LAPORAN AKHIR TAHUN
PENELITIAN STRATEGIS NASIONAL INSTITUSI
(PSNI)

PENGEMBANGAN KONSORSIUM *EXCELZYME* LOKAL UNTUK PENINGKATAN AKTIVITAS BIO-PRODUK DALAM APLIKASI PADA AGRO-INDUSTRI

TAHUN KE – 1 DARI RENCANA 3 TAHUN

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DIBIAYAI OLEH:
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KEMENTERIAN RISET, TEKNOLOGI, DAN PENDIDIKAN TINGGI
SESUAI DENGAN PERJANJIAN PENDANAAN PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
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DAFTAR ISI

PRAKATA

Dengan mengucapkan puji syukur kehadirat Allah S.W.T, laporan kemajuan penelitian STRANAS Institusi ini dapat terselesaikan dengan baik. Manfaat *Excelzyme* telah pula didiseminasikan kepada industri PT Petrosida, Gresik.

Penelitian ini juga melibatkan 2 staf peneliti muda pada Laboratorium Proteomik, Lembaga Penyakit Tropis Universitas Airlangga, 1 orang mahasiswa Program Doktor, 1 orang mahasiswa Program Magister Kimia, Departemen Kimia, dan 3 orang mahasiswa Program Sarjana, Departemen Kimia, Fakultas Sains dan Teknologi, Universitas Airlangga.

Peneliti berharap semoga penelitian ini bermanfaat bagi kemandirian bangsa dalam memproduksi enzim local secara komersial.

Surabaya, 14 Nopember 2018

Ketua Peneliti

RINGKASAN

Bioresource Technology berbasis biomassa asal limbah pertanian merupakan biomassa yang menyimpan sumber energi tinggi karena kaya akan polisakarida. Teknologi ini menggunakan enzim sebagai biokatalisator, yang ramah lingkungan dan mampu mengolah limbah pertanian kaya akan lignoselulosa menjadi produk bermanfaat, diantaranya sebagai bahan baku pakan ternak dan bio-energi. Mengingat enzim kelompok lignoselulolitik masih sangat tergantung dengan produk import, maka biodiversitas mikrobial yang sangat berlimpah di Indonesia menjadi peluang untuk dapatnya memproduksi enzim kelompok lignoselulolitik secara mandiri di Indonesia.

Limbah pertanian sebagian besar kaya akan materi lignoselulosa yang terdiri dari kandungan selulosa (45-50%), hemiselulosa (35-40%) dan lignin (10-20%). Kandungan polisakarida yang cukup tinggi tersebut merupakan bahan baku murah serta bersifat dapat diperbaharui (*renewable*) yang dapat dimanfaatkan sebagai bahan baku pakan ternak, prebiotik, dan pupuk organik mengatasi permasalahan ketahanan pangan selain mengurangi efek pemanasan global. Namun demikian, permasalahan yang terjadi adalah masih rendahnya nilai nutrisi dari limbah pertanian tersebut di atas baik sebagai campuran bahan pakan ternak maupun pupuk. Pengolahan secara enzimatis terhadap limbah pertanian tersebut diatas akan mampu meningkatkan mutu pakan ternak dan pupuk organik. Namun demikian selama ini produk enzim masih import dan harga di pasaran sangat mahal berkisar Rp.150.000-300.000/kg. Perlu dikembangkan produk enzim lokal yang mampu bersaing dengan produk enzim import.

Kelompok studi proteomik Universitas Airlangga berhasil mengembangkan *Excelzyme* (Patent merk No.IDM000293741, tahun 2011) asal isolat lokal Indonesia, serta telah mampu memproduksi *Excelzyme* bekerja sama dengan PT Petrosida, Gresik dalam skala pilot. (Patent formulasi No IDP000040523, tahun 2016). Aplikasi Excelzyme khususnya yang berperan aktif pada limbah pertanian kaya akan polisakarida dalam bidang agro-industri mulai dilaksanakan sejak tahun 2009. Namun demikian , hasil menunjukkan masih perlunya dikembangkan konsorsium kelompok lignoselulolitik lainnya, antara lain selulolitik, pektinolitik, dan lakase ke dalam *Excelzyme*. Pada tahun 2015-2017, tim telah berhasil mengisolasi dan mengidentifikasi mikrobial termofilik lokal penghasil selulolitik, pektimolitik dam lakase termofilik. Dalam penelitian Strategis Nasional ini akan mengoptimalkan formulasi konsorsium excelzyme untuk meningkatkan aktivitas excelzyme dalam aplikasinya pada agro-industri. Berkaitan dengan hal tersebut, maka tim akan mengoptimalkan pengklasifikasian *Excelzyme* menjadi 3 jenis produk, yaitu *Excelzyme* 1 untuk aplikasi dalam proses *bio-deinking/bio-bleaching*, *Excelzyme* 2 untuk aplikasi dalam campuran pakan ternak ruminansia, dan *Excelzyme* 3 untuk aplikasi campuran pupuk organik.

Telah berhasil diisolasi enzim pectinase, selulase dan lakase termofilik di sumber air panas Gunung Pancar, Bogor. Kultur campuran juga telah dilakukan dengan melakukan campuran enzim rekombinan selulase dan xilosidase (GBtXyl43B) menggunakan system batch flask. Hasil menunjukkan bahwa kedua campuran enzim tersebut dapat diekspresikan dengan aktivitas yang tidak berbeda dengan enzim tunggal masing-masing. Uji pendahuluan ini selanjutnya akan diujicobakan pada skala bioreactor 1,5 mL.

BAB 1. PENDAHULUAN

1.1. Latar belakang

Sektor pertanian merupakan penyumbang kedua terjadinya pemanasan global di bumi ini. Sementara itu, pertanian adalah sumber daya alam utama bagi kelangsungan hidup manusia. Oleh karena itu, hasil pengolahan pertanian yang dikenal dengan biomassa berupa hasil panen dan limbah hasil pengolahan pertanian sebaiknya dikelola dengan baik dan maksimal bagi kelestarian alam.

Bioresource Technology berbasis biomassa merupakan teknologi ramah lingkungan yang dapat mengolah limbah pertanian berbasis lignoselulosa yang kaya akan kandungan selulosa, dan hemiselulosa secara enzimatis sehingga dapat dimanfaatkan sebagai bahan baku industri kimia, bio-energi, pakan ternak, dan prebiotik. Pengolahan limbah petanian kaya polisakarida secara enzimatis sangat dipilih mengingat bersifat ramah lingkungan. Pengolahan biomassa berbasis lignoselulosa tersebut memerlukan aktivitas konsorsium kelompok enzim lignoselulolitik, diantaranya kelompok xilanolitik, pektinolitik, selulolitik, dan lakase. Kendala yang dialamai Indonesia saat ini adalah kebutuhan enzim tersebut masih diimport dari luar negeri, sehingga perlu eksplorasi sumber alam biodiversitas mikrobial dengan kandungan enzim tersebut secara lokal untuk kemandirian produk Indonesia.

Kelompok studi proteomik Universitas Airlangga telah berhasil mengembangkan enzim kelompok xilanolitik asal isolat termofilik lokal Indonesia yang dinamakan *Excelzyme* (Patent merk No.IDM000293741). *Excelzyme* telah mampu diaplikasikan pada agro-industri, diantaranya pengolahan limbah kelapa sawit, tongkol jagung, enceng gondok, jerami padi menjadi bahan pakan ternak dan pupuk organik (Kurniati, *et.a*;,2016 dan Purwani, *et.al*., 2016). Namun demikian, perlu tahapan lanjutan untuk meningkatkan aktivitas *excelzyme* tersebut dengan memformulasikan konsorsium lignoselulolitik ke dalam *Excelzyme* sehingga aktivitas dan hasil pengolahan biomassa lebih optimal.

Tahapan konsorsium akan dilakukan dalam jangka waktu 2 tahun penelitian ini, meliputi (1). Mengoleksi enzim-enzim selulolitik, pektinolitik , dan lakase dari isolat termofilik lokal yang diisolasi dari sumber air panas Gunung Pancar Bogor dan studi pendahuluan telah berhasil mengoleksi 4 isolat terpilih yang mengekspresikan ke empat kelompok enzim lignoselulolitik, (2). Memproduksi dan Memformulasikan konsorsium ke empat enzim tersebut ke dalam *Excelzyme*, (3). Mengaplikasikan *Excelzyme* konsorsium ke dalam biomassa agro-industri.

Penerapan bio-produk *Excelzyme* konsorsium akan dilakukan dengan mitra industri dengan rincian sebagai berikut (1). proses produksi *Excelzyme* dilakukan oleh PT Petrosida,

sedangkan proses pemasaran dan aplikasi pada agro-industri akan dilakukan oleh mitra PT Star Specialty Chemicals Indonesia. Mengingat kerjasama produksi dengan PT Petrosida telah dilakukan pada riset RAPID (tahun terakhir 2017) dan telah menjadi rutinitas kegiatan produksi, maka pada penelitian Stategis Nasional ini akan bermitra dengan PT Star Specialty Chemicals Indonesia untuk target pemasaran dan aplikasi pada agro-industri.

1.2. Perumusan Masalah

a. Ketersediaan enzim selama ini dipasok dari luar negeri
b. Kebutuhan enzim konsorsium sebagai bahan yang ramah lingkungan belum digunakan dalam skala industri
c. *Excelzyme* mampu diaplikasikan untuk meningkatkan mutu pakan ternak dan pupuk organik, namun hasil pengolahannya di agro-industri belum maksimal

1.3. Tujuan

a. memproduksi kelompok enzim lignoselulolitik, yang terdiri dari selulase, pektinase, dan lakase asal isolat lokal termofilik sebagai konsorsium ke dalam *excelzyme*
b. memformulasikan campuran *excelzyme* dan konsorsiumnya sebagai campuran bahan bio-produk agro-indstri
c. menentukan mutu produk *excelzyme* melalui penentuan karakter *excelzyme* dalam campuran konsorsium
d. mengaplikasikan bio-produk konsorsium *excelzyme* kepada masyarakat pengguna

1.4. Manfaat Khusus

a. Mengoptimalkan enzim lokal *Excelzyme* dalam bentuk konsorsium yang diisolasi dari sumber biodiversitas mikrobial termofilik asal Indonesia
b. Meningkatkan nilai ekonomis produk biomassa hasil samping pertanian yang kaya akan sumber energi polisakarida
c. Meningkatkan kemandirian dan daya saing produk *Excelzyme* konsorsium lokal untuk pemenuhan kebutuhan domestik, khususnya pada agro-industri

1.5. Urgensi (Keutamaan) penelitian dalam mengatasi masalah, strategi skala nasional

a. Keutamaan bagi industri

Hasil riset penggunaan konsorsium *excelzyme* untuk aplikasi agro-industri, khususnya meningkatkan mutu pakan dan hasil pertanian akan membantu industri terkait dalam meningkatkan kualitas produknya sehingga mempunyai nilai manfaat yang relatif tinggi bahkan dapat mempromosikan produknya kepada pemangku kepentingan nasional

maupun internasional terutama di negara tropis. Sedangkan efek langsung terhadap industri mitra akan dirasakan dengan meningkatnya omzet penjualan enzim lokal dengan harga yang dapat bersaing dengan produk import yang berdampak peningkatan kegiatan industri dan ekonomi nasional dalam meningkatkan nila manfaat biodiversitas mikrobial Indonesia mengantisipasi perubahan iklim global serta ketahanan pangan nasional.

b. Keutamaan bagi ekonomi

Bio-Produk enzim *Excelzyme* lokal akan mampu bersaing dengan rpoduk enzim sejenis yang import, sehingga meringankan pelaku agro-industri, diantaranya para petani dan peternak dalam ketersediaan produk pertanian dan peternakan. Peningkatana hasil mutu ternak dan hasil panen setelah penggunaan enzim akan meningkatkan nilai jual bagi petani dan peternak dalam memasarkan ternak dan hasil panennya. Hal ini diharapkan akan meningkatkan pendapatan petani dan peternak. Selain itu rendahnya harga gabah akan dapat teratasi mengingat gabah ini dapat dimanfaatkan sebagai bahan baku alternatif pakan. Peningkatan pendapatan petani akan memicu jalannya roda perekonomian nasional. Perlu diketahui bahwa Indonesia masih didominasi oleh kegiatan pertanian dan peternakan.

c. Keutamaan bagi masyarakat

Problema kekurangan pangan yang menjadi ancaman masyarakat di Indonesia maupun di negara lainnya akan dapat diantisipasi dengan memanfaatkan semua aspek pemanfaatan limbah pertanian menjadi produk yang bermanfaat yang diolah secara enzimatis menggunakan sumber biodiversitas mikrobial asal indonesia. Kesadaran masyarakat untuk bertindak ceroboh dalam membakar biomassa hasil pertanian juga dapat dikurangi, sehingga efek pemanasan global dapat diminimalkan.

