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PREDICTING THE CLEAVAGE SITES OF MULTIPLE PROTEASES FAMILIES ON RICE ALPHA AMYLASE ISOZYME 3D SEQUENCE

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Abstract. Proteases is a proteolytic enzyme that often determined the crucial process in degradation pathway occurred in all of organisms. Prediction of novel protease is important action to design the protease inhibitor. In the secretion of rice αAmy3 protein in outside cells will be followed by secretion of recombinant protein target and proteolytic enzymes together, which means potentially also degraded the recombinant protein target In this study, the proteases was detected in rice αAMY 3D protein sequences. Our study resulted the 3 major proteases appeared in rice αAMY 3D protein sequences, they were cysteine proteases, serine proteases and metalloproteases. Based on the literature, such proteases also appeared in rice suspension cells. Design the inhibitor for such proteases will be suggested for reduction of proteases level.
Keywords: proteases, in silico, PROSPER, rice cells.

INTRODUCTION

Proteases is the machinery enzyme which able to degrade protein in all of organisms. Many of proteases have their own biochemical and regulatory characteristics. Proteolytic enzymes, or proteases, role as overall metabolic controller and key point in transduction pathways by directing the activation or hydrolysis of proteins implicated in regulatory process, or by contributing to the eliminate the misfolded proteins and the selective recycling of amino acids from short-lived proteins (Mandal et al. 2016). In plant, as metabolic controller, they plays important role during development stage and stress condition. The finding of proteases for the initiation and completion of animal apoptosis, and caspase enzymatic activities in plant cell extracts, is linked the plant proteases to program cell death (Cai and Gallois 2015). Therefore, the proteases study in plant gain interest, due to it will affect the yield reduction in plant production and the diversity in the nature.

Proteolysis degradation is one of the challenges for optimized accumulation of recombinant proteins in the plant cell system. The reduction of recombinant protein levels in plant cell cultures has been described as a proteases effect and affect the plant metabolic pathway (Yusibov et al. 2016). Other candidate biopharmaceuticals, such as, human serum albumin (Sun et al. 2011) and cytokines (Sirko et al. 2011) also showed the proteolytic processing when they are produced in plant cells. The identification of proteases that are responsible for the degradation of a given recombinant protein is the new challenges, due to the difficulties to find the proteolytic enzymes candidate among several hundred proteolytic enzymes in plant (Simova-Stoilova et al. 2010). Identification of unknown proteases degraded the recombinant protein targeted become important issue, in order to tackle the reduction of recombinant protein yield.

The finding of several recombinant protein degradations in its host, led many people to find the specific type of proteases, in order to know the best treatment for prevent or reduce the proteases level in the host. There are many methods applied to identify the proteases in plant cells, including mass spectrometry technique (Giansanti et al. 2016) and *in silico* methods (Song et al. 2012). *in silico* method has known as a useful alternative approach to provide good insights into complex proteases interaction. Besides, computational tools will reduce the cost in experiment for identify the proteases. Therefore, in this study, the bioinformatics approaches will be applied in order to find

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the candidate of proteolytic enzymes in plant. The aim of this study was to predict the proteases appeared in α-amylase (αAMY) 3D sequences, one of common protein secreted along with recombinant protein in rice suspension cell systems.

METHOD

Data Collection

The rice αAMY 3D protein sequences was obtained from Uniprot (entry name: AMY3D_ORYSJ and the accession number: P27933). The total 38 kDa sequences of rice αAMY 3D showed in Figure 1.

Proteases Prediction

The proteases prediction conducted by using PROSPER (Song et al. 2012). PROSPER is a prediction tool for *in silico* prediction of protease substrates and their cleavage sites based on the webserver, for twenty-four different protease types, covering four major protease families-Aspartic (A), Cysteine (C), Metallo (M) and Serine (S). The protein sequence of rice αAMY 3D was input in the webserver. The notification of prediction results will be sent to email soon after the protein sequences insertion to webserver. The computer specification to run this software are Intel Celeron CPU N3050 @ 1.60GHz, RAM 2 GB, 64-bit Operating System and x64-based processor.

