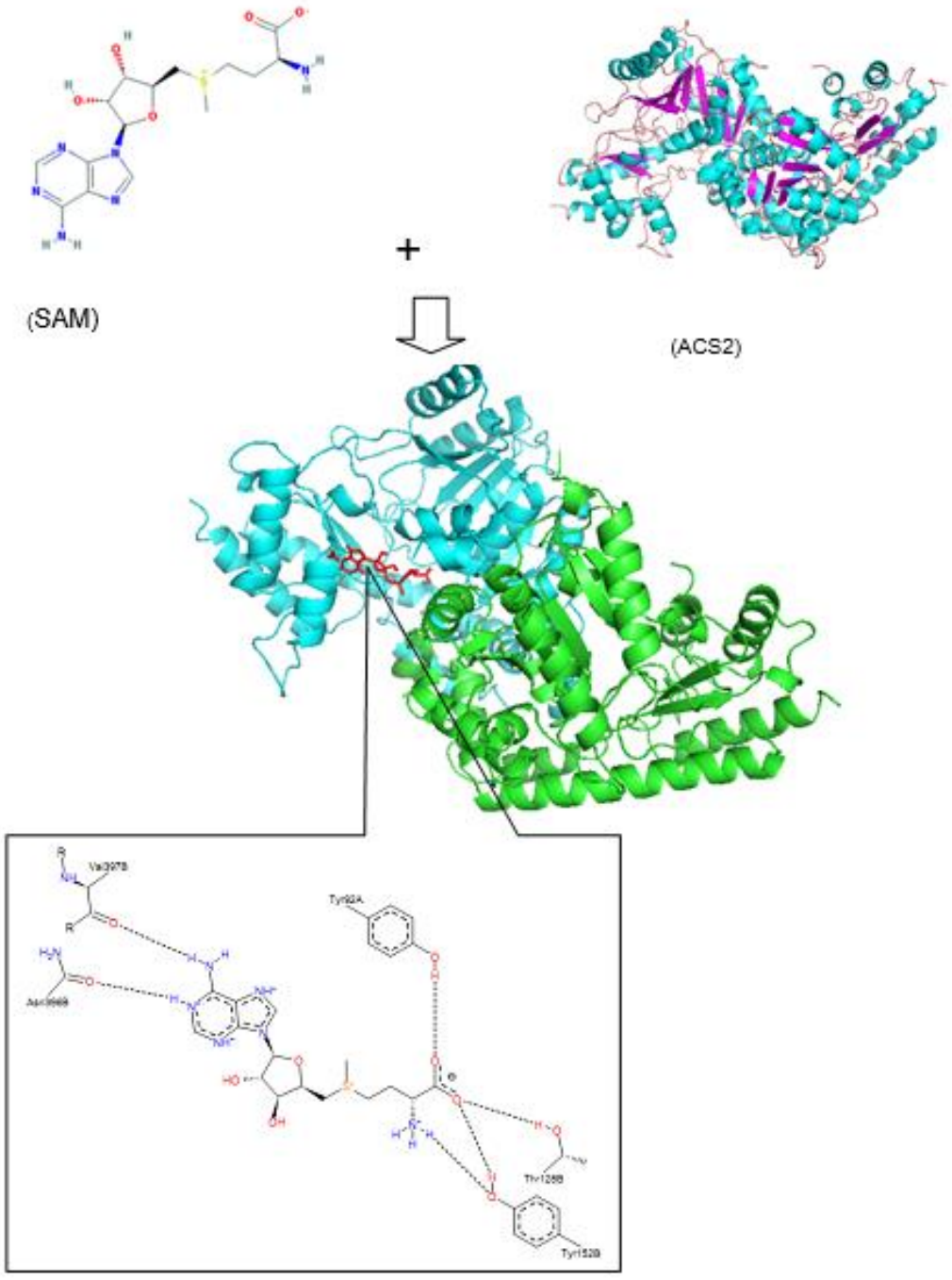

Figure 2. Binding interaction between SAM and ACS2, all showed the hydrogen binding. Asparagine position 396 chain B, valine 397 chain B, thyrosine 152 chain B, threonine 128 chain B and thyrosine 92 chain A. (Chain B=cyan, chain A=green, SAM=red). SAM taken from Pubchem database (Pubchem, 2016).

Table 1. Binding region of active sites ACS2 with SAM

No.	Amino acid	Position	Chain	Binding

1	Thyrosine	92	A	Hydrogen
2	Threonine	128	B	Hydrogen
3	Thyrosine	152	B	Hydrogen
4	Asparagin	396	B	Hydrogen
5	Valine	397	B	Hydrogen

Interaction binding enzyme of ACS2 with SAM of chili pepper (*Capsicum chinense)* or tomato (*Solanum lycopersicum*) consisted of hydrogen binding which is valine397B residue and asparagin396B residue role as acceptor hydrogen but thyrosine92A as a donor. Hydrogen binding provided stability of the binding interaction. According to Kuchel Philip and Ralston, (2002) hydrogen binding give stability contribution of protein about 2 kJ mol^{-1} until 7,5 kJ mol^{-1}. According to Pace *et al*. (2014), hydrogen bond on folding contributes 1.1 ± 0.8 kcal/mol to protein stability. In this study suggested that modeling using tomato can be applied to *Capsicum chinense* due to high similarity of a sequence about 91%. In this study was proposed that active sites ACS apple that is reported Capitani *et al*. (1999) probably different kind of enzyme of ACS that compared ACS2 chili pepper or tomato because this enzyme encoded not only by single genes but multiple genes as reported by (Peng *et al*., 2005).

4. Conclusion

In this study conclude that modeling docking in silico enzyme of ACS2 of *Capsicum chinense* can be done using a sequence from Tomato with similarity about 91%. In this study determine 5 active sites of the enzyme ACS2 that is asparagin396B, thyrosine152B, threonine128B, valine397B, and thyrosine92A.

Author Contributions

Muhammad Rizza Pahlevi: Conceptualization, Methodology, Software and Data curation, Writing- Original draft preparation. **Eunike Sri Puspaningsih**: Visualization, Investigation. **Eka Sudibya** and**, Juniarti Wulan Lestari**: Supervision. **Prayitno Ribut Suwasono**: Software, Validation. **Hani Widhianata**: Writing- Reviewing and Editing.

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Declaration of Conflicting Interests

None

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