PENGGUNAAN SISA UDANG UNTUK PENGHASILAN KITINASE OLEH *Trichoderma virens* MENGGUNAKAN FERMENTASI KEADAAN PEPEJAL

RACHMAWATY

Tesis ini dikemukakan sebagai memenuhi
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Dikhaskan untuk yang saya sayangi dan sentiasa menyokong saya

Ayah dan mama,

Suami saya Ferry eko cahyono

Anak-anak saya, ahli keluarga dan kawan-kawan

Orang-orang yang memberi inspirasi kepada saya untuk penyelesain pengajian ini

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ABSTRAK

Penghasilan kitinase oleh *Trichoderma virens* menggunakan sisa udang sebagai substrat telah dikaji melalui fermentasi keadaan pepejal dengan kandungan lembapan pada 70%. Enam kaedah prarawatan yang berbeza iaitu prarawatan ketuhar, gelombang mikro, pendidihan dan penghancuran, pengeringan suria, dan kimia telah dilakukan terhadap sisa udang dengan sisa udang tanpa prarawatan dijadikan sebagai kawalan. Aktiviti kitinase tertinggi diperoleh daripada prarawatan gelombang mikro pada hari ketiga fermentasi dengan aktiviti kitinase pada 0.194 U/g IDS, 3.2 kali lebih tinggi berbanding kawalan (0.06 U/g IDS). Kajian terhadap kesan sumber nitrogen terhadap penghasilan kitinase menggunakan reka bentuk faktorial umum menunjukkan yang ammonium sulfat dengan 30.29 mM nitrogen memberi kesan yang signifikan berbanding ekstrak yis dengan 7.43 mM nitrogen. Reka bentuk faktorial 2-peringkat, masa eraman, suhu, dan kelembapan substrat juga memberi kesan yang signifikan kepada penghasilan kitinase. Reka bentuk komposit berpusat (RBKB) digunakan dalam mengoptimumkan keadaan bagi penghasilan kitinase sisa udang melalui fermentasi keadaan pepejal. Penghasilan kitinase didapati meningkat 2.46 kali ganda (0.487 U/g IDS) pada keadaan optimum iaitu pada suhu 27.9 $^{\circ}$C, kelembapan substrat54.5% dan enam hari masa pengeraman. Bagi pencirian separa kitinase, suhu dan pH optimum masing-masing adalah pada 60°C dan pH 3.0. Kitinase mengekalkan 72% aktiviti pada suhu 70°C. Walau bagaimanapun, kehilangan jumlah aktiviti kitinase berlaku selepas 60 minit pengeraman pada suhu 70°C dan 80°C dengan sisa aktiviti masing-masing 48% dan 28%. Kitinase lebih stabil dalam pH berasid daripada pH beralkali. Berat molekul kitinase adalah 50 dan 42 kDa bagi endokitinase, 33 dan 25 kDa bagi eksokitinase dan 18 kDa bagi protease. Pengekstrakan kitinase mentah dari *Trichoderma virens* dapat merencatkan pertumbuhan *Ganoderma boninense*.

ABSTRACT

The chitinase production by *Trichoderma virens* using shrimp waste as a substrate was studied in solid state fermentation with 70% of moisture content. Six different pretreatment methods namely oven pretreatment, microwave pretreatment, boiling and crushing pretreatment, sun-dried pretreatment and chemical pretreatment were conducted on shrimp waste with non-treated shrimp waste as a control. The highest chitinase activity was obtained from microwave pretreatment on the third day of fermentation with chitinase activity of 0.194 U/g IDS, 3.2 fold higher than the untreated shrimp waste (0.06 U/g IDS). Study on the effect of nitrogen source on chitinase production using general factorial design showed that ammonium sulphate with 30.29 mM nitrogen gave significant effect compared toyeast extract with 7.43 mM nitrogen. Two level factorial design, incubation time, temperature, and substrate moisture also have a significant impact on the production of chitinase. Central composite design (CCD) was used in optimizing the conditions for chitinase production of shrimp waste by solid-state fermentation. Chitinase production was found to have increased 2.46 times (0.487 U/g IDS) at optimum condition: temperature of 27.9 ° C, 54.5% of substrate moisture and six days of incubation time. The optimal degradation showed an improvement of chitinase production of 2.46 fold as compared to before optimization using CCD. For partial characterization of chitinase, the optimum temperature and pH are at 60 °C and pH 3.0, respectively. Chitinase retained 72% of its activity at 70 °C. However, the loss of the chitinase activity occurred after 60 minutes of incubation at 70 °C and 80 °C with residual activity are 48% and 28%, respectively. Chitinase was more stable in acidic than in alkaline pH. The molecular weight of chitinase was 50 and 42 kDa for endochitinase, 33 and 25 kDa for eksokitinase and 18 kDa for protease. Extraction of crude chitinase from *Trichoderma virens* can inhibit the growth of *Ganoderma boninense*.