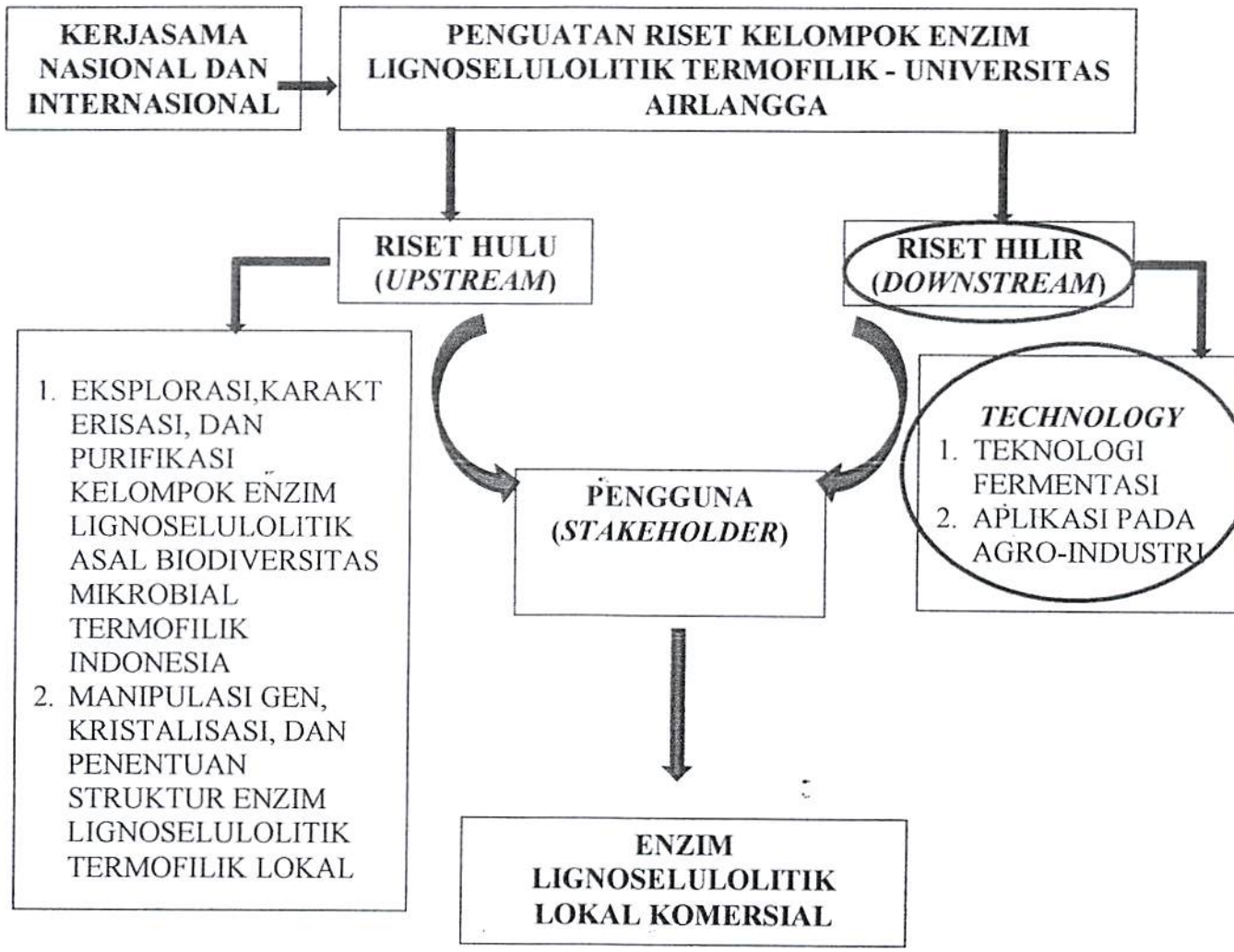
Rencana Target Capaian Tahunan

No.	Jenis Luaran				Indikator Capaian	
	Kategori	Sub-Kategori	Wajib	Tambahan	TS	TS+1
1.	Artikel ilmiah dimuat di jurnal	Internasional bereputasi	2	1	Draft, submitted	Review, accepted
		Nasional Terakreditasi	Tidak ada	Tidak ada	Tidak ada	Tidak ada
2.	Artikel ilmiah dimuat di prosiding	Internasional Terindeks	1	Tidak ada	submitted	Review, accepted
		Nasional	Tidak ada	Tidak ada	Tidak ada	Tidak ada
3.	Invited speaker dalam	Internasional	Tidak	2	Sudah	Sudah

	temu ilmiah		ada		dilaksanakan	dilaksanakan
		Nasional	Tidak ada	Tidak ada	Tidak ada	Tidak ada
4.	Visiting Lecturer	Internasional	Tidak ada	2	Sudah dilaksanakan	Sudah dilaksanakan
5.	Hak Kekayaan Intelektual (HKI)	Paten	Tidak ada	Tidak ada	Tidak ada	Tidak ada
		Paten sederhana	1	Tidak ada	Draft	terdaftar
		Hak Cipta	Tidak ada	Tidak ada	Tidak ada	Tidak ada
		Merek dagang	1	-	terdaftar	granted
		Rahasia dagang	Tidak ada	Tidak ada	Tidak ada	Tidak ada
		Desain Produk Industri	Tidak ada	Tidak ada	Tidak ada	Tidak ada
		Indikasi Geografis	Tidak ada	Tidak ada	Tidak ada	Tidak ada
		Perlindungan Varietas Tanaman	Tidak ada	Tidak ada	Tidak ada	Tidak ada
		Perlindungan Topografi Sirkuit Terpadu	Tidak ada	Tidak ada	Tidak ada	Tidak ada
6.	Teknologi Tepat Guna		1	Tidak ada	terdaftar	proses
7.	Model/Purwarupa/Desain/Karya seni/ Rekayasa Sosial		Tidak ada	Tidak ada	Tidak ada	Tidak ada
8.	Bahan Ajar		Tidak ada	2	draft	produk
9.	Tingkat Kesiapan Teknologi (TKT)		1	Tidak ada	Skala 4	Skala 5

BAB 2. TINJAUAN PUSTAKA

2.1. *State of the art* bidang penelitian



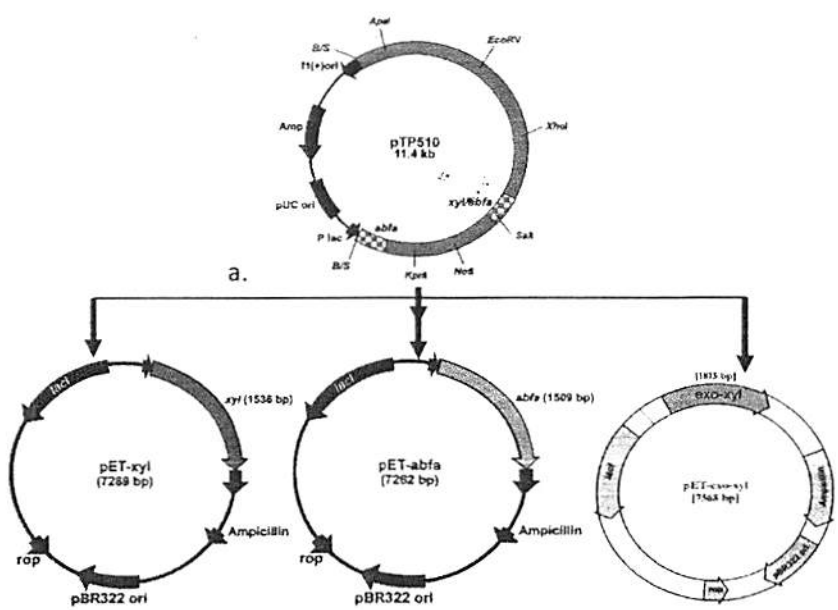
Gambar 1. *State of the art* bidang penelitian
○: yang dikerjakan dalam penelitian Strategis Nasional

2.2. Studi Pendahuluan yang sudah dilaksanakan

Eksplorasi, isolasi, pemurnian dan karakterisasi kompleks enzim xilanolitik asal isolat lokal termofilik *Geobacillus thermoleovorans* IT-08 telah berhasil kami lakukan (Puspaningsih, NNT, 2002). Isolat *Geobacillus thermoleovorans* IT-08 diisolasi dari sumber air panas Gunung Pancar, Bogor (Tan, I. 2001). Peningkatan potensi enzim xilanolitik yang terkandung dalam isolat *G. thermoleovorans* IT-08 selanjutnya dilakukan dengan mengisolasi gen-gen penyandi enzim xilanolitik ke dalam vektor kloning dengan teknik *shotgun*. Tiga gen

penyandi enzim xilanolitik termofilik asal *G. thermoleovorans* IT-08 berhasil diklonkan ke dalam sel inang *Escherichia coli* DH5α. DNA plasmid rekombinan dinamakan pTP510 yang selanjutnya telah pula dipetakan dan ditentukan runutan nukleotidanya (Puspaningsih, N.N.T, 2003-2005). Selain itu ketiga gen tersebut telah diterima di *GenBank* dengan *Accession Number* : DQ387047 [exo-xilanase (*exo-xyl*)], DQ345777 [β-xilosidase (*xyl*)], dan DQ387047 [α-L-arabinofuranosidase (*abfa*)]. Ketiga gen penyandi yang terinsersi dalam pTP510, telah pula berhasil dipisahkan dari kompleks xilanase di pTP510 dan diklonkan ke dalam vektor ekspresi pET-101D/TOPO menghasilkan pET-*xyl* , pET-*abfa* (Puspaningsih, NNT. Dan tim., 2006), dan pET-*exoxyl* (Puspaningsih,NNT., dan tim, 2009). Peta pTP510, pET-*xyl*, pET-*abfa*, dan pET-*exoxyl* terlihat pada Gambar 3. Selanutnya pET-*exoxyl* telah berhasil dieksplorasi lebih lanjut dan berhasil dikarakterisasi sebagai Xilosidase B dengan kandungan domain CBM dan domain katalitik xilosidase (Ratnadewi dkk, 2013). Hasil analisis BLAST menunjukkan bahwa enzim β-xilosidase A dan β-xilosidase B termasuk *family GH*43, sedangkan enzim α-L-arabinofuranosidase masuk dalam *family GH* 51. Untuk melengkapi koleksi enzim xilanolitik asal isolat lokal Indonesia, maka telah pula berhasil diisolasi gen penyandi enzim endo-xilanase asal *Bacillus subtilis* PC-01 yang diisolasi dari sumber air panas Pacet, Jawa Timur (Puspaningsih, N.N.T dan tim, 2005). Enzim endo-xilanase tersebut termasuk dalam *family* GH111.

Keunikan yang ditemukan dalam hasil eksplorasi tingkat gen di atas menunjukkan bahwa gen penyandi *xyl* dan *abfa* mempunyai ukuran gen yang hampir sama (*xyl* 1536 pb dan *abfa* 1509 pb), demikian pula dengan massa molekul relatif untuk keduanya sekitar 57 kDa, serta menunjukkan aktivitas bifungsional. Perbedaan ciri kedua enzim tersebut adalah pada suhu optimumn



Gambar 2. Peta pTP510 dan turunanya (pET-*xyl*, pET-*abfa*, dan pET-*exoxyl*) [Puspaningsih, N.N.T. (2003) dan tim (2005, 2009)]