Data Analysis

The characterization of predicted proteases data was conducted in cleavage sites of multiple protease families. The data was then analyzed by descriptive analysis, and compared with the literature available from scientific journal using google, a search engine.

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        10         20         30         40         50
MKNTSSLCLL LLVVLCSLTC NSGQAQVLFQ GFNWESWKQQ GGWYNMLKGQ
        60         70         80         90        100
VDDIAKAGVT HVWLPPPSHS VAPQGYMPGR LYDLDASKYG TAAELKSLIA
       110        120        130        140        150
AFHGKGVQCV ADVVINHRCA EKKDARGVYC VFEGGTPDDR LDWGPGMICS
       160        170        180        190        200
DDTQYSDGTG HRDTGEGFGA APDIDHLNPR VQRELTDWLN WLKSDVGFDG
       210        220        230        240        250
WRLDFAKGYS TDIAKMYVES CKPGFVVAEI WNSLSYNGDG KPAANQDQGR
       260        270        280        290        300
QELVNWVNAV GGPAMTFDFT TKGLLQAGVQ GELWRLRDGN GKAAGMIGWL
       310        320        330        340        350
PEKAVTFVDN HDTGSTQKLW PFPSDKVMQG YAYILTHPGV PCIFYDHMFD
       360        370        380        390        400
WNLKQEITAL AAIRERNGIN AGSKLRIVVA DADAYVAVVD EKVMVKIGTR
       410        420        430
YDVGNAVPSD FHQTVHGKDY SVWEKGSLRV PAGRHL
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Figure 1. The protein sequences of rice αAMY 3D

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RESULTS AND DISCUSSION

The current major rice suspension system uses a highly inducible α-Amylase promoter to control protein expression. The αAmy3 promoter gene was characterized as the more responsive promoter to sugar starvation (Hong et al. 2012). However, the sugar starvation condition often resulted in secreted of proteolytic enzymes (Kim et al. 2008). The physical changes also can occurred in certain condition in the plant (Marpaung et al. 2019). The secretion of αAmy3 protein in outside cells will be followed by secretion of recombinant protein target and proteolytic enzymes together. In other words, there are several proteases targeting to αAmy3 protein will also appeared in the medium together with recombinant protein target, which possibly to degrade the recombinant protein. Identification of type of proteases targeting to αAmy3 protein will allow knowing any possible proteases will attack the recombinant protein target in the medium of rice suspension cells. Therefore, understanding the type of proteases will allow the best treatment for yield reduction in rice suspension cells.

In this study, the proteases cleavage site was predicted using PROSPER in the 38 KDa sequences. Based on the output, there are several proteases cleavage the αAMY 3D sites (Fig 2.). There are 82 total predicted proteases will appeared in αAMY 3D sequences, among the superfamilies proteases. The serine proteases (38 unit) were the most appeared in the αAMY 3D sequences (Fig 3), followed by metalloproteases (31 unit) and cysteine proteases (7 unit) (Table 1). The studies on serine protease inhibitors for inhibition the chymotrypsin and trypsin-like proteases become focused recently, due to the majority of protease activity in plant cells was from serine proteases (Pillay et al. 2014). Serine inhibitor has been used to tackle the unwanted serine proteases in transformed rice cell suspension cultures (Kim et al. 2007). In that study, overexpressing the chymotrypsin and trypsin inhibitor, a type of serine proteases inhibitor, resulted on reduction of protease activity approximately 23% compare to non-transformed cells. The metalloproteases also become concern in plant research. The matrix metalloproteinases in tomato has been known as cell death controller (Zimmermann et al. 2016).

The proteases activities in rice suspension cultured cells in medium during sucrose starvation has been characterized, and found the metalloprotease is one of protease secreted to the rice medium (Ho et al. 2008). Meanwhile, the major of extracellular protein secreted to medium was cysteine protease via sucrose starvation (Kim et al. 2008). Protease inhibitor cocktail and overexpression of proteases inhibitor could be the solution in order to reduce the unwanted proteases in rice suspension cells.

Table 1. Comparison Between Predicted Proteases and Experimental Identified in αAmy3D sequences

Type of Predicted Protease	Prediction Cleavage Sites (unit)	Experiment on Proteases Identification	Refference
Cysteine protease	7	Cysteine and Endopeptidase	Kim et al., 2008
Metalloprotease	31	60-70 KDa and 110 KDa Metallo Proteases	Ho et al., 2008
Serine protease	38	Chemotrypsin and Trypsin	Kim et al., 2007

CONCLUSION

Designing novel inhibitor as controller for regulate the protease function, prediction of putative protease substrates is a critical action. In this study, the 3 major proteases, including cysteine protease, serine protease and metalloprotease have been predicted appeared on αAmy3D protein sequences.

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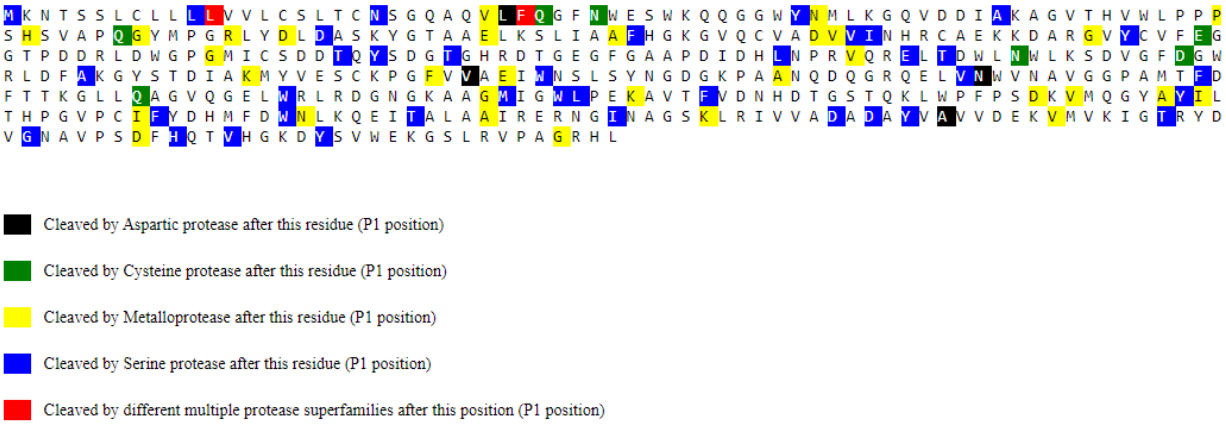

Figure 2. Predicted cleavage sites of protease in αAMY 3D sequences

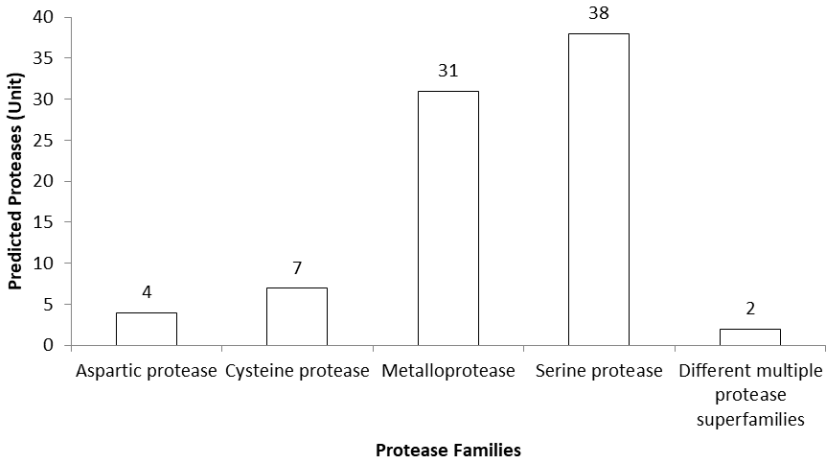

Figure 3. Comparison the amount of proteases appeared in αAMY 3D sequences

The secreted αAmy3D protein in rice suspension cells medium possibly bring such proteases, and able to degrade the recombinant protein target. Our study suggest for further treatment, such as design the proteases inhibitor, either in cocktail form or expressing the gene inside the cells.

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