

**Purifikasi Parsial dan Karakterisasi Xilanase
dari Mutan *Aspergillus niger* FNCC 6114
Hasil Induksi Radiasi Gamma
pada Fermentasi Padat
Menggunakan Dedak Gandum**

SKRIPSI

Untuk memenuhi sebagian persyaratan
mencapai derajat Sarjana S-1 pada Program Studi Biologi



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Setelah membaca, meneliti, memberikan petunjuk dan mengoreksi serta mengadakan perbaikan seperlunya, kami sebagai pembimbing internal menyatakan bahwa skripsi sarjana mahasiswa atas nama:

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Bersama dengan surat ini, kami berharap skripsi/proyek akhir sarjana atas nama mahasiswa tersebut dapat segera diperiksa. Terima kasih atas perhatian Anda.

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MOTTO

“For indeed, with hardship will be ease. Indeed, with hardship will be ease”

(QS. Al-Insyirah:5-6)

“A lesson without pain is meaningless. That’s because no one can gain without sacrificing something. But by enduring that pain and overcoming”

(Edward Elric)

“Giving up halfway is worse than never trying at all”

(Misato Katsuragi)

“If you don’t take risks, you can’t create a future”

(Monkey D. Luffy)

“Whatever you lose, you’ll find it again. But what you throw away, you’ll never get it back”

(Kenshin Himura)

“When you hit the point of no return, that’s the moment it truly becomes a journey. If you can still turn back, it’s not really a journey”

(Hinata Miyake)

“When you get into a tight place, and everything goes against you, till it seems as though you could not hang on a minute longer, never give up then, for that is just the place and time that the tide will turn.”

(Harriet Beecher Stowe)

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Yogyakarta, 18 Mei 2026



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Menggunakan Dedak Gandum**

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Abstrak

Kebutuhan industri akan xilanase yang dapat diproduksi secara berkelanjutan mendorong pencarian mikroorganisme unggul dan substrat berbiaya rendah. Penelitian ini mengevaluasi pengaruh mutasi acak akibat radiasi gamma pada *Aspergillus niger* FNCC 6114 terhadap produksi xilanase melalui fermentasi padat menggunakan dedak gandum. Tiga isolat (*Wild type*, Mutan 3.6, dan Mutan 3.7) dipurifikasi secara parsial dengan presipitasi amonium sulfat 80% dan dialisis, kemudian dikarakterisasi aktivitas enzimnya. Mutan 3.7 menghasilkan aktivitas tertinggi, baik pada ekstrak kasar (≈ 21.540 U/mL) maupun setelah purifikasi ($\approx 2.988.825$ U/mL), dengan aktivitas spesifik 9.510 U/mg protein. Karakterisasi menunjukkan kondisi optimum pada pH 4 (12.482 U/mL; 39.721 U/mg) dan suhu 60 °C (15.270 U/mL; 48.586 U/mg). SDS-PAGE menunjukkan pita protein target 20–40 kDa yang sesuai dengan xilanase GH10/GH11, dengan Mutan 3.7 memiliki pita non-target paling sedikit. Hasil ini menunjukkan Mutan 3.7 sebagai isolat terbaik untuk produksi xilanase menggunakan dedak gandum.

Kata Kunci: *Aspergillus niger*, dedak gandum, mutagenesis acak, radiasi gamma, xilanase

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**Partial Purification and Characterization of
Xylanase from *Aspergillus niger* FNCC 6114 Mutant
Induced by Gamma Radiation
in Solid-State Fermentation
Using Wheat Bran as Substrate**

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Abstract

The industrial demand for sustainably produced xylanase has encouraged the exploration of superior microorganisms and low-cost substrates. This study evaluated whether gamma irradiation-induced random mutagenesis in *Aspergillus niger* FNCC 6114 could improve xylanase production during solid-state fermentation using wheat bran. Three isolates (*Wild type*, Mutant 3.6, and Mutant 3.7) were cultivated, and crude enzymes were partially purified by ammonium sulfate precipitation (80% saturation) followed by dialysis. Mutant 3.7 showed the highest xylanase activity in both the crude extract ($\approx 21,540$ U/mL) and after partial purification ($\approx 2,988,825$ U/mL), with a specific activity of 9,510 U/mg protein. Characterization of Mutant 3.7 revealed optimum activity at pH 4 (12,482 U/mL; 39,721 U/mg protein) and 60°C (15,270 U/mL; 48,586 U/mg protein). SDS-PAGE detected protein bands at 20–40 kDa, consistent with GH10 and GH11 xylanases, while Mutant 3.7 showed the greatest xylanase enrichment with fewer non-target proteins. This research proves that Mutant 3.7 was the most promising isolate for xylanase production from wheat bran under solid-state fermentation.