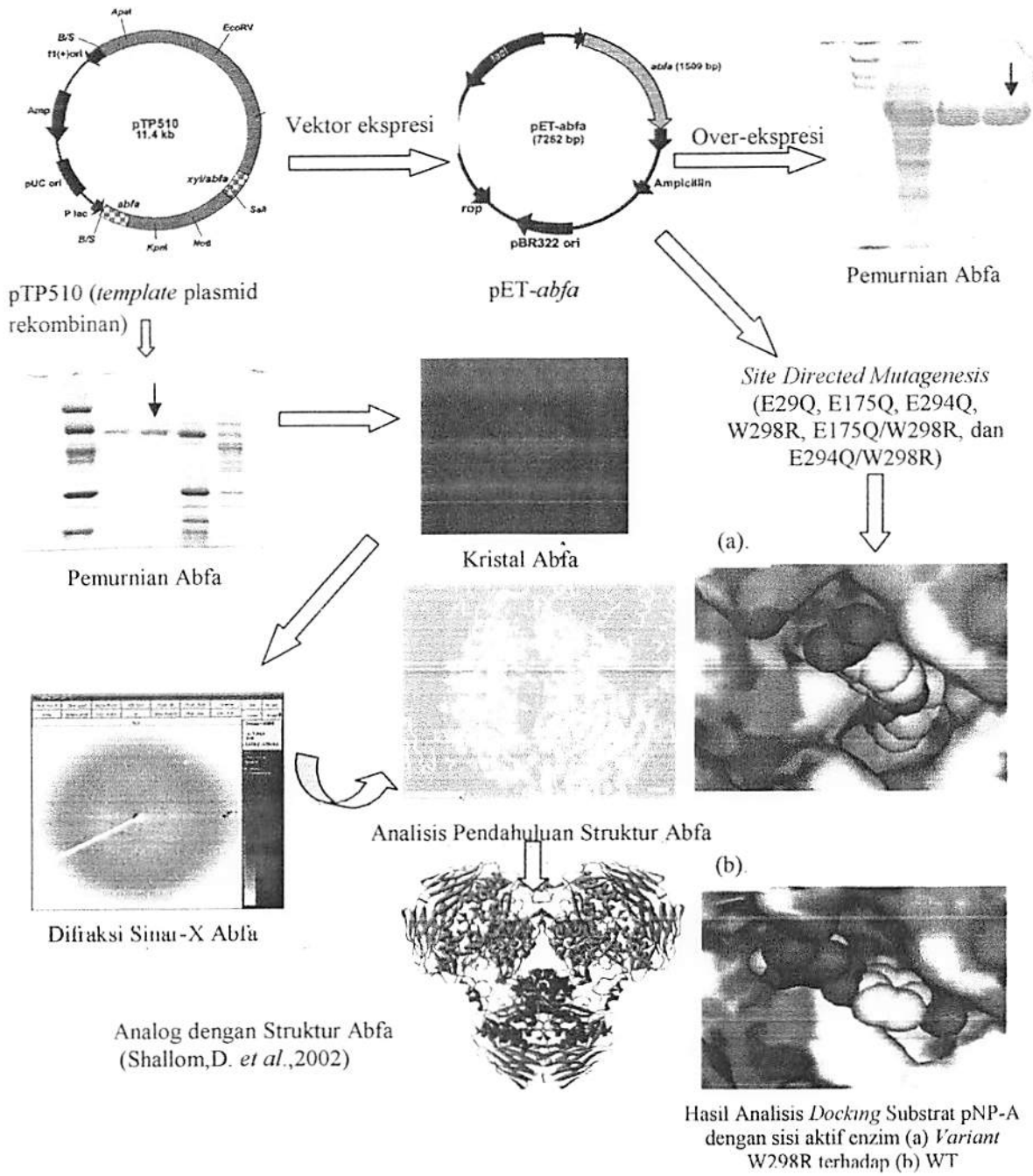
Keunikan pada karakteristik kedua enzim Xyl dan Abfa melahirkan suatu inovasi peneliti untuk meningkatkan tahapan penelitian kearah proteomik. Kedua enzim Xyl dan Abfa rekombinan selanjutnya memasuki tahapan kristalisasi dan analisis difraksi sinar-X pada tahun 2005 hingga 2007 dan berhasil menentukan struktur keduanya. Peningkatan aktivitas juga dilakukan melalui tahapan rekayasa protein. Tahapan analisis proteomik *Excelzyme* tipe α-L-arabinofuranosidase (Abfa) terlihat pada Gambar 2.

Spesifisitas konsorsium enzim xilanolitik yang diekspresikan oleh *E.coli* DH5α (pTP510) maupun enzim Xyl, Exoxyl dan Abfa tunggal asal *E.coli* BL21 (pET-*xyl*, pET-exoxyl dan pET-*abfa*) dalam mendegradasi beberapa substrat xilan komersial diantaranya *oat spelt xylan*, *birchwood*, *wheat-arabinoxylan*, *rye*, dan *arabinan* menunjukkan bahwa terdapat sinergisme tahapan hidrolisis berbagai jenis xilan. Data yang menarik adalah Abfa mampu menghidrolisis substrat xilan pada ikatan arabinosil-xilosa (α-1,2) maupun terhadap substrat arabinan pada ikatan arabinosil-arabinosil (α-1,5). Fenomena ini menunjukkan adanya kemampuan Abfa untuk mendegradasi substrat xilan alam, seperti hemiselulosa asal jagung, gandum, dan padi yang selanjutnya telah dibuktikan dari hasil uji aktivitas enzim Abfa terhadap limbah tongkol jagung, jerami padi, dan ampas tebu.

Pada tahun 2013 telah berhasil ditemukan bahwa enzim novel exo-xilanase merupakan enzim xilosidase (Xyl) GH43B yang unik dan memiliki kebaharuan pada domai *Carbohydrate Binding Module nya (CBM)* yang berbeda dengan Xyl43A yang juga dikandung dalam pTP510 dari *G.thermoleovorans* IT-08 yang sama. Keunikan ini akan menyempurnakan peran *Excelzyme* dalam aplikasi pengolahan limbah pertanian. CBM berperan mengikat subtrat tidak larut dan menjadi potenzi *Excelzyme* dalam efisiensi pengolahan substrat tidak larut seperti oat spelt xilan dan limbah pertanian non-soluble (Ratnadewi dkk., 2013)

Sejak kelompok studi proteomik Universitas Airlangga berhasil mengembangkan *Excelzyme* asal isolat lokal Indonesia, khususnya yang berperan aktif pada limbah pertanian kaya akan lignoselulosa maka tantangan dan inovasi untuk mampu mengaplikasikan enzim temuan dalam bidang agro-industri mulai dilaksanakan sejak tahun 2007. Berkaitan dengan hal tersebut, maka tim mengklasifikasikan *Excelzyme* menjadi 3 jenis produk, yaitu *Excelzyme* 1 untuk aplikasi dalam proses *bio-deinking/bio-bleaching*, *Excelzyme* 2 untuk aplikasi dalam campuran pakan ternak ruminansia, dan *Excelzyme* 3 untuk aplikasi campuran pupuk organik. Namun demikian optimalisasi aktivitas *Excelzyme* tersebut yang hanya

mengandung kelompok enzim xilanolitik saja masih perlu ditingkatkan melalui penambahan konsorsium kelompok lignoselulolitik lainnya, antara lain kelompok enzim selulolitik, pektinolitik, dan lakase.



Gambar 3. Analisis Proteomik Excelzyme tipe α-L-arabinofuranosidase (Abfa) (Puspaningsih, N.N.T dan tim, 2005-2010)

Pada tahun 2015, Kelompok Studi Proteomik , Universitas Airlangga telah berhasil mengisolasi dan mengkarakterisasi mikrobial termofilik hasil isolasi dari sumber air panas Gunung Pancar menghasilkan koleksi 3 kelompok enzim lignoselulolitik selain xilanolitik

yang telah ditemukan sebelumnya (Puspaningsih, 2004), antara lain enzim selulolitik, pektinolitik , dan lakase. Mikrobial termofilik terpilih penghasil enzim tersebut juga telah berhasil diidentifikasi, yaitu *Anoxybacillus flavithermus* and *Geobacillus kastopilus* (Laras *et.al*, 2017).

Kegiatan difusi melalui tahapan diseminasi hasil percobaan telah dilakukan bagi pengusaha pakan ternak lokal khususnya di Jawa Timur dan juga bagi kelompok peternak maupun petani (dalam hal komposting) melalui pelatihan.

2.3. Peta Jalan Riset dan Teknologi

Peta jalan riset dan teknologi *Excelzyme* telah disusun oleh peneliti dan tertuang dalam Buku *Road Map* Penelitian Universitas Airlangga tahun 2007-2015 (Gambar 4.). Pengembangan riset dan teknologi *Excelzyme* dilakukan melalui dua pendekatan, yaitu saintifik dan aplikasi yang dilaksanakan berkolaborasi dengan institusi dalam negeri maupun luar negeri (sesuai *state of the art* bidang penelitian).

Kerjasama penelitian yang sudah berlangsung serta peta jalannya selama ini antara :

- **Mie University, Jepang**

1. *JSPS-short course program* pada tahun 2003 selama 2 bulan dan menghasilkan klaster gen penyandi enzim xilanolitik rekombinan asal isolat lokal yang terekspresi di *Escherichioa coli* DH5α, dinamakan pTP510. Klaster gen tersebut telah diterima di GenBank dengan Accession No. DQ387047, DQ345777, dan DQ387046.
2. Kunjungan tim dari Mie University ke Universitas Airlangga sebagai pembicara tamu dalam acara berikut.

a. Seminar *Lignocelluloses Biomass* , sebagai pembicara tamu adalah Prof. Dr. Kunio Ohmiya pada tanggal 5 April 2005 (Pada tahun 2006 beliau sudah pensiun dan digantikan oleh Prof. Dr. Kazoo Sakka sampai sekarang)

b. The International Conference and Workshop on Basic Science (ICOWOBAS), sebagai pembicara tamu adalah Prof. Dr. Kazuo Sakka pada tanggal 6 Agustus 2007.

c. *The Second Asean Biochemistry Workshop*, sebagai pembicara tamu adalah Prof. Dr. Kazuo Sakka pada tanggal 7-8 Agustus 2007.

3. Kunjungan tim Universitas Airlangga (a.n. Ni Nyoman Tri Puspaningsih) ke Mie University ,Jepang sebagai pembicara dalam acara rutin Mie Bioforum setiap 5 tahun sebagai berikut.

a. *Mie Bioforum Conference on lignocellulose* pada tanggal 10-14 November 2003 di Ishe-shima, Mie, Jepang (biaya oleh Unesco)

b. *Mie Bioforum 2008 on lignocellulose* pada tanggal 1-5 September 2008 dan *Mie Bioforum 2014* di Spain Mura, Mie, Japan (bantuan biaya oleh BKLN-Dikti/2008 dan bantuan biaya Universitas Airlangga/2014)

   Hasil kunjungan menambah wawasan keilmuan bagi peneliti, mengingat peserta Mie Bioforum berasal dari berbagai Negara di Asia, Eropa, dan Amerika.

4. Pengiriman dua mahasiswa S3 MIPA Universitas Airlangga a.n. I Nengah Wirajana,SSI, MSi ke Mie University melalui program *sandwich* Dikti selama 4 bulan (tahun 2009) dan A.A. Istri Ratnadewi (2012). Penelitian yang dikerjakan terkait dengan kelompok enzim xilanase yang dikembangkan oleh ketua peneliti yang sebagian topik melibatkan peneliti mitra.

- **University of Groningen**

1. Kerjasama penelitian dimulai pada tahun 2006 melalui perolehan dana internasional kerajaan Belanda *KNAW-mobility program* yang pendanaannya dilanjutkan pada tahun 2007, 2008, dan 2009 dengan topik utama pada analisis struktur dan fungsi enzim xilanolitik yang merupakan kelanjutan *joint research* dengan Jepang
2. Dalam serangkaian kegiatan kerjasama dengan University of Groningen untuk pertama kalinya *The First Asean Biochemistry Seminar and Workshop* diselenggarakan di Surabaya dan melibatkan berbagai pembicara dari berbagai perguruan tinggi nasional maupun tingkat Asean. Pada tahun 2007, The second Asean Biochemistry Seminar and Workshop dilaksanakan bersama-sama dengan The International Conference and Workshop on Basic Science (ICOWOBAS) yang merupakan joint conference antara Fakultas Sains dan teknologi Unair dengan Universirti Teknologi Malaysia. Pada tahun 2009, *The third Asean Biochemistry Seminar* diselenggarakan untuk pertama kalinya di luar Surabaya, yaitu di Malaysia bersama-sama dengan *Annual Meeting of Malaysian Society of Biochemistry and Molecular Biology*. Hal ini menunjukkan pencetus awal Asean Biochemistry Seminar and Workshop oleh research group telah berkembang dan disejajarkan dengan *International Conference* yang telah ada. Pengembangan *joint conference* ini telah melahirkan *tripartite collaboration* antara Malaysia (Universiti Teknologi Malaysia, Universiti Kebangsaan Malaysia), Belanda (University of Groningen) dan Universitas Airlangga
3. Telah ditandatanganinya Letter of Intent (LoI) antara University of Groningen dan Universitas Airlangga, khususnya Fakultas MIPA (sebelum menjadi Fakultas Sains dan Teknologi)

4. Pengiriman satu mahasiswa S1 dalam pertukaran mahasiswa tahun 2010 dan satu mahasiswa S3 dalam *sandwich program* pada tahun 2012
5. Joint International Collaboration dengan nama *Molecular and Cellular Life Sciences* yang pada periode kedua ini melibatkan University of Groningen, sedangkan pada kegiatan pertama merupakan kolaborasi antara Universitas Airlangga dengan Osaka University, jepang. Kegiatan akan diadakan pada tanggal 17 dan 18 Juli 2017.