KANDUNGAN

SENARAI JADUAL

SENARAI RAJAH

SENARAI SINGKATAN

$(GlcNAc)_2$	-	Diasetilkitobiosa
$(NH_4)_2SO_4$	-	Ammonium sulfat
Al	-	Aluminium
ANOVA	-	Analisis varian
ATCC	-	Jenis kultur koleksi Amerika
BCA	-	Agen Biokawalan
BGK	-	Bran gandum komersial
BSA	-	Albumin serum bovin
BSR	-	Pereputan pangkal batang
Ca	-	Kalsium
$CaCl_2.2H_2O$	-	Kalsium klorid dehidrat
Cl	-	Klor
Co	-	Kobalt
CoA	-	Koenzim A
Cu	-	Tembaga
DNS	-	Asid dinitrosalisilik
Fe	-	Ferum
FKP	-	Fermentasi keadaan pepejal
FmT	-	Fermentasi tenggelam
GlcNAc	-	N-asetil-glukosamin
HCl	-	Hidroklorik asid
K	-	Kalium
KCl	-	Kalium klorida
KH_2PO_4	-	Kalium dihidrogen fosfat
Mg	-	Magnesium
$MgSO_4.7H_2O$	-	Magnesium Sulfat heptahidrat
Mn	-	Mangan

Mo	-	Molibdenum
N_2	-	Nitrogen
Na	-	Natrium
NAG	-	N-asetil-glukosamin
NaOH	-	Natrium hidroksida
NH_3	-	Ammonia
NO_3	-	Nitrat
OFAT	-	Satu faktor dalam satu masa
P	-	Fosfor
PAGE	-	Elektroforesis gel poliakrilamid
PDA	-	Agar dekstrosa kentang
PIRG	-	Peratusan perencatan jejari pertumbuhan
RBKP	-	Reka bentuk komposit pusat
RSM	-	Kaedah gerak balas permukaan
S	-	Sulfur
SDS	-	Natrium dodesil sulfat
SEM	-	Mikroskop elektron penskanan
Si	-	Silikon
SKKUP	-	Sisa kitin kulit udang pepejal
T	-	Titanium
TCA	-	Trikloroasetik
Zn	-	Zink

SENARAI SIMBOL

µg	-	Mikrogram
µL	-	Mikroliter
µmol	-	Mikromol
^{0}C	-	Darjah selsius
g	-	Gram
g/L	-	Gram per liter
h	-	Hari
i/i	-	Isi padu per isi padu
j/j	-	Jisim per jisim
kDa	-	Kilo dalton
kg	-	Kilogram
min	-	Minit
mL	-	Mililiter
mm	-	Milimeter
mM	-	Milimolar
nm	-	Nanometer
OD	-	Ketumpatan optik
psm	-	Putaran seminit
U/g IDS	-	Unit per gram substrat kering awal

SENARAI LAMPIRAN

BAB 1

PENGENALAN

1.1 Latar Belakang Kajian

Setiap tahun, Malaysia menghasilkan antara 60-70% sisa udang berdasarkan berat kering daripada pemprosesan makanan bagi eksport makanan laut. Sisa udang kaya dengan kitin iaitu homopolimer N-asetil-D glukosamina (GlcNAc) yang dihubungkan oleh ikatan β-1,4. Kitin terkandung sebanyak 30-40% dalam sisa udang selain dua lagi kandungan utama sisa udang iaitu protein (10-30% (j/j)) dan kalsium karbonat (10-30% (j/j)). Terbitan kitin mempunyai nilai ekonomi yang tinggi berdasarkan aktiviti biologi dan aplikasi agrokimianya (Muzarelli *et al*., 2012). Walau bagaimanapun, sisa udang sukar diurai secara semulajadi dan menjadi antara penyumbang utama kepada pencemaran alam sekitar.

Kini, penulenan dan pengubahsuaian kitin daripada sisa udang kepada produk karbohidrat bernilai tambah melibatkan rawatan yang menggunakan bahan kimia selain hidrolisis tak terkawal. Cara ini hanya membawa kepada penghasilan produk sampingan yang tidak diingini serta kos penulenan yang tinggi untuk menyingkirkan protein dan kalsium karbonat (Chaiharn *et al*., 2013).

Banyak bakteria dan kulat yang dapat menghasilkan enzim kitinolitik luar sel yang dikenali sebagai kitinase (E.C. 3.2.1.14). Enzim kitinase mampu menukarkan kitin kepada sebatian yang bermanfaat kepada industri seperti glukosamina (Das *et al*., 2012). Kitinase mempunyai peranan yang penting dalam kawalan biologi perosak dan penyakit (Kumar *et al*., 2012). Selain itu, kitinase juga digunakan dalam penyelidikan biologi untuk menghasilkan protoplas kulat bagi menguraikan dinding sel kulat, dan dalam penjagaan kesihatan manusia untuk menghasilkan penyediaan oftalmik (Narayana dan Vijayalakshimi, 2009). Penggunaan kitinase untuk mengawal penyakit pada tumbuhan yang disebabkan oleh pelbagai kulat fitopatogen, serangga, nematod, dan juga penghasilan pelbagai oligomer kitin juga semakin mendapat sambutan (Huang *et al*., 2005; De la vega *et al*., 2006; Chang *et al*., 2007). Namun, kos pengeluaran kitinase yang tinggi telah meningkatkan keperluan untuk mencari strain yang dapat menghasilkan kitinase dalam dengan banyak secara kos efektif (Mabuchi *et al.*, 2000).

Penyelidikan terdahulu telah menunjukkan keupayaan beberapa spesies kulat seperti *Trichoderma* sp. dan *Aspergillus* sp. (Felse dan Panda, 2000; Noppakarn *et al*., 2002), dan bakteria serta aktinomiset seperti *Bacillus subtilis* (Wang *et al*., 2006), *B. cereus* (Chang *et al*., 2007) dan *Streptomyces* (Akagi *et al*., 2006) dalam menghasilkan kitinase. Keupayaan ini menjadikan sisa udang yang kaya dengan kitin sebagai sumber substrat (sumber karbon) terbaik kepada mikrorganisma-mikroorganisma ini untuk menghasilkan kitinase (Green *et al*., 2005; Chang *et al*., 2010), sekaligus membantu dalam menyelesaikan masalah pengurusan sisa selain memberi manfaat komersial (Chaiharn *et al*., 2013).

Fermentasi keadaan pepejal (FKP) adalah teknologi yang membolehkan pertumbuhan mikroorganisma tanpa kehadiran air bebas (Digankumar *et al.*, 2010). FKP merupakan kaedah penghasilan enzim yang menarik kerana kaedah ini mempunyai kelebihan dari segi penjanaan efluen yang rendah, penggunaan alatan fermentasi yang ringkas, dan produk terhasil boleh terus digunakan. Tambahan pula, kaedah kultur tenggelam sebelum ini lebih digemari untuk penghasilan beberapa enzim industri seperti amilase, selulase, hemiselulase, protease, dan xilanase (Chang *et al*., 2007).

Pada tahun-tahun kebelakangan ini, penggunaan kaedah FKP semakin menarik minat ramai penyelidik kerana beberapa kajian yang melibatkan enzim (Wang dan Yang, 2007), perasa (Ferron *et al*., 1996), pewarna (Nimnoi dan Lumyong, 2011), dan beberapa bahan lain yang penting kepada industri makanan telah menunjukkan yang FKP dapat memberikan hasil yang lebih tinggi (Couto dan Sanroman, 2006) berbanding fermentasi tenggelam (FmT). Tambahan pula, FKP lebih kos efektif kerana menggunakan sisa sebagai sumber (Robinson and Nigam, 2003).

Kajian tentang kos bagi kedua-dua kaedah FKP dan FmT telah dibuat oleh Castilho *et al.* (2000). Dalam kajiannya, Castillo *et al.* melakukan analisis ekonomi yang terperinci terhadap penghasilan lipase oleh *Penicillium restrictum* dan mendapati jumlah pelaburan modal yang diperlukan FmT adalah 78% lebih tinggi daripada FKP untuk skala penghasilan 100 m^3 lipase setiap tahun. Kos keseluruhan produk bagi FKP juga adalah 47% lebih rendah daripada harga jualan.

Kajian-kajian ini menunjukkan bahawa kelebihan utama bagi proses FKP ialah penggunaan bahan mentah yang sangat murah sebagai substrat utama. Penggunaan sisa bahan laut bukan sahaja menyelesaikan masalah alam sekitar malah mengurangkan kos penghasilan kitinase oleh mikrob. Oleh itu, FKP sememangnya merupakan kaedah yang berkesan dalam penggunaan sisa pepejal kaya nutrien sebagai substrat. Memandangkan sisa makanan dan agrikultur mengandungi karbohidrat dan nutrien lain yang tinggi sisa-sisa ini boleh digunakan sebagai substrat untuk menghasilkan bahan kimia dan enzim secara pukal menggunakan teknik FKP (Singhania *et al.*, 2009).

Dalam kajian ini, *Trichoderma virens* digunakan sebagai penghasil kitinase dengan menguraikan kitin daripada sisa udang sebagai substrat dalam proses fermentasi keadaan pepejal.

1.2. **Objektif Kajian**

1. Memilih kaedah prarawatan terbaik bagi penghasilan kitinase yang tinggi oleh *Trichoderma virens* menggunakan sisa udang secara fermentasi keadaan pepejal (FKP).
2. Mengkaji kesan sumber nitrogen terhadap penghasilan kitinase oleh *Trichoderma virens* menggunakan reka bentuk faktorial umum (FU) dalam proses FKP.
3. Menyaring faktor-faktor yang mempengaruhi penghasilan kitinase oleh *Trichoderma virens* menggunakan faktorial dua peringkat (FDP) dalam proses FKP.
4. Mengoptimumkan faktor-faktor yang mempengaruhi penghasilan kitinase oleh *Trichoderma virens* menggunakan reka bentuk komposit berpusat (RBKB) dalam proses FKP.
5. Pencirian kitinase mentah yang dihasilkan oleh *Trichoderma virens*.
6. Mengkaji potensi penggunaan kitinase mentah yang dihasilkan oleh *Trichoderma virens* sebagai agen antikulat kepada *Ganoderma boninense*.

1.2 Skop Kajian

Skop kajian ini memfokuskan kepada prarawatan sisa udang bagi penghasilan kitinase oleh *Trichoderma virens* secara FKP. Pengaruh fizikal dan faktor persekitaran telah dikenal pasti menggunakan reka bentuk FU dan reka bentuk FDP, manakala pengoptimuman penghasilan kitinase secara FKP telah dijalankan menggunakan RBKP. Pencirian kitinase dilakukan dengan mengkaji keoptimuman dan kestabilan kitinase mentah pada suhu dan pH yang berbeza, dan potensi kitinase mentah dalam merencat *Ganoderma boninense*.

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