Keywords: *Aspergillus niger*, gamma irradiation, random mutagenesis, solid-state fermentation, wheat bran, xylanase

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DAFTAR ISI

HALAMAN JUDUL	i
HALAMAN PENGESAHAN	ii
SURAT PERNYATAAN KEASLIAN SKRIPSI	iii
SURAT PERSETUJUAN	iv
MOTTO	v
PERSEMBAHAN	vi
KATA PENGANTAR	vii
DAFTAR ISI	xiii
DAFTAR TABEL	xv
DAFTAR GAMBAR	xvi
DAFTAR LAMPIRAN	xvii
BAB I PENDAHULUAN	1
A. Latar Belakang	1
B. Rumusan Masalah	3
C. Tujuan Penelitian	4
D. Manfaat Penelitian	5
BAB II TINJAUAN PUSTAKA	6
A. <i>Aspergillus niger</i> dan Potensinya sebagai Produsen Xilanase	6
B. Dedak Gandum sebagai Substrat Produksi Xilanase	8
C. Fermentasi Padat (<i>Solid State Fermentation</i>) untuk Produksi Xilanase	9
D. Sumber dan Manfaat Xilanase dalam Industri	10
E. Purifikasi Enzim	13
F. Karakterisasi Aktivitas Enzim	14
BAB III METODE PENELITIAN	16
A. Waktu dan Tempat Pelaksanaan	16
B. Alat dan Bahan	16
1. Karakterisasi Komponen Senyawa Dedak Gandum sebagai Substrat dengan Metode AOAC	17
2. Preparasi Media Mandels dan Buffer Sitrat	18
3. Preparasi Inokulum <i>A. niger</i>	19
4. Produksi Xilanase dengan Metode Fermentasi Padat	20
5. Ekstraksi Xilanase dengan Buffer Sitrat	20
6. Purifikasi Parsial Enzim dengan Amonium Sulfat (NH ₄) ₂ SO ₄	21
7. Pengeringan Beku (Liofilisasi) Enzim Kasar dan Terpurifikasi Parsial	22
8. Penentuan Aktivitas Xilanase dengan Metode DNS	22
9. Penentuan Kadar Protein Enzim dengan Metode Lowry	25
10. Penapisan Profil Molekul Enzim dengan Metode SDS-PAGE	27
11. Uji Signifikansi Aktivitas Xilanase	28
BAB IV HASIL DAN PEMBAHASAN	29
A. Karakterisasi Komponen Senyawa Dedak Gandum sebagai Substrat	29
B. Penapisan Xilanase untuk Pemilihan Mutan Terbaik pada <i>A. niger</i> Wild type FNCC 6114, Mutan 3.6 dan Mutan 3.7	31
C. Karakterisasi Xilanase Terpurifikasi Parsial dari <i>A. niger</i> Mutan 3.7 pada Berbagai pH	40

D. Karakterisasi Xilanase Terpurifikasi Parsial dari <i>A. niger</i> Mutan 3.7 pada Berbagai Suhu.....	44
E. Analisis Profil Protein Xilanase dari <i>Wild type A. niger</i> FNCC 6114, Mutan 3.6, dan Mutan 3.7 Hasil SDS-PAGE	47
BAB V PENUTUP	52
A. Kesimpulan	52
B. Saran	52
DAFTAR PUSTAKA.....	54
LAMPIRAN	90



DAFTAR TABEL

Tabel 1.	Komposisi Kimia Dedak Substrat Dedak Gandum	29
Tabel 2.	Hasil Penapisan Aktivitas Xilanase dari EK dan EP	36
Tabel 3.	Aktivitas Xilanase dari EP Mutan 3.7 pada Berbagai pH.....	40
Tabel 4.	Aktivitas Xilanase dari EP Mutan 3.7 pada Berbagai Suhu.....	44
Tabel 5.	Profil Berat Molekul dan Dugaan Identifikasi EP Mutan 3.7.....	48