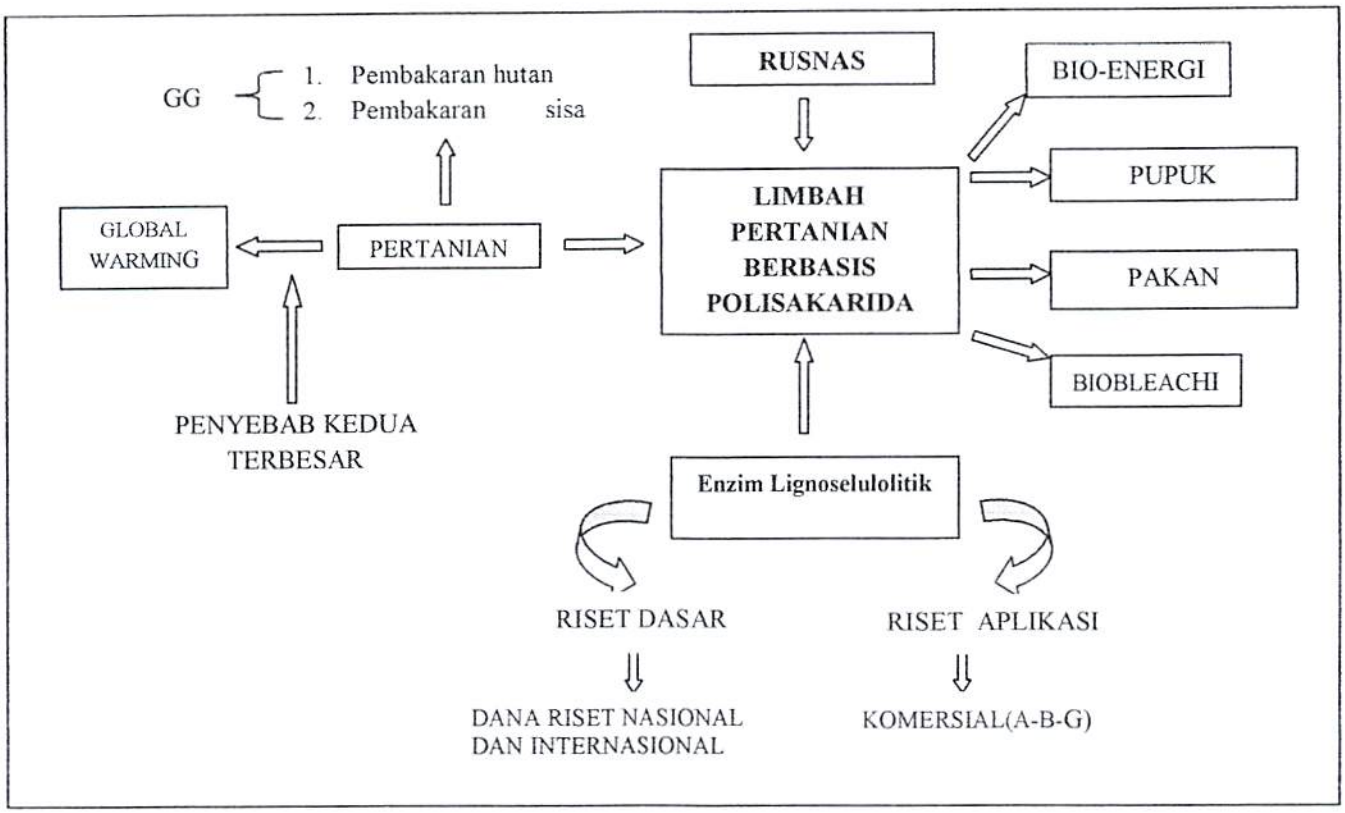
- **Osaka University, Jepang**

1. Sejak tahun 2013 jaringan kerjasama penelitian telah diperluas dengan Institute of Protein Research untuk pengembangan riset proteomic yang tidak hanya focus pada struktur dan fungsi protein, namun juga perangkat spektroskopi dan protein modelling sebagai terobosan baru pengembangan riset rekayasa protein. Pada awal kerjasama ini telah dikirimkan dua orang staf peneliti muda ke laboratorium tersebut dengan biaya Osaka University (2013)
2. Joint International Collaboration *Molecular and Cellular Life Sciences* pertama antara Universitas Airlangga dan Osaka University, tanggal 7-8 Mei 2015. Selanjutnya pada periode kedua ini melibatkan University of Groningen, yang akan diadakan pada tanggal 17 dan 18 Juli 2017.
3. Joint Research melalui skema HKLN tahun 2016 dengan mengirimkan satu staf dosen muda atas nama Ni Nyoman Purwani, S.Si., M.Si. selama 1 bulan untuk studi awal kristalisasi CBM36

- **Kerjasama dengan PT PUPUK KALTIM, PT PETROKIMIA GRESIK, PT PETROSIDA dalam aplikasi EXCELZYME sebagai campuran pupuk organic**
- **Laboratory of Enzyme Technology, Biotec-NSTDA, Thailand**

  Pada tahun 2015, Kerjasama disepakati melalui penandatanganan MoA antara Laboratorium Proteomik, Universitas Airlangga dengan Biotec-NSTDA , Thailand. Kerjasama ini akan diperuntukkan untuk mengembangkan riset hilirisasi pada bidang riset yang sama, yaitu produksi dan aplikasi kelompok enzim lignoselulolitik melalui program *academic mobility* bagi para mahasiswa maupun peneliti atau dosen muda kelompok riset proteomik Universitas Airlangga.

  Pada saat ini satu mahasiswa program magister sedang melaksanakan sebagian penelitian tesis nya di Biotec-NSTDA, Thailand



Gambar 4. ***Road map*** **Pengembangan Riset** ***Excelzyme*** **(Sumber: Puspaningsih, N.N.T dalam** ***Road Map*** **Penelitian Universitas Airlanga, BPP 2007-2015)**

BAB 3. METODE PENELITIAN

Sampel Penelitian

a. Isolat yang digunakan : pETGH5 (5) dan GbtXylGH43B (B)
b. Bakteri penghasil pektinase , lakase, dan selulase hasil eksplorasi dari sumber air panas Gunung Pancar Bogor, yaitu : *Anoxybacillus flavithermus* TP-01, *Geobacillus kaustophilus* TP-02

Bahan Penelitan

Semua bahan yang digunakan dalam penelitian ini memiliki kualitas pro-analis (p.a) kecuali disebutkan lain.

Pertumbuhan sel inang *E. coli* BL21 dilakukan pada media Luria Bertani (LB) dan media terdefinisi modifikasi (MTM). Media LB cair memiliki komposisi (b/v), *bacto-triptone* 1%, *yeast extract* 0,5% dan NaCl 1%. Media MTM sebagai media pertumbuhan untuk optimasi memiliki komposisi (b/v), KH_2PO_4 0,16%, $Na_2HPO_4.2H_2O$ 0,66%, $MgSO_4.7H_2O$ 0,2% serta D(+)-glukosa 1,5% dan $(NH_4)_2SO_4$ 0,1% dan pelarut berupa ekstrak taoge. Ekstrak taoge diperoleh dengan memanaskan 5% taoge hingga memiliki volume akhir separuh

volume awal. Kanamicin digunakan sebagai antibiotik untuk pertumbuhan sel inang *E. coli* BL21 rekombinan pETGH5 (5) dan pETXylGH43B (B).

Reagen DNS yang digunakan untuk analisis aktivitas enzim dan penentuan konsentrasi gula pereduksi terbuat dari campuran asam 3,5-dinitro salisilat, NaOH, kalium natrium tartrat, fenol dan natrium sulfit. Asam sitrat ($C_6H_8O_7$) dan $Na_2HPO_4.7H_2O$ digunakan sebagai bahan pembuat buffer fosfat sitrat pH 6. *Beechwood xylan* dan *carboxy methyl cellulose* (CMC). digunakan sebagai substrat untuk uji aktivitas enzim dengan metode DNS. D(+)-xilosa dan D(+)-glukosa digunakan sebagai standar produk yang terbentuk dalam penentuan aktivitas enzim.

Alat Penelitan

Peralatan yang digunakan berupa peralatan gelas dan non-gelas serta peralatan instrumen. Spektrofotometer UV-Vis (Shimadzu UV-1800), *Vortex* (Labinco L46), *Ultrasonic Homogenizer* (JY 92-IIDN), *Refrigerated Centrifuge* (Hermle Z 400 K), *Incubator Shaker* (Gerhard), *Autoclave* (TOMY), Oven (Memmert), *Water Bath* (GEMMYCO YCW-010), *Laminar Air Flow Cabinet*, timbangan analitik (Ohaus).

Metode Penelitian

Preparasi Inokulum

Satu ose biakan *E. coli* BL21 rekombinan pETGH5 (5) dan GbtXylGH43B (B) diambil dari media padat dan masing-masing diinokulasikan ke dalam tabung yang berisi 5 mL media inokulum yang telah ditambahkan 2,5 µL kanamicin (100 mg/mL). Inokulum diinkubasi dalam *shaker incubator* dengan kecepatan 150 rpm selama 16-18 jam pada suhu 37^{0}C. Sebelumnya, media disterilisasi menggunakan *autoclave* selama 15 menit pada suhu 121°C. Media inokulum berupa MTM digunakan untuk produksi dengan media MTM dan LB untuk produksi dengan LB cair.

Bakteri terseleksi penghasil selulase, lakase dan pectinase yang telah berhasil diidentifikasi sebagai *Anoxybacillus flavithermus* TP-01 dan *Geobacillus kaustophilus* TP-02 selanjutnya ditumbuhkan ke dalam media LB dan diinkubasi sesuai metode standard.

Produksi enzim

Produksi enzim menggunakan media LB dan MTM. Satu persen (v/v) masing-masing inokulum isolat 5, B dan 5+B dimasukkan ke dalam Erlenmeyer 500 mL yang berisi 100 mL media produksi MTM dan media LB cair. Sebelumnya, ke dalam masing-masing media produksi ditambahkan 50 µL kanamicin (100 mg/mL). Kultur diinkubasi pada temperatur 37°C dengan kecepatan 150 rpm selama ±2 jam. Dilakukan penambahan 100 µL IPTG (1 M) setelah inkubasi atau pada OD_{600} antara 0,4 – 0,8 dan dilanjutkan kembali

dinkubasi hingga ±4 jam. Kemudian kultur dipanen dengan sentrifugasi pada suhu 4°C 3500 rpm selama 15 menit. Pelet dilarutkan dengan 5 mL bufer *phosphat citrate* (PC) 6 dan dilisis dengan sonikator 2 menit 80%, kemudian disentrifugasi pada suhu 4°C 3500 rpm selama 15 menit. Supernatan merupakan enzim yang akan ditentukan aktivitasnya.

Uji aktivitas enzim

a. Kultur Campuran

Penentuan aktivitas enzim xilanolitik dengan substrat 1% (b/v) *beechwood xylan* dan aktivitas enzim selulase dengan substrat *carboxy methyl cellulose* (CMC) dalam bufer *phosfat citrate* (PC) 6. Uji aktivitas enzim xilanolitik dan selulase terhadap substrat dilakukan *duplo* pengukuran dengan metode DNS. Aktivitas enzim ditentukan dengan mengukur banyaknya gula pereduksi yang dihasilkan dari hidrolisis substrat. Seratus mikroliter substrat ditambah 100 µL enzim diinkubasi pada temperatur 55°C selama 30 menit. Hasil inkubasi ditambah dengan 600 µL DNS, didihkan dalam penangas air selama 15 menit, kemudian segera didinginkan dalam penangas es selama 20 menit dan diamkan pada suhu ruang selama 30 menit. Absorbansi dibaca pada λ 550 nm. Kontrol yang digunakan diperlakukan sama dengan kondisi di atas tanpa ada proses inkubasi.

Standar xilosa dan glukosa dibuat dengan berbagai variasi konsentrasi 0,1-1 mg/mL dari larutan stok 10 mg/mL. Sebanyak 250 µL larutan standar ditambah dengan 750 µL pereaksi DNS kemudian dikocok kuat atau divortex. Campuran dimasukkan ke dalam penangas air dan dipanaskan selama 15 menit. Kemudian segera didinginkan dalam penangas es selama 20 menit dan diamkan pada suhu ruang selama 30 menit. Absorbansi dibaca pada panjang gelombang, λ 550 nm. Satu unit aktivitas enzim didefinisikan sebagai jumlah enzim yang melepaskan 1 µmol gula reduksi per menit pada kondisi percobaan.

$$Aktivitas\ per\ mL\ ekstrak = \frac{[xilosa]\ per\ ml\ lar\ uji\ x\ Vol\ lar\ uji\ x\ f.pengenceran}{Mr\ xilosa\ atau\ glukosax\ Waktu\ inkubasi\ x\ Vol\ enzim}$$

b. Enzim Pektinase

Aktivitas enzim pektinase ditentukan dari banyaknya gula pereduksi (asam galakturonat) sebagai produk dari hasil reaksi antara enzim dan substrat setelah diinkubasi (Rohishoh, 2012; Zheng, 2000; Li, 2015). Gula pereduksi yang dihasikan diukur dengan menggunakan reagen asam 3,5-dinitrosalisilatatau disebut metode DNS (Miller, 1959). Larutan standar yang digunakan adalah asam galakturonat .

Satu unit enzim didefinisikan sebagai jumlah enzim yang dibutuhkan untuk menghasilkan 1µmol asam galakturonat dalam waktu satu menit pada kondisi percobaan. Aktivitas enzim pektinase diukur berdasarkan rumus unit aktivitas sebagai berikut:

$$\text{Unit aktivitas} = \frac{\text{konsentrasiasamgalakturonatx volume total x Fp}}{\text{waktu inkubasi x volume enzim x BM asam galakturonat}}$$

c. Enzim Selulase

Aktivitas *crude* enzim selulase ditentukan dengan cara menentukan jumlah glukosa yang terbentuk, yaitu dengan menggunakan reagen *3,5-dinitrosalicylic acid* (Miller, 1959). Substrat yang digunakan adalah 1 % karboksil metal sellulase dalam buffer fosfat sitrat pH 7,0. Campuran tersebut diinkubasi pada suhu 60°C selama 30 menit. Reaksi dihentikan menggunakan larutan DNS sebanyak 600 µL dan dipanasakan selama 15 menit bersama-sama dengan control (mengandung 100 µL enzim yang tealh di nonaktifkan ditambah 600 µL DNS dan 100 µL substrat) kemudian segera didinginkan dalam air es selama 20 menit. Selanjutnya, campuran didinginkan, setelah dingin diukur absorbansinya pada λ 550 nm.

d. Enzim lakase

Uji aktivitas lakase dilakukan berdasarkan metode Bourbonnais dan Paice (1990). Prinsip uji ini adalah sebagai berikut : pewarna non-phenol ***Asam 2,2'-azinobis-di-(3-ethylbenzthiazolinesulphonate)*** (ABTS) dioksidasi oleh lakase menjadi radikal kation ($ABTS^{+}$) yang lebih stabil. Konsentrasi radikal kation yang berwarna biru kehijauan (dibaca pada panjang gelombang 420 nm) berkolerasi dengan aktivitas lakase (Bar, 2001)

Aktivitas lakase dinyatakan sebagai International Unit (IU) per liter. Aktivitas enzim sebesar 1 IU didefinisikan sebagai jumlah enzim yang mengoksidasi 1 µmol ABTS tiap menit. Aktivitas lakase dihitung dengan menggunakan persamaan sebagai berikut :

e. $$aktivitas\,(IU/l) = 2\left(\frac{V}{v\,x\,\varepsilon\,x\,d}\right) x\,\Delta A.\min^{-1}\,x\,1000$$

f. Dengan :

g. $\Delta A.\min^{-1}$ = perubahan absorbansi tiap menit pada 420 nm

h. ε = *extinction coefficient* ABTS pada 420 nm = 36 $mM^{-1}cm^{-1}$

i. V = volume total reaksi (ml)

j. v = volume enzim (ml)

k. d = *Light path of cuvette* (cm)

Analisis hasil pemurnian dengan SDS-PAGE

Elektroforesis protein dengan SDS-PAGE menggunakan 2 macam gel poliakrilamid yaitu gel pemisah (*separating gel*) 12% dan gel penahan *(stacking gel)* 5%. Setelah itu set alat elektroforesis Mini Protean II (BioRad) dipasang dan *running buffer* dituangkan ke dalam bejana elektroforesis. Sampel disiapkan dengan memasukkan 20 µL sampel dan 5 µL sampel buffer 5X. Campuran dimasukkan ke dalam penangas air mendidih selama 5 menit. Selanjutnya sampel dimasukkan ke dalam sumur gel penahan dengan bantuan pipet mikro. Proses elektroforesis dimulai dengan menyalakan *power suplay* dan mengatur tegangan menjadi 100 volt. Elektroforesis dihentikan ketika pergerakan protein sudah mencapai sekitar 1 cm dari batas bawah gel pemisah.

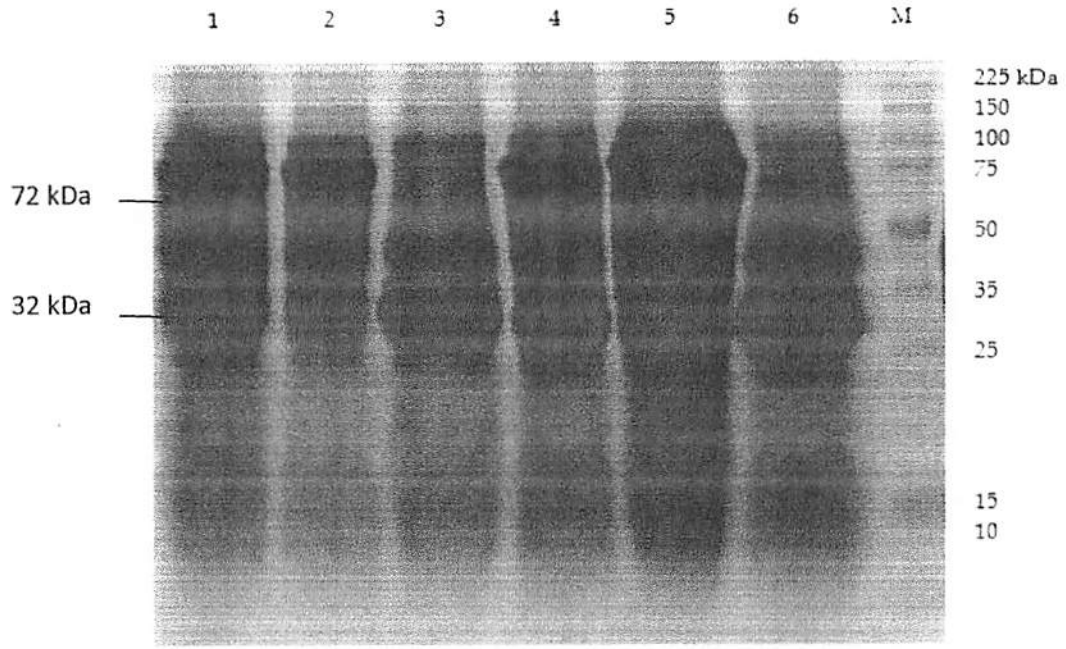
Gel hasil elektroforesis dilepas dari cetakan dan jarak migrasi bromofenol biru diukur dari batas atas gel pemisah. Penampakan pita protein dilakukan dengan merendam gel SDS-PAGE kedalam larutan *staining* selama 1 jam sambil digoyang konstan menggunakan mesin penggoyang. Setelah 1 jam, gel dipindah ke larutan *destaining* untuk memudarkan kelebihan warna pada gel. Proses *destaining* dilakukan sampai diperoleh pita-pita protein berwarna biru dengan latar belakang warna jernih. Berat molekul protein sampel ditentukan dengan membandingkannya dengan berat molekul dari *protein marker.*

BAB 4. Hasil Penelitian

Enzim selulase dan xilanolitik dihasilkan dari isolat *E. coli* BL21 rekombinan pETGH5 (5) dan GbtXylGH43B (B). Data aktivitas enzim terlihat di Tabel 1. Hasil analisis berat molekul ditentukan dengan SDS-PAGE sesuai dengan Gambar 1. Enzim selulase memiliki berat molekul 32 kDa dan enzim xilanolitik 72 kDa.

Tabel 1. Data aktivitas enzim

Media	Isolat	Selulase (U/mL)	Xilanolitik (U/mL)
LB	5	2,49	-
	B	-	1,21
	5+B	2,81	1,22
MTM	5	2,84	-
	B	-	1,26
	5+B	2,61	1,38

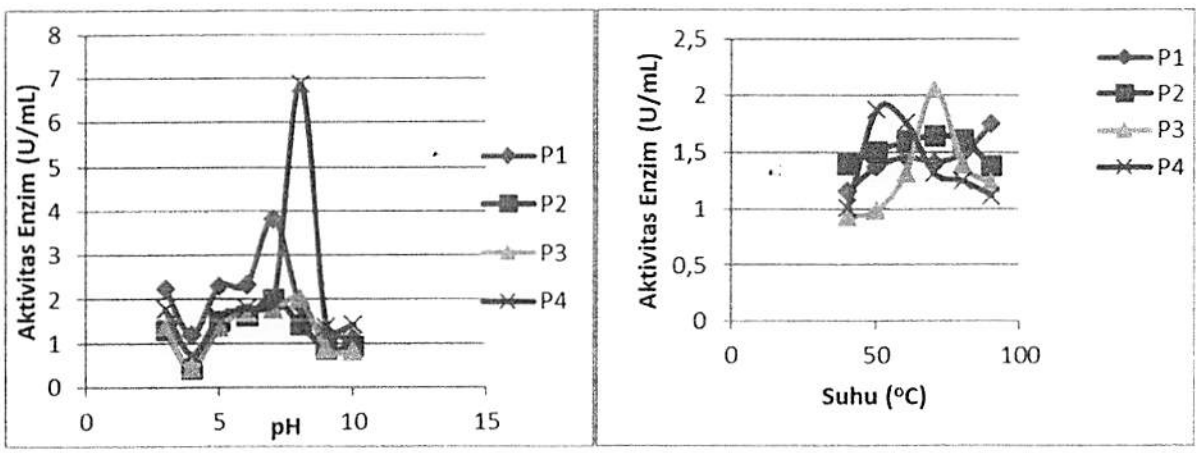


Gambar 1. Hasil elektroforegram SDS-PAGE : 1). 5 (LB), 2). B (LB), 3). 5+B (LB), 4). 5 (MTM), 5). B (MTM), 6). 5+B (MTM).

Karakterisasi Enzim selulase, pectinase dan lakase termofilik

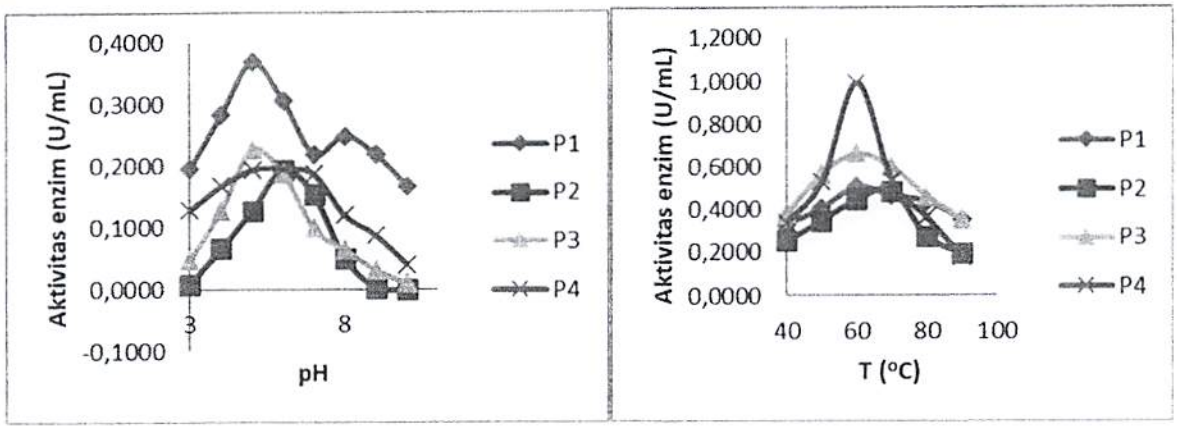
Hasil Optimasi pH dan suhu optimum

Enzim Selulase



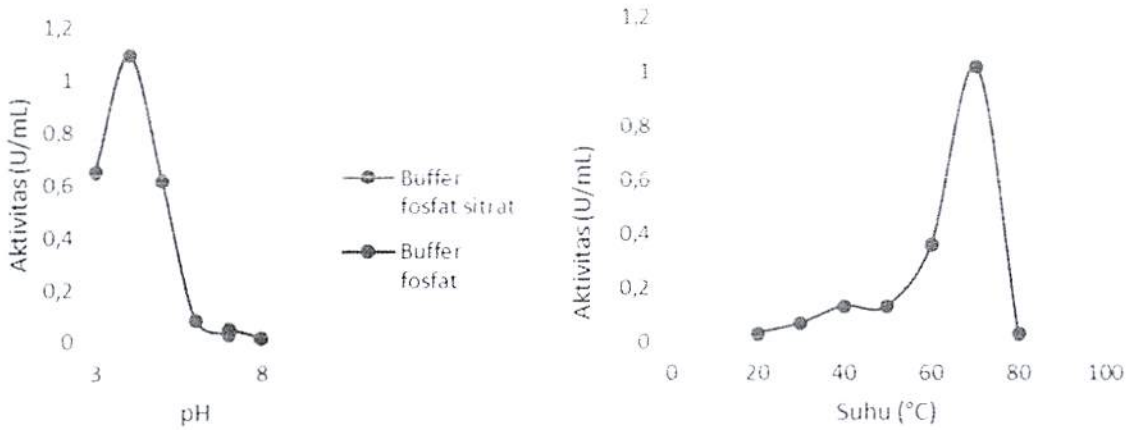
Gambar 2. pH dan suhu optimum enzim selulase dari 4 isolat terpilih

Enzim Pektinase



Gambar 3. pH dan suhu optimum enzim pectinase

Enzim Lakase



Gambar 4. pH dan suhu optimum enzim lakase

Hasil karakterisasi pH dan suhu optimum enzim selulase, pektinase dan lakase pada Gambar 2, 3 dan 4 menunjukkan rentang pH optimum berada masih pada rentang pH asam sampai dengan netral. Sedangkan hasil penentuan suhu optimum menunjukkan rentang suhu optimum masih pada rentang suhu pada enzim termofilik. Hasil tersebut menjadi peluang dan tantangan bagi penelti untuk mendapatkan sifat biokimia enzim yang berada pada rentang lebih basa bila akan diaplikasikan pada industry pulp and paper.

BAB 5. KESIMPULAN DAN SARAN

5.1. Kesimpulan

1. Eksplorasi dan perluasan jenis enzim lignoselulase telah berhasil diisolasi enzim selualase, lakase dan pectinase dari bakteri termofilik Anoxybacillus flavithermus TP-01 dan geobacillus kaustophilus TP-02

2. Kultur campuran selulase dan xilanolitik telah berhasil diproduksi bersamaan dalam satu shake flask dan mampu terekspresi dengan baik

6.2. Saran

1. Optimalisasi produksi kultur campuran masih perlu dianalisis dengan system terkendali bioreactor
2. Peningkatan sifat biokimia kelompok lignoselulase perlu dilakukan

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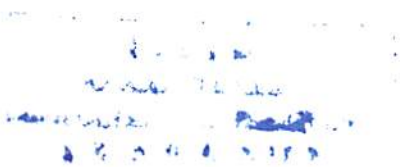

th IUBMB Congress & 15th FAOBMB Congress (IUBMB SEOUL 2018)

ne 4 (Mon) ~ 8 (Fri), 2018 | COEX, Seoul, Korea

April 16, 2018

Ni Nyoman Tri Puspaningsih
Universitas Airlangga
Department of Chemistry
Indonesia

Dear Prof. Ni Nyoman Tri Puspaningsih

Subject: Invitation to the IUBMB SEOUL 2018 Congress

On behalf of the IUBMB SEOUL 2018 Organizing Committee, we are pleased to invite you to the 24th IUBMB Congress & 15th FAOBMB Congress (IUBMB SEOUL 2018), which will be held at COEX in Seoul, Korea between June 4 and 8, 2018. This triennial congress under the theme of '*Integrating Science for Bio-Health Innovation*' will provide a unique opportunity for expert review of international advances at the cutting edge of biochemistry and molecular biology.

As you all know, the IUBMB Congress is one of the biggest international research conferences in basic biological science. Therefore, in this congress, there will be more than 25 symposia covering various research fields from virus to human cancers. Numerous renowned scientists from all over the world including Nobel laureates will be invited for plenary as well as special lectures. In addition, numerous workshops or satellite meetings introducing novel biotechnologies will also be given. In these symposia and lectures, new findings in both basic research and applied technology will be introduced and discussed. Thus, the main purpose of the IUBMB congress is to provide a platform for biomedical researchers or students to socialize and exchange valuable information.

We look forward to seeing you in Seoul, Korea.

Sincerely

Uhtaek Oh
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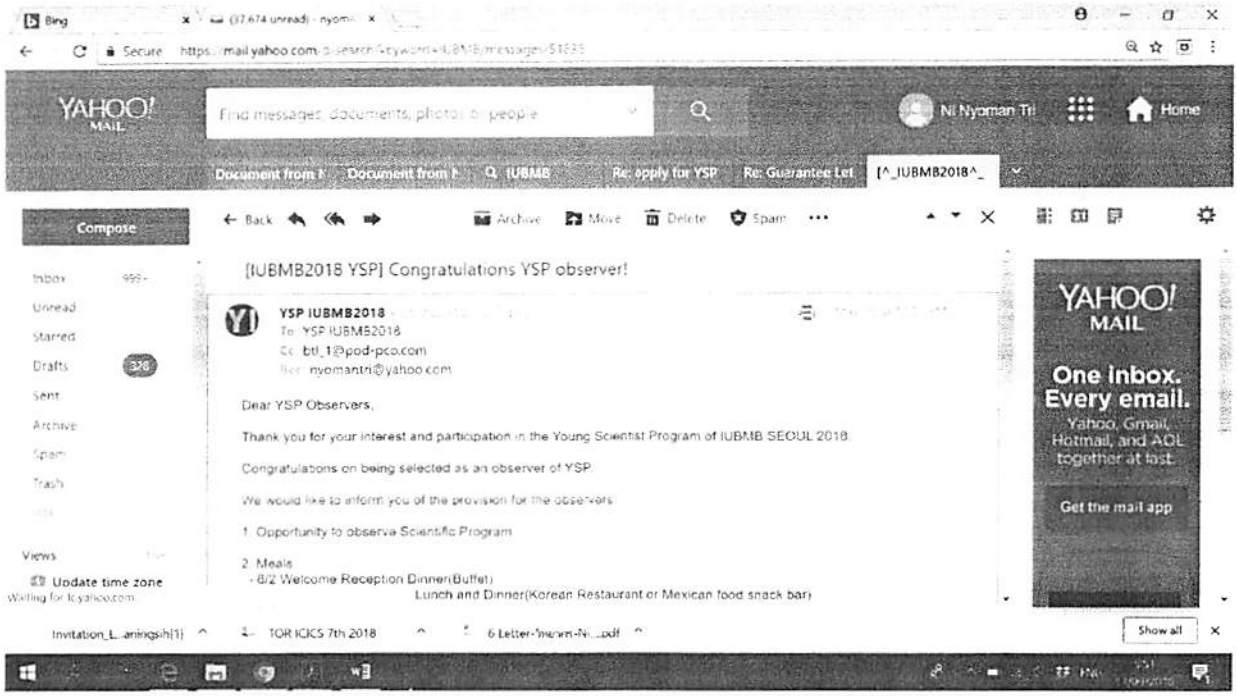
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THE 7th INTERNATIONAL CONFERENCE OF
THE INDONESIAN CHEMICAL SOCIETY (ICICS) 2018
IN PAPUA

Jayapura, 8 April 2018

Dear Prof. Ni Nyoman Tri Puspaningsih

It is my honor and pleasure to invite you as a one of the invited speakers to the 7th International Conference of The Indonesian Chemical Society (ICICS) that will be held in Jayapura, Papua, Indonesia, on September 26-27, 2018.

Indonesia is a country consists of number of islands and provinces, including Papua Province. Papua is the largest island in Indonesia, rich in bio-diversity from both land and sea. Geographically, Papua island is located on tectonic plate, causes it rich on non-biological natural resources such as oil, gas, minerals and mining materials, including gold, copper, coal, limestone, quartz sand, clay, sand iron, and silica. Despite of these abundant, there have been limited number of researches done to explore the potency of Papua's natural wealth.

We would be delighted to have you present at this conference to hear of your thoughts and experiences of Biochemistry field. We truly believe that we will be able to enhance our knowledge from the finding of the research you will share.

I am sure that you will consider to attend this conference and confident that your lecturing will contribute to success this conference. Please find hereby attached the Invitation Letter and Terms of Reference of the Conference and look forward to welcome you in this conference.

Respectfully Yours,

Yohanis Ngili
Conference Chairperson
In ICICS 7th Papua.

RSVP: icics7papua@gmail.com
Our conference website: https://icics2018.fmipa.uncen.ac.id

Department of Chemical and Biological Engineering,
College of Engineering,
Korea University,
Seoul, 136-701,
Republic of Korea

INVITATION LETTER
September 10~14, 2018

The 12th Korea-ASEAN Joint Symposium on Biomass Utilization and Renewable Energy

Ni Nyoman Tri Puspaningsih

[1]Departement of Chemistry, Faculty of Science and Technology,
[2]Proteomic Laboratory, Institute of Tropical Disease , Universitas Airlangga, Kampus C Mulyorejo, Surabaya, East Java 60115, Indonesia.

Ni Nyoman Tri Puspaningsih

We are pleased to invite you to present a paper on "BUILDING RESEARCH CONSORTIUM ON LIGNOCELLULASES TO DEVELOP GREEN INDUSTRY" at the 12th Korea-ASEAN Joint Symposium on Biomass Utilization and Renewable Energy (September 10-14, 2018) in Korea University, Seoul, Korea.

Your participation is very important to the success and continuity of the program. We hope you will be able to attend and look forward to seeing you at the Symposium.

Thank you very much for your cooperation.

Sincerely yours,

Prof. Seung Wook Kim Ph.D
Chairman of the Symposium
Organizing Committee
Korea University

Telephone: +82-2-3290-3300
FAX: +82-2-926-6102
E-mail: kimsw@korea.ac.kr

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http://ambc.mardi.gov.my

Date: 8 March 2018

Dr Ni Nyoman Tri Puspaningsih
Department of Chemistry, Faculty of Science and Technology,
Universitas Airlangga,
Kampus C Mulyorejo,
Surabaya, East Java 60115,
Indonesia

Dear Sir / Madam,
Participation Status in ASEAN Microbial Biotechnology Conference, 24-26 April 2018, Pullman Hotel Kuching, Sarawak, Malaysia.

We are pleased to inform you that your abstract titled:-
Biochemical Properties and In Silico Analysis of α-L-arabinofuranosidase Variant and Its Expression in *Escherichia coli* Extracellular Secretion System

has been accepted for oral presentation

Please make payment before 15 March 2018 to enable us to reserve the slot for you. We believe that your participation will help towards the success of our Conference. If you have any enquiry please do not hesitate to contact us at technical.ambc2018@mardi.gov.my

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Ms One Asmarani,
Proteomic Laboratory, Institute of Tropical Disease,
Universitas Airlangga, Kampus C UNAIR,
Mulyorejo Surabaya 60115,
East Java Indonesia

Dear Sir / Madam,
Participation Status in ASEAN Microbial Biotechnology Conference, 24-26 April 2018, Pullman Hotel Kuching, Sarawak, Malaysia.

We are pleased to inform you that your abstract titled:-
CELL IMMOBILIZATION OF *Streptomyces griseus AND* IT'S VARIANT FOR FRUCTOSE PRODUCTION

is accepted for poster presentation	[X]
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Date: 8 March 2018

Ms Lailatul Fithri
Proteomic Laboratory, Institute of Tropical Disease,
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Universitas Airlangga,
Kampus C-UNAIR,
Mulyorejo, Surabaya 60115

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BIOCHEMICAL CHARACTERIZATION OF LACCASE ISOLATED FROM PALM OIL TRUNK FUNGI AND ITS ACTIVITY TO NATURAL LIGNOCELLULOSE

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Date: 10 March 2018

Ms Anita Kurniati,
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Institute of Tropical Disease,
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Ms Sylvia Aulia Rahmah,
Proteomic Laboratory,
Institute of Tropical Disease,
Universitas Airlangga, Kampus C Mulyorejo 60115,
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Date: 10 March 2018

Ms Ni Nyoman Purwani,
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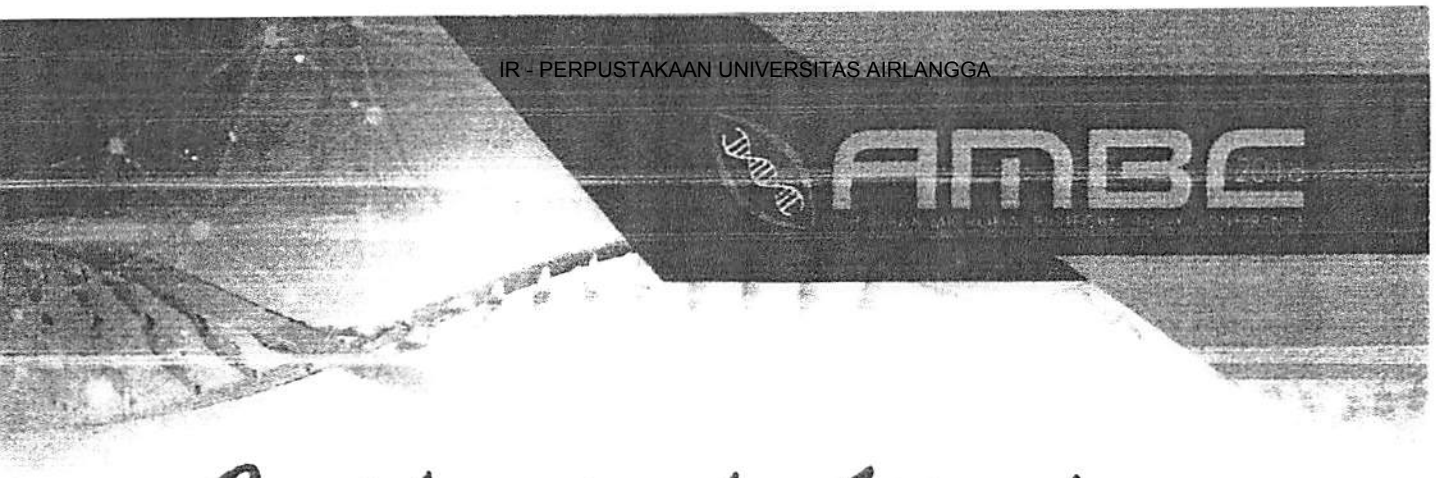

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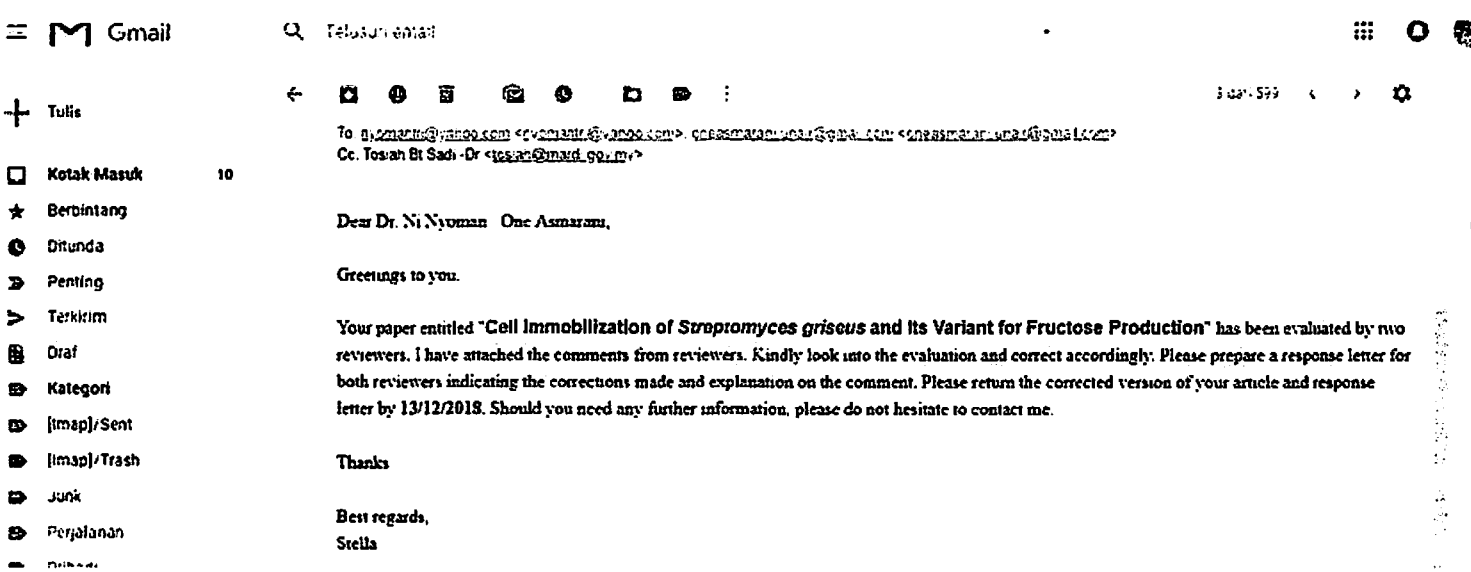
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To
Cc. Tosah Bt Sadi -Dr <tosah@mardi.gov.my>
Dear Dr. Ni Nyoman One Asmarani,
Greetings to you.
Your paper entitled "Cell Immobilization of Streptomyces griseus and Its Variant for Fructose Production" has been evaluated by two reviewers. I have attached the comments from reviewers. Kindly look into the evaluation and correct accordingly. Please prepare a response letter for both reviewers indicating the corrections made and explanation on the comment. Please return the corrected version of your article and response letter by 13/12/2018. Should you need any further information, please do not hesitate to contact me.
Thanks
Best regards,
Stella

Malaysian Journal of Microbiology, Vol xx(x) xxxx, pp. xxx-xxx
DOI: http://dx.doi.org/10.21161/mjm.xxxxx

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Heating Cell Immobilization of *Streptomyces griseus* and Its Variant for Economical Fructose Production

One Asmarani[1], Januar Rachmawati[2], Suci Rahayu[2], Sylvia Aulia Rahmah[1], Purkan[2], Ni Nyoman Tri Puspaningsih[1,2,3*]

[1]Proteomic Laboratory, Institute of Tropical Disease, [2]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, Kampus C UNAIR, Mulyorejo Surabaya 60115, East Java Indonesia
[3]Department of Bioinformatic & Biomedical Science, Asia University, Taiwan
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Received XXX, Received in revised form XXX, Accepted XXX

ABSTRACT

Aims: This study determined the optimum temperature for cell immobilization, the optimum time of fructose production by immobilized cell, and immobilized cell stability against repeated use in fructose production.
Methodology and results: Research on cell immobilization of *Streptomyces griseus* and the variant have been done. The *Streptomyces griseus* variant was resulted from UV mutation. The variant was able to produce fructose as hydrolysis product 3 times as much after 30 minutes. Heating was done at 50, 60, 70, 80 and 90 °C. The fructose production was performed at intervals of 4 hours for 32 hours. The results showed that the optimum cell immobillization temperature of *Streptomyces griseus* and its variant was 80 °C. The optimum time of fructose production by immobilizing cell of *Streptomyces griseus* was 28 hours and its variant cells was 24 hours. Immobilized cells of *Streptomyces griseus* can be reused for 6x28 hours to produce fructose compared to variants cells was 5x24 hours, respectively.
Conclusion, significance and impact of study: This study reported that immobilized cells of *Streptomyces griseus* can be reused and its variant were highly advantageous in the production of fructose. As a result reduced the cost of industrial process, and the fructose produced is also still high.

Keywords: cell immobilization, fructose, Streptomyces griseus, variant

INTRODUCTION

The high demands for sugars and the development of enzyme technology have increased the production of sweeteners, especially for glucose and fructose (Roberto *et al.*, 2009). Fructose is sweeter than glucose and more safety for sugar metabolism. Moreover, D-fructose plays an important role as a diabetic sweetener because it is slowly reabsorbed by the stomach and does not influence the glucose level in the blood (Bhosale *et al.*, 1996). Fructose can be produced from starch through the enzymatic hydrolysis and isomerization. The hydrolysis product of glucose then isomerized by glucose isomerase to produce HFS (High Fructose Syrup). HFS is used worldwide as a sweetener in canned goods, baked goods, processed foods, dairy products, and carbonated beverages. According to Vuilleumier, S. (1993) HFS is prefer used for food industry because it does not pose the problem of crystallization as sucrose does. The glucose isomerase can be isolated from *Streptomyces*, *Bacillus*, *Actinoplanes* and *Arthrobacter* species.

The study using *Streptomyces griseus* was done by: (a) Puspaningsih *et al.* (1992), to simplify the fructose production stage of starch through the process of co-immobilization of glucoamylase enzyme with alginate matrix; (b) Purkan *et al.* (1997), to optimize glucose isomerase production by the addition of xylose and glucose inducers, and (c) Widianti (1999), to increase the production of glucose isomerase by UV mutation. The mutation revealed that *Streptomyces griseus* variant were able to produce glucose isomerase 3 times as much after 30 minutes.

*Corresponding author

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DOI: http://dx.doi.org/10.21161/mjm.xxxxx

The conversion of glucose to fructose by using free GI (Glucose Isomerase) enzyme is not economical because the enzyme is intra-cellular and also high-cost of free enzyme, when used in the solution is not recoverable. This problem could be overcome if the enzyme is immobilized. Current production of high-fructose syrups generally uses immobilized, rather than soluble enzymes. According to Saxena, V. (2016) the advantage of immobilized GI enzyme, it is used for a longer duration due to its long life in the support material and the ability to be reused. Research by Hobbs, L. (2009) showed that using immobilized enzyme technology, it is possible to produce high-fructose syrups containing 42%, 55% or 90% fructose.

The immobilization of whole microbial cells and their applications to bioprocessing have been of interest for nearly thirty years. Immobilization of whole cells for extra-cellular enzyme production offers several advantages, such as the ease to separate cell mass from the bulk liquid for possible reuse, facilitating continuous operation over a prolonged period, enhancing reactor productivity and ensuring higher efficiency of catalysis (Kar and Ray, 2008). Further, the use of immobilized cells as industrial catalysts can be advantageous compared to batch fermentation process (Adinaraya *et al.*, 2005).

Recently, the immobilized whole cell has been regarded as an alternative method of enzyme immobilization. Immobilization of enzymes is a tedious and time-consuming process. Moreover, the cost of isolation and purification of enzymes is high which is not necessary in whole cell immobilization (Yang *et al.*,1988; Elakkiya *et al.*, 2016.). By heating the microbial cells on cell immobilization, it can act as a matrix that isolates enzymes for repeated isomerization reactions (Takasaki, 1969). Heating cell immobilization will be done in this study using *Streptomyces griseus* and its variant, to produce fructose.

The aims of this research were to determine the optimum temperature for cell immobilization by cell heating, the optimum time of fructose production by immobilized cell, and immobile cell stability against repeated use in fructose production.

MATERIALS AND METHODS

Microorganism and culture

The strain of *Streptomyces griseus* and its variant was obtained from the Biochemistry Laboratory, Department of Chemistry, Faculty of Science and Technology Universitas Airlangga. All chemicals were analytical grade purchased from Merck (Germany), Becton Dickinson (France) and Sigma Aldrich (USA).

Solid medium consist of 1% D-glucose, 0.02% $MgSO_4.7H_2O$, 0.5% yeast extract and 1.7% bacto agar. The medium were sterilized by autoclave at 121 °C for 15 minutes. The culture plates were incubated for 5-7 days at 30 °C. For GI production, the spores were transferred to 100 mL of liquid medium consisting of 0.5% yeast extract, 0.3% peptone, 0.3% casein, 0.02% $MgSO_4.7H_2O$, and 1% xylose. After incubation under constant shaking (175 rpm) for 42 hours at 30 °C, the pellet cell was collected by centrifugation at 3000 rpm for 15 minutes and washed with 0.85% NaCl.

Cell immobilization of *Streptomyces griseus* and its variant

The pellet cells of *Streptomyces griseus* and its variant were suspended into phosphate buffer pH 7. Each 5 mL of cell suspension in a covered reaction tube was heated at 80 °C for 24 hours in a water bath. The pellet cell was collected by centrifugation at 3000 rpm for 5 minutes. Those pellet cell was immobilized cells that was ready to be tested for activity. The activity was determined by incubating the immobilized cell and 5 mL of the substrate (0.1 M D-glucose and 0.01 M $MgSO_4.7H_2O$ in phosphate buffer pH 7) in a closed reaction tube with stirring at 65 °C for 5 minutes and determined its concentration by cysteine-carbazole method (Bhatia and Prabhu, 1980).

Determination of optimum temperature of immobilized *Streptomyces griseus* cell and its variant

Pellet cells of *Streptomyces griseus* and its variant were suspended into phosphate buffer pH 7. Each 5 mL of cell suspension in a covered reaction tube was heated at different temperature (50, 60, 70, 80, dan 90 °C) for 24 hours in a water bath. Cell was immobilized cells, then separated by centrifugation at 3000 rpm for 20 minutes and was ready for activity detection. The activity was measured by incubating the immobilized cell and 5 mL of the substrate (0.1 M D-glucose and 0.01 M $MgSO_4.7H_2O$ in phosphate buffer pH 7) in a closed reaction tube with stirring at 65 °C for 5 minutes and determined its concentration by cysteine-carbazole method (Bhatia and Prabhu, 1980).

Determination of optimum time for fructose production by immobilized cell

Optimum time for fructose production was measured by incubating immobilized cell (product of optimum temperature of immobilized cell) and 10 ml substrate in a covered Erlenmeyer with stirring at 65 °C. The fructose production was taken for each 4 hours and determined its concentration by the cysteine-carbazole method (Bhatia and Prabhu, 1980).

Stability of immobilized cell

The stability of repeated usage of immobilized cell was determined by incubating of immobilized cells (products of optimum immobilization temperature) and 5 ml of the substrate in covered Erlenmeyer with stirring at 65 °C. The optimum time of fructose production has been fixed by length of time. In the present study, the length of time is used to incubate reaction mixture. Further,

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immobilized cell was washed with phosphate buffer pH 7 and can be reused for next application until GI activity in immobilized cells was low. This can be seen from the concentration of fructose by the method of cysteine-carbazole (Bhatia and Prabhu, 1980).

Determination of fructose concentration

The fructose concentration was determined by the modified method of cysteine-carbazole (Dische and Borenfreud, 1951). This method is specific for the fructose concentration of 10-60 µg/mL. The reaction was done by mixing of fructose with 1 mL of 2% L-cysteine HCl follow by addition of 5 mL 75% H_2SO_4 and stirred. The reaction is carried out at a temperature of 4 °C. Then 0.15 mL of 0.12% carbazole was added into solution and stirred. The reaction mixture was heated at 40 °C for 30 minutes, moved to 4 °C, then kept the reaction at room temperature for 4 minutes. The colour formation was measured by absorbance at wavelength range 560-565 nm. The standard fructose curve was made with varying concentrations of 5-30 µg/mL

RESULTS AND DISCUSSION

Production of GI enzymes in *Streptomyces griseus* cells and its variant

The GI enzyme is intra-cellular and inductive enzyme (Bhatia and Prabhu, 1980). To produce a maximum GI enzyme, an appropriate inducer is required. Purkan *et al.* (1997) reported that the xylose inducer increased GI production compared to the glucose and glucose-xylose mixed inducers. Beyond as an inducer, xylose also as a carbon source, while nitrogen sources are obtained from peptone, casein hydrolysate and yeast extract. Phosphate is used to synthesize nucleic acids and as a source of energy. Magnesium and Sodium are used for cell wall synthesis, nucleic acid and maintaining membrane structure (Crueger and Crueger, 1989). Mg^{++} also serves as an activator (Godfrey and Reichelt, 1983)

Puspaningsih *et al.* (1992) determined the growth curve of *Streptomyces griseus* using dry weight measurement. The result showed that the maximum dry weight cell growth of *Streptomyces griseus* was 42 hours, while *Streptomyces griseus* variant can be harvested after 10 hours respectively (Widianti, 1999). At the time of the cell harvest, the GI activity of *Streptomyces griseus* was the highest. The growth difference between *Streptomyces griseus* and its variant occurs due to the errors on translation process, thus causing important proteins/enzymes needed for cell metabolism during the growth phase are not functional (Puspaningsih, 1995).

Determination of optimum temperature of immobilized *Streptomyces griseus* cell and its variant by heating

The breakdown of cells without destruction of the enzyme makes the GI more expensive (Bailey and Ollis, 1986). Therefore, in this study we used microbial cells, for immobilization process that will be more simple and economical (Jack and Zajic, 1977).

Determination of optimum immobilization temperature is important for generating high GI activity in immobilized cells. The amount of activity can be known from the concentration of fructose as the product. Cell immobilization has some advantages when compared with free cell culture. The reaction speed can be accelerated, a high dilution rate can be used in continuous fermentation without cell washing, and it is less susceptible to the effect of inhibitory compounds and nutrient depletion (Marques *et al.*, 2006). The main advantage of immobilization confers to whole cells are low sensitivity to temperature and pH when compared to free enzymes (Lee *et al.*, 1984). The process of immobilization was done by heating. Takasaki (1969) reported that the type of microbial cell immobilization by heating without chemical treatment. When *Streptomyces sp* cells having high GI activity was heated at 60-85 °C for 2-20 minutes, the GI will be retained within the cell. It was stated that the enzyme trapped in the cell would not come out of the cell, although the cells were heated for a long time and the conditions were suitable for the extraction of enzymes from cells with autolysis (Chibata, 1978). Heating causes inactivation of other enzyme intrusions that contribute to autolysis and retains GI enzymes, thus immobile cells may be used repeatedly (Bhatia and Prabhu, 1980).

In this study, immobilized cell temperature of *Streptomyces griseus* varied between 50-90 °C. The result of optimum immobilization temperature can be seen in Table 1 and Figure 1, while the optimum immobilization temperature of *Streptomyces griseus* cell and its variant were 80 °C.

Table 1. Data of immobilization temperature variation by heating

Temperature (°C)	Fructose concentration (µg/mL)	
	Streptomyces griseus	Variant
50	37.7164	63.9398
60	43.3582	94.9716
70	43.6716	96.5479
80	51.7313	101.6708
90	43.1940	95.3657

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Figure 1: Curve of immobilization temperature variation by heating

Determination of optimum time of fructose production by immobilized cell

The enzymatic production of fructose can be obtained by isomerization of glucose by isomerase glucose enzyme (GI) (Bailey and Ollis, 1986). The determination of the optimum time of fructose production is very important in producing high fructose levels. In this condition, the GI in immobilized cells will work on the optimum activity in converting glucose to fructose.

The immobilized cell was contacted with the substrate at 65°C with stirring. Each 4-hour interval of fructose is formed to determine its concentration. The optimum timing result of fructose production by immobilized cell can be seen in Table 2 and Figure 2. It showed that the optimum time of fructose production of *Streptomyces griseus* immobilized cell was 28 hours and its variant was 24 hours. GI catalyze the reversible reaction of glucose to fructose, and reverse fructose to glucose until reach the certain equilibrium point (Wiseman, 1985).

Table 2: Data of optimum time of fructose production by immobilized cell

Fructose Production time (hours)	Fructose concentration (µg/mL)	
	Streptomyces griseus	Variant
4	57.9851	121.7686
8	81.0746	139.1078
12	100.4478	152.9064
16	122.4129	167.0870
20	135.8806	176.9388
24	151.4228	200.1892
28	172.9851	184.0322
32	94.4029	-

Stability of immobilized cells

The immobilized cell stability test for repeated use was conducted to find out how long the immobile cell can be used for the isomerization reaction of glucose to fructose. The results of stability testing of immobilized cells against repeated use in fructose production can be seen in Table 3 and Figure 3. The immobilized cells of *Streptomyces griseus* cell can be used repeatedly for fructose production for 6 x 28 hours, and its variant for 5 x 24 hours.

Figure 2: Curve of optimum time of fructose production by immobilized cell

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Table 3: Data of repeatedly used of immobillized cell

Number of use	Fructose concentration (µg/mL)	
	Streptomyces griseus	Variant
1	179.4925	124.3301
2	167.6119	122.1627
3	142.4179	121.1775
4	135.9701	116.2516
5	131.0746	93.7894
6	87.4925	90.0457

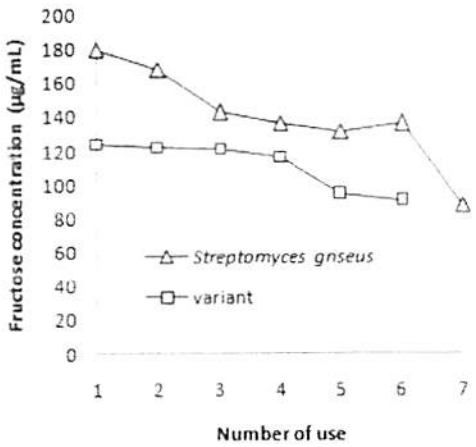

Figure 3: Curve of repeatedly used of immobillized cell.

From the Fig. 3, we observed that whenever immobilized cell were used, the resulting activity were decreases. This was assumed due to changes in the conformation of the enzyme's active center due to too frequent contact with the substrate. In the first application, the conformation of the active center of the enzyme is allegedly slightly altered, the greater the permanent damage occurs when too often contacted with the substrate. This situation causes the decline in the activity (Tyasrini, 1994). The results of this study indicated that the reused immobilized cells is very advantageous in the production of fructose, because in addition to reduce costs, the fructose produced is also still high.

CONCLUSION

Cell immobilization of *Streptomyces griseus* and its variant by heating is potentially used for fructose production.

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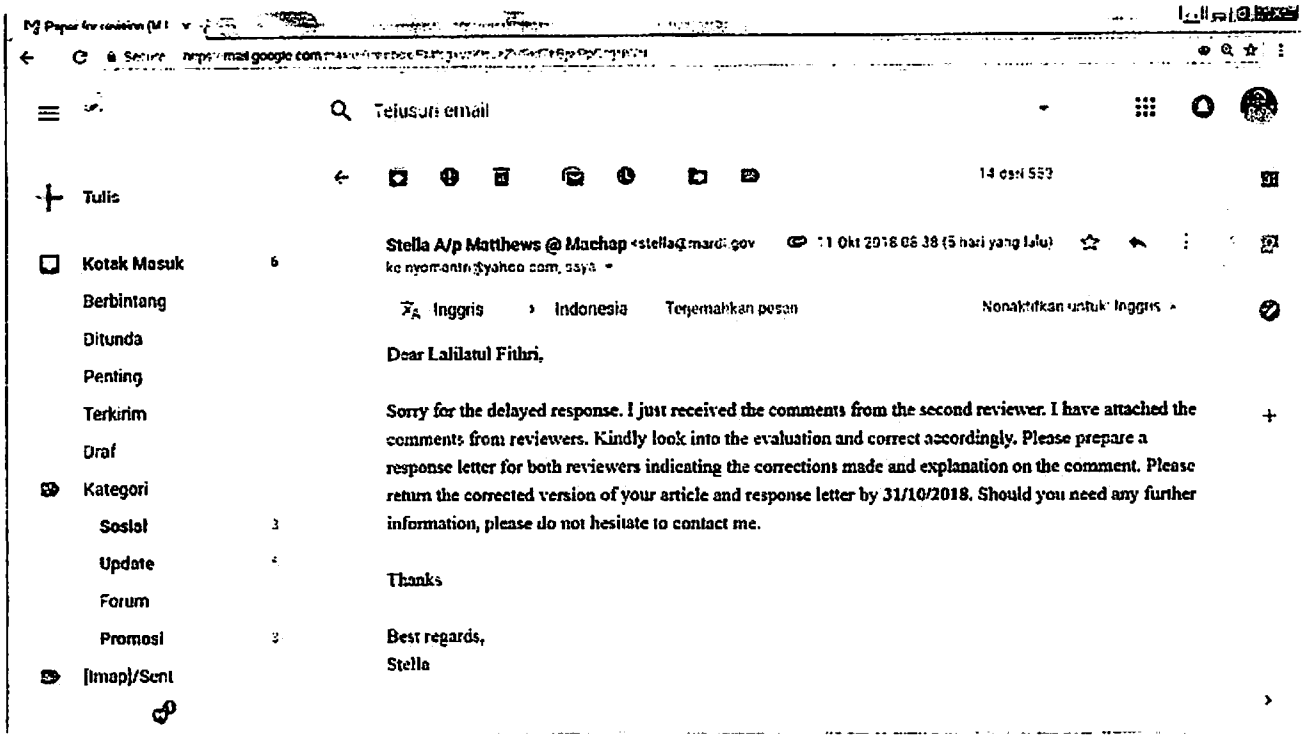

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Characterization of Fungal Laccase Isolated from Oil Palm Empty Fruit Bunches (OPEFB) and Its Activity to Cereal of Agriculture Waste

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ABSTRACT

Aims: Indonesia as an agricultural country has great potential in the agricultural sector. As a consequence, in recent years there has been a growing and persisting demand towards lignocellulosic waste. Laccase is metalloenzyme that can degrade lignin. Oil palm empty fruit bunches (OPEFB) is one of the lignin-containing waste. Laccase was isolated from the fungi of oil palm empty fruit bunches. The present study is designed to isolate and determine the activity of laccase from fungi of oil palm empty fruit bunch, also partial purifications with ammonium sulphate and observed the natural substrate surface profile after laccase adding treatment by Scanning Electron Microscope (SEM).
Methodology and Result: The laccase activity, temperature and pH optimum, temperature and pH stability characterization that obtained from fungi were measured by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid). Fungal laccase has been partial purificated with ammonium sulphate. The result showed that laccase has successfully isolated and characterized from A, B, C, and D fungi of oil palm empty fruit bunches. The characterization of fungal laccase showed that the optimum temperature between 40-50 °C and optimum pH between 5-7. Furthermore, the purification level of the Fungus lacc-A, B, C, and D showed 1.547, 1.769, 1.996 and 3.044 times, respectively. The results showed that natural substrate profile have more efficient than activity of crude extract. Surface profile of cereal waste of corn cobs and rice straw as a part of natural lignocellulose waste that has been treated by laccase is damaged, hollow, and broken, its indicated that laccase succesfully catalysis the lignin degradation.
Conclusion, Significance and Impact of Study: The Fungus lacc-D has the highest level of laccase concentration than other fungus and its activity to cereal agriculture waste is can degrade lignin on the substrate.

Keyword: *laccase; lignin; fungi; Scanning Microscope Electron*

INTRODUCTION

Indonesia as an agricultural country has a great potential in the agricultural sector. Every year there is a rapid development in the agricultural processing industry. Palm oil is one of the export commodities from the plantation sector which has an important role in the economy in Indonesia. Based on BPS data for 2016, Indonesia's palm oil production reaches 31.40 million tons per year. As much as 25-26% of the total palm oil production is empty bunches which are by products (Dian, 2018), and only as much as 10% of the oil palm empty fruit bunches waste has been used for boiler or compost raw materials, and the rest is still in the form of waste.

Oil palm empty fruit bunches (OPEFB) contain lignin of 25.83% (Sudiyani, 2009), this component is high enough so that it can be ascertained that the fungus contained from oil palm empty fruit bunchs contains laccase which can integrate the lignin content. Fungi that found in woody plants have been reported to be able to degrade lignin (Arora and Gill, 2000), so it can be ascertained that fungi are one of the organisms that have active enzyme laccase.

In recent years, many studies have been developed to overcome the abundance of lignocellulosic waste. Lignocellulosics are one of the renewable plant resource (da silva *et al.*, 2013). By converting these lignocellulosic waste into useful materials, such as organic fertilizer,

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prebiotic, and alternative energy of bioethanol will increase the value-added of lignocellulose. However, the utilization of these wastes has not shown the maximum results, such as the utilization of rice straw waste into bioethanol.

In general, to overcome the high content of lignin is to pretreatment with chemicals that are acidic solutions or alkaline solutions. However, the use of chemicals in lignin degradation pretreatment can cause new problems for the environment. If the handling is not perfect, then these chemicals can pollute the environment. In order to overcome the problem, there is an alternative method by using degradation enzyme it can degrade lignin. ie laccase.

Laccase is commercial by the name EC 1.10.3.2, p-diphenol oxidase. This enzyme can be isolated from fungi or mushroom (Dwivedi, *et al*, 2011). Laccases were metalloenzyme that acts as lignin degradation on biocatalytic process (Kameshwar and Qin, 2016). Due to this attention, the laccase is used in the pulp, paper and textile industries to obtain efficient and environmentally-friendly product because this enzyme only requires oxygen and produces water as the only side product (Riva, 2006). In addition, laccase catalyse the oxidation process of phenolic and non-phenolic aromatic compounds effectively and spontaneously by reducing molecular oxygen to water. Laccase is oxidizing enzyme group. It can oxidize various substrates such as diphenol, substituted methoxy-phenol, aliphatic and aromatic amines to radical form and reduce oxygen to water (Muthukumarasamy dan Murugan, 2014).

The utilization of laccase is an alternative to overcome lignin interference of lignocellulosic waste. The laccase enzyme works specifically to degrade lignin. Lignin degradation causes the lignin complex with hemicellulose and cellulose disconnected. After the lignin complex with hemicellulose and cellulose disconnected followed by hemicellulose and cellulose degradation by xylanase and celluiase (Chandra *et al*, 2015). In addition to the specificity of enzyme performance, another reason for using laccase as an alternative solution is the rate of reaction. Moreover, enzyme-catalyzed reