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Purification Crude Extracellular Protease of *Saccharomycopsis fibuligera* strain R64 Isolated from *Tape'* Indonesian Fermented Food

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Abstract: The research aims to purify crude extracellular protease produced by *Saccharomycopsis fibuligera* strain R64 that isolated from *tape*' Indonesian fermented food. *Saccharomycopsis fibuligera* strain R64 was tested on Malt Extract Agar (MEA) that added 3% skim milk powder to discover the proteolytic activity. Extracellular protease purified with ammonium sulfate precipitation, dialysis and gel filtration chromatography with Sephadex G-100. Bradford method used to measure protein content, protease activity measured with Walter method and SDS-PAGE used to measure the molecule weight of protease. Results shown that isolated yeasts give 2-5 mm of clear zones on MEA that added with skim milk powder. Precipitations with 45% ammonium sulfate give the best protein content of 5.05 mg/mL and protease activity of 0.051 U/mL. After dialysis the protein content the protease activity were increased to 5.57 mg/mL and 0.0631 U/mL. Moreover, the gel filtration chromatography with sephadex G-100 yields 0.023 U/mL proteolytic activities, 2.1 mg/mL protein contents with 97.4 kD protease molecular weight.

Keywords: Crude extracellular protease, purification, *Saccharomycopsis fibuligera* strain R64, *tape*'

INTRODUCTION

Tape' is the most popular of many kinds Indonesian fermented foods that made from cassava. Dried mixed starter which called *ragi tape*' that naturally contains filamentous fungi, yeast and bacteria intentionally added to make it Sujaya *et al.* (2002). Both simple and complex sugars used by microorganisms that live on the *tape*' as their carbon source especially yeasts (Lewis and Young, 1990).

Number of carbon sources makes *tape*' potential as yeasts habitat. Roostita *et al*. (2011) counted 2×10^6 cfu/g yeasts population which shown antimicrobial activities in *tape*'. Beside antimicrobial activities and specific characteristics such as ethanol and CO_2 that makes refreshing aroma, yeasts also generate proteolytic activity by producing extracellular protease (Roostita and Fleet, 1996). *Saccharomycopsis fibuligera* strain R64 were one of isolated yeast from *tape'* that produced extracellular protease with optimum pH of 5 and temperature of 25°C (Roostita *et al*., 2012).

The extracellular protease produced by yeasts is well known and many people utilized it for their activities. Yeasts extracellular protease has potential in beer and wine stabilization (Ogrydziak, 1993). Proteolytic enzymes have some important role in medicine such as food digestion, protein turnover, blood coagulation, embryonic development and cell division (Reid, 2012). Moreover, the enzymes were an important group in scientific, medical research and biotechnology (Rawlings *et al*., 2009).Enzyme purity will affect the proteolytic activity that needed. Therefore the research was done to purify crude extracellular protease produced by *Saccharo mycosis fibuliger a*s train R64 that isolated from *tape*'.

MATERIALS AND METHODS

Saccharomycopsis fibuligera strain R64 proteolytic activity tested on Malt Extract Agar (MEA) that added 3% skim milk powder. The extracellular proteases produced by *Saccharomycopsis fibuligera* strain R64 isolated by separating cells and the growth media. Extracellular protease isolate purified with ammonium sulfate precipitation, dialysis and gel filtration chromatography. Bradford method used to measure protein content, protease activity measured with Walter method and SDS-PAGE used to measure the molecule weight of protease (Walker, 2002).

RESULTS AND DISCUSSION

Saccharomycopsis fibuligera strain R64 isolated from *tape'* were tested and gave clear zones until 5 mm. Clear zone produced by yeasts proteolytic activities that degrade casein substrate added to MEA (Fig. 1). Although yeast shave internal proteolytic activities, external proteolytic activities generated by yeasts

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Fig. 1: Proteolytic activity of selected yeasts isolated from Cassava *tape*'

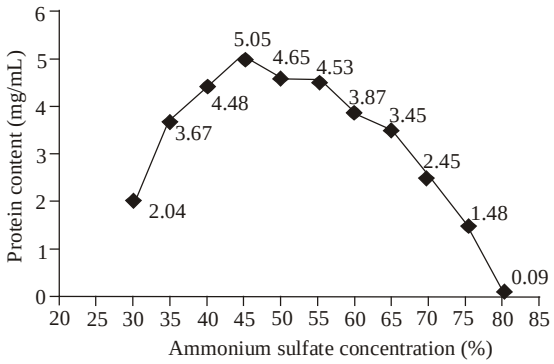

Fig. 2: Protein content of ammonium sulfate precipitation

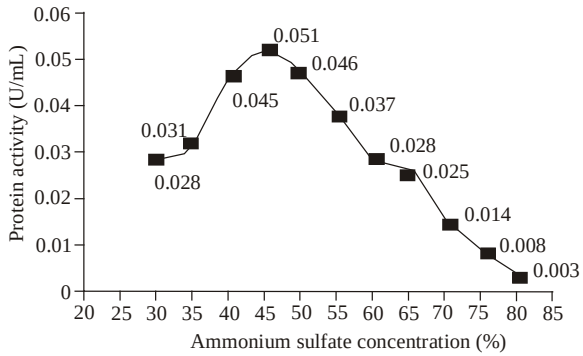

Fig. 3: Proteolytic activity of ammonium sulfate precipitation

extracellular proteases also equally good and more easily determined (Roostita, 2004). Extracellular protease determined from supernatant shown 4.12 mg/ml protein content and 0.049 U/mL proteolytic activities.