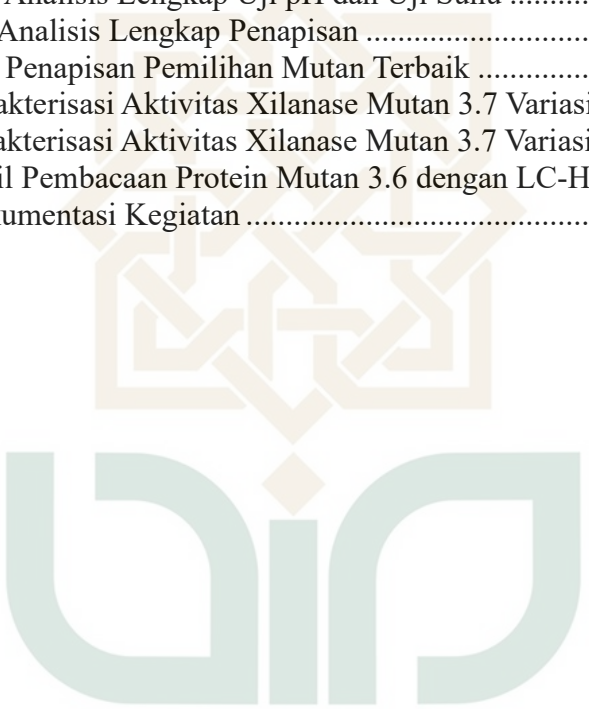


DAFTAR GAMBAR

Gambar 1. Tren Riset <i>A. niger</i> sebagai Produsen Enzim.....	7
Gambar 2. Struktur Senyawa Penyusun Dedak Gandum	9
Gambar 3. Diagram Degradasi Xilan oleh <i>A. niger</i> An76.....	12
Gambar 4. Skema Uji Lowry	14
Gambar 5. Skema Reaksi Reduksi DNS dengan Gula Pereduksi	15
Gambar 6. Pengamatan Pertumbuhan Inokulum	33
Gambar 7. Skema Pertumbuhan Fungi Filamen.....	34
Gambar 8. Skema Induksi Mutasi oleh Radiasi Gamma	38
Gambar 9. Aktivitas Relatif Xilanase terhadap Variasi pH	41
Gambar 10. Aktivitas Relatif Xilanase terhadap Variasi Suhu	45
Gambar 11. Analisis profil protein dengan SDS-PAGE	47

DAFTAR LAMPIRAN

Lampiran 1. Kurva Standar Xilosa pH 5.3	90
Lampiran 2. Kurva Standar Xilosa pH 3	91
Lampiran 3. Kurva Standar Xilosa pH 5	92
Lampiran 4. Kurva Standar Xilosa pH 6	93
Lampiran 5. Kurva Standar Xilosa pH 7	94
Lampiran 6. Kurva Standar Xilosa pH 8	95
Lampiran 7. Hasil Analisis Lengkap Uji pH dan Uji Suhu	96
Lampiran 8. Hasil Analisis Lengkap Penapisan	96
Lampiran 9. Hasil Penapisan Pemilihan Mutan Terbaik	98
Lampiran 10. Karakterisasi Aktivitas Xilanase Mutan 3.7 Variasi pH	99
Lampiran 11. Karakterisasi Aktivitas Xilanase Mutan 3.7 Variasi Suhu	100
Lampiran 12. Hasil Pembacaan Protein Mutan 3.6 dengan LC-HRMS	101
Lampiran 13. Dokumentasi Kegiatan	102



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BAB I

PENDAHULUAN

A. Latar Belakang

Kebutuhan manusia akan sandang, pangan, tekstil, dan banyak kebutuhan pokok lainnya mendorong sektor industri untuk mencari solusi akan proses produksi yang menerapkan konsep keberlanjutan dan efisiensi di dalamnya (Adegbeye *et al.*, 2020; Nguyen *et al.*, 2025; Zhang *et al.*, 2022). Enzim merupakan jawaban dari kebutuhan industri untuk konsep berkelanjutan, karena merupakan katalisator biologis yang dapat diperoleh dengan pendekatan bioproses (Farhan *et al.*, 2025; Monica dan Uppuluri, 2025). Riset mengenai peran enzim sebagai agen bioproses skala industrial telah menjadi *research trend* sejak tahun 1979 dikembangkan oleh Chibata (1979). Penggunaan enzim sebagai agen bioproses industrial sangatlah beragam, mulai dari sektor pengelolaan limbah air (Bayramoglu *et al.*, 2012; Sondhi *et al.*, 2014), tekstil (Lima *et al.*, 2022; Ntovolou *et al.*, 2025; Sankarraj dan Nallathambi, 2018; Yu *et al.*, 2015), hingga yang terpopuler menurut beberapa studi literatur adalah sektor pangan (Motta *et al.*, 2023; Siddikey *et al.*, 2025; Yang, 2023; Zhang *et al.*, 2018).

Salah satu enzim yang kerap digunakan pada skala industri adalah xilanase, yang berfungsi sebagai pengurai xilan, yang merupakan komponen utama hemiselulosa dalam dinding sel tumbuhan, menjadi xilo-oligosakarida dan xilosa (Kim *et al.*, 2023; Pérez *et al.*, 2022; Rodríguez *et al.*, 2025). Xilanase populer dalam sektor pangan, umumnya dimanfaatkan sebagai pengurai arabinoksilan dalam adonan gandum untuk memperbaiki tekstur dan volume roti (Wang *et al.*, 2018). Penelitian pangan fungsional terkini juga mengembangkan xilanase sebagai klarifikator (penjernih) jus buah (Ristović *et al.*, 2026; Wang *et al.*, 2023; Zhang *et al.*, 2023). Tren riset xilanase dalam sektor pangan mengarah pada kemampuannya dalam menghidrolisis xilan menjadi produk-produk berupa xilo-oligosakarida, xilobiosa, dan xilotriosa dan yang memiliki manfaat sebagai pemanis rendah kalori dan dapat

mendukung kesehatan pencernaan karena perannya sebagai senyawa prebiotik (Berger *et al.*, 2021; Nieto-Domínguez *et al.*, 2017; Su *et al.*, 2021; Zheng *et al.*, 2020), dan xilosa yang potensial sebagai bahan dasar untuk pembuatan xilitol sebagai pemanis rendah kalori untuk terapi diabetes (Amo *et al.*, 2011; Rehman *et al.*, 2018).

Seiring dengan tingginya perkembangan tren penggunaan xilanase, pendekatan produksi yang menjunjung efisiensi produksi dan *sustainability* menjadi sebuah kebutuhan (Guerrero *et al.*, 2025; Lodhi *et al.*, 2024). Fermentasi padat atau dalam bahasa inggris *Solid State Fermentation* (SSF) menggunakan mikroorganisme seperti *Aspergillus niger* merupakan salah satu pendekatan yang potensial untuk produksi xilanase karena memiliki efisiensi enzimatik yang tinggi serta relatif aman diterapkan pada skala industri (Afiah Razali *et al.*, 2021; Ali *et al.*, 2024; Azzouz *et al.*, 2021; Ntana *et al.*, 2020). Keberhasilan metode fermentasi padat sangatlah dipengaruhi oleh pemilihan substrat yang sesuai (Kalaiselvan *et al.*, 2025; Kumar & Kanwar, 2012). Dalam hal ini, dedak gandum merupakan limbah agroindustri yang kaya akan serat kasar yang terdiri atas selulosa, hemiselulosa dan lignin, yang sesuai dengan kriteria produksi xilanase dengan metode fermentasi padat tersebut (Saroj *et al.*, 2025). Secara kimiawi, dedak gandum mengandung komponen lignoselulosa yang terdiri dari sekitar 11,7-13,1% selulosa (De Bondt *et al.*, 2020) dan sekitar 49,7% hemiselulosa (Sánchez-Bastardo *et al.*, 2017), dengan arabinoksilan sebagai komponen dominan yang menjadikannya sumber karbon potensial bagi mikroorganisme penghasil xilanase (Ali *et al.*, 2024; Ameen, 2023; Glaser *et al.*, 2023; Merali *et al.*, 2015; Vinuthana *et al.*, 2026).

Kandungan serat kasar yang tinggi pada dedak gandum berpotensi sebagai substrat fermentasi dalam memproduksi enzim, sekaligus dapat membantu mengurangi pencemaran lingkungan akibat limbah organik yang tidak dimanfaatkan secara optimum (Ali *et al.*, 2024; Marzo-Gago *et al.*, 2022; Mittermeier *et al.*, 2024). Berdasarkan karakteristik tersebut, dedak gandum telah banyak dikaji sebagai substrat potensial dalam produksi xilanase melalui fermentasi padat. Riset pendahuluan oleh Ali *et al.*, (2024) menyebutkan

bahwa, *A. niger* isolat ISL-9 (Culture bank *Institute of Industrial Biotechnology* (IIB) milik GC University Lahore) mampu menghasilkan aktivitas endoxilanase sebesar 21,87 U/g substrat kering menggunakan dedak gandum sebagai substrat tunggal pada kondisi fermentasi padat. Penelitian terbaru oleh Fauziah *et al.* (2026) menunjukkan bahwa perlakuan mutasi acak pada *A. niger* FNCC 6114 dengan kode 3.6 dan 3.7 dengan iradiasi gamma dosis 300 Gy berhasil memperoleh aktivitas spesifik xilanase yang meningkat secara berurutan sebesar 1,59 kali (12.879,28 U/mg) dan 1,70 kali (13.784,67 U/mg) dibandingkan dengan isolat *Wild type*-nya (8.103,62 U/mg) yang diproduksi dengan metode fermentasi terendam (*sub-merged fermentation* (SmF)).

Temuan tersebut membuka peluang untuk mengkaji lebih lanjut potensi isolat kedua Mutan *A. niger* FNCC 6114 tersebut dalam produksi xilanase dengan metode fermentasi padat menggunakan substrat dedak gandum. Oleh karena itu, penelitian ini dilakukan untuk mengetahui aktivitas xilanase pada ekstrak kasar dan terpurifikasi parsial dari isolat *Wild type A. niger* FNCC 6114 dan isolat Mutan (Mutan 3.6 dan Mutan 3.7), mengkarakterisasi xilanase terpurifikasi parsial dari isolat terpilih serta melakukan *pre-eliminary research* untuk memperoleh gambaran awal mengenai profil protein yang terekspresi pada Mutan tersebut.

Penelitian ini diharapkan dapat menghasilkan informasi mengenai potensi produksi xilanase melalui fermentasi padat berbasis dedak gandum serta karakteristik xilanase dari isolat mutan terpilih. Informasi tersebut dapat menjadi landasan ilmiah bagi pengembangan xilanase lokal untuk berbagai aplikasi industri.

B. Rumusan Masalah

Berdasarkan latar belakang yang telah dijelaskan, rumusan masalah dalam penelitian ini adalah sebagai berikut:

1. Bagaimana efektivitas purifikasi parsial menggunakan metode pengendapan dengan amonium sulfat (*salting out*) dengan kejenuhan 80% dan dialisis dalam meningkatkan kemurnian enzim xilanase dari isolat

Wild type A. niger FNCC 6114 serta Mutan 3.6 dan 3.7 pada fermentasi padat menggunakan dedak gandum?

2. Mutan *A. niger* FNCC 6114 manakah yang menunjukkan aktivitas, total aktivitas dan aktivitas spesifik xilanase tertinggi?
3. Bagaimana pengaruh variasi pH substrat terhadap aktivitas xilanase dan aktivitas spesifik xilanase pada sampel terpurifikasi parsial dari Mutan terpilih?
4. Bagaimana pengaruh variasi suhu terhadap aktivitas xilanase dan aktivitas spesifik xilanase pada kondisi pH substrat optimum dari sampel terpurifikasi parsial Mutan terpilih?
5. Bagaimana karakteristik protein xilanase hasil pemurnian parsial dari isolat *Wild type*, Mutan 3.6, dan Mutan 3.7 berdasarkan tingkat kemurnian serta profil berat molekulnya dalam mengidentifikasi keberadaan xilanase famili GH10 dan GH11?

C. Tujuan Penelitian

Berdasarkan latar belakang yang telah dijelaskan, tujuan dari penelitian ini adalah sebagai berikut:

1. Menganalisis efektivitas pemurnian parsial menggunakan metode pengendapan amonium sulfat (*salting out*) dengan kejenuhan 80% dan dialisis dalam meningkatkan kemurnian enzim xilanase yang dihasilkan oleh isolat *Wild type Aspergillus niger* FNCC 6114, Mutan 3.6, dan Mutan 3.7 pada fermentasi padat menggunakan dedak gandum.
2. Menganalisis nilai aktivitas, total aktivitas, dan aktivitas spesifik xilanase pada ekstrak hasil pemurnian parsial untuk menentukan isolat Mutan *A. niger* FNCC 6114 yang menunjukkan aktivitas tertinggi.
3. Menganalisis pengaruh variasi pH substrat terhadap aktivitas xilanase dan aktivitas spesifik xilanase pada ekstrak hasil pemurnian parsial dari isolat Mutan terpilih.

4. Menganalisis pengaruh variasi suhu terhadap aktivitas xilanase dan aktivitas spesifik xilanase pada kondisi pH substrat optimum menggunakan ekstrak hasil pemurnian parsial dari isolat Mutan terpilih.
5. Menganalisis karakteristik protein xilanase hasil pemurnian parsial dari isolat *Wild type*, Mutan 3.6, dan Mutan 3.7 berdasarkan tingkat kemurnian serta profil berat molekulnya untuk mengidentifikasi keberadaan enzim target yang sesuai dengan xilanase famili GH10 dan GH11.

D. Manfaat Penelitian

Manfaat penelitian ini adalah memberikan informasi ilmiah dan data komprehensif mengenai kemampuan Mutan *Aspergillus niger* FNCC 6114 terpilih dalam menghasilkan enzim xilanase melalui fermentasi padat menggunakan dedak gandum, termasuk karakteristik esensial enzim seperti kondisi pH optimum, suhu optimum, serta perkiraan profil berat molekulnya. Penelitian ini juga dapat menjadi dasar dalam pemilihan dedak gandum sebagai alternatif substrat yang murah dan mudah diperoleh. Dalam skala yang lebih luas, hasil penelitian ini diharapkan dapat menjadi acuan bagi perkembangan industri bioteknologi, khususnya dalam produksi xilanase secara komersial, mendukung perluasan pemanfaatan enzim pada industri pangan, serta mendorong riset lanjutan dalam pengembangan isolat-isolat unggul dengan kemampuan produksi enzim yang lebih tinggi.

BAB V

PENUTUP

A. Kesimpulan

Berdasarkan hasil penelitian ini, maka dapat disimpulkan:

1. Purifikasi parsial menggunakan metode pengendapan amonium sulfat dengan kejenuhan 80% dan dialisis berhasil meningkatkan aktivitas spesifik xilanase.
2. Isolat Mutan 3.7 menunjukkan kemampuan produksi ekstrak dengan aktivitas xilanase, total aktivitas dan aktivitas xilanase spesifik tertinggi dibandingkan isolat *Wild type* dan Mutan 3.6.
3. Ekstrak terpurifikasi parsial Mutan 3.7 optimum bekerja pada substrat dengan pH 4 yang masih stabil secara berturut-turut pada pH 7,8,3,5 dan 6.
4. Ekstrak terpurifikasi parsial Mutan 3.7 optimum bekerja pada substrat dengan pH 4 di suhu 60°C yang masih stabil secara berturut-turut pada suhu 50°C, 70°C, 30°C, dan 40°C.
5. Ekstrak terpurifikasi parsial Mutan 3.7 memperoleh tingkat kemurnian tertinggi yang ditunjukkan dengan munculnya pita protein target yang sesuai dengan xilanase GH10 dan GH11 dan minim akan pita protein non-target dibandingkan ekstrak *Wild type* dan Mutan 3.6.

B. Saran

Berdasarkan hasil penelitian ini, dirumuskan saran sebagai berikut:

1. Penelitian lanjutan mengenai optimasi produksi xilanase Mutan 3.7 pada dengan fermentasi padat yang mencakup variasi jumlah inokulum, kelembapan substrat, durasi fermentasi, serta temperatur dan pH inkubasi untuk memperoleh sekresi dan aktivitas enzim yang maksimum.
2. Purifikasi lebih lanjut hingga diperoleh enzim dengan kemurnian tinggi, menggunakan metode kromatografi kolom atau inhibisi terhadap protein yang berpotensi menghambat kinerja sistem xilanolitik ekstrak.

3. Karakterisasi lanjutan meliputi studi kinetik (K_m , V_{max}), analisis stabilitas termal dan pH jangka panjang, serta uji substrat spesifik terhadap berbagai xilan kompleks.
4. Analisis proteomik pada ekstrak teprurifikasi parsial Mutan 3.7, Mutan 3.6 dan *Wild type* dengan pendekatan kuantitatif LC-MS/MS untuk mengidentifikasi protein xilanolitik inti, protein aksesori, dan jalur metabolik yang berperan dalam peningkatan produksi enzim.
5. Analisis genomik dengan *whole genome sequencing* (WGS) dan transkriptomik pada isolat Mutan 3.7 dan *Wild type* untuk mengetahui gen yang berbeda ekspresinya serta varian genetik spesifik yang berpotensi berperan dalam fenotipe unggul Mutan 3.7, sehingga dapat diidentifikasi jalur molekuler dan mekanisme biologis yang mendasari perbedaan karakteristik antara Mutan dan *Wild type*.
6. Penelitian lanjutan menggunakan substrat dari limbah lokal Indonesia yang tinggi hemiselulosa, seperti tandan kelapa sawit.
7. Pengaplikasian xilanase produksi Mutan 3.7 pada bioproses pangan, seperti klasifikasi jus buah dengan nilai fungsional tinggi karena kemampuannya menghidrolisis xilan menjadi xilooligasakarida.

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