Supernatant concentrated using ammonium sulfate to obtain higher purity. Precipitation of ammonium sulfate based on the solubility of proteins that polar interact with the water molecules, ionic interactions of proteins with salt sand equal repel power of proteins. Solubility of proteins at particular pH and temperature increases depend on the increase of salt concentration (salting in) and addition of certain salts will cause

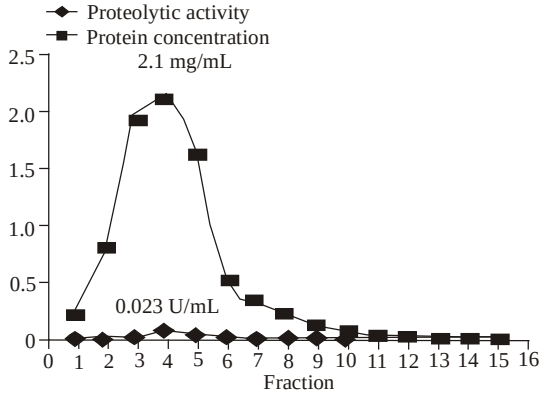

Fig. 4: Proteolytic activity of gel filtration sephadex G-100

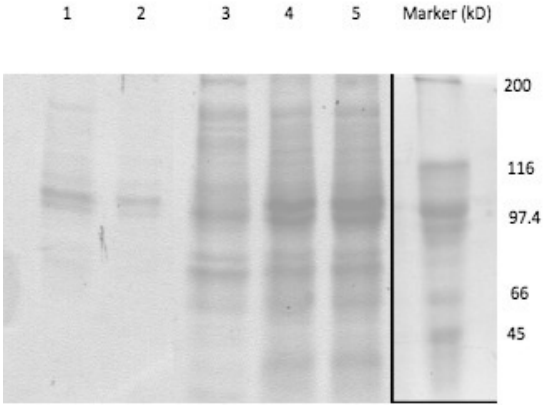

Fig. 5: SDS-PAGE (1 and 2) Fraction 4 Sephadex G-100, (3) Supernatant, (4) Precipitation 45% $(NH_4)_2SO_4$, (5) Dialysis

decreased protein solubility (salting out), more over protein solubility will affect solution ionic strength. Water molecules bonded to the salt ions eventually led to withdrawal of many water sheaths surrounds the surface of the protein and causing the protein to interact, aggregate and then precipitate (Burgess, 2008).

Figure 2 and 3 showed that precipitation of 45% ammonium sulfate gives best proteolytic activity of 0.051 U/mL and protein contents of 5.05 mg/mL. Moreover, 45% ammonium sulfate shown highest protein precipitation. The increase of ammonium sulfate level more than 45% resulting in high saturation, therefore the precipitation and proteolytic activity was low. Excess mineral and ammonium sulfate in crude enzyme removed using dialysis method which was done approximately 60 min to avoid decreased of the proteolytic activity. Dialysis gives proteolytic activity 0.049 U/mL and the protein contents of 4.87 mg/mL.

Electrophoresis aims to separate compounds that are not desired. The results of gel electrophoresis by Sephadex G-100 (Fig. 4) yielded widened protein peaks and shown the highest proteolytic activity on fraction 4 with specific activity of 0.023 U/mL and 2.1 mg/mL protein contents. Purification by Sephadex G-100 provides a level of purity 20.1 times of crude extract with acquisition of 10.20%. The acquisition levels

below 50% presumably caused by proteins that have hyaluronidase activity lower than other proteins excreted by cells. Loss of proteins during purification can occur due to autolysis (Scopes, 1994).

The results of SDS-PAGE (Fig. 5) showed that the Sephadex G-100 (1 and 2) gives clearer bands than supernatant (3), 45% $(NH_4)_2SO_4$ Precipitation (4) and Dialysis (5). The higher level of enzyme protein purity, there action between the enzymeprote in with coomassie brilliant blue would be optimum. Remaining bands at SephadexG-100 (1 and 2) column showed high proteolytic activity with molecular weight of 97.4 kD.

CONCLUSION

Tape' potential as habitat of extracellular protease producing yeasts. *Saccharomycopsis fibuligera* strain R64as extracellular protease producing yeasts isolated from *tape*' shown proteolytic activity until 5 mm of clear zones. Crude extracellular protease at supernatant isolated and showed 4.12 mg/mL protein content and 0.049 U/mL proteolytic activities, precipitations with 45% ammonium sulfate gives protein content of 5.05 mg/mL and proteolytic activity of 0.051U/mL. Dialysis provide the increased of protein content of 5.57 mg/mL and proteolytic activity of 0.0631U/mL. Moreover, the gel filtration chromatography with sephadex G-100 yields 0.023 U/mL proteolytic activities, 2.1 mg/mL protein contents with 97.4 kD protease molecular weight.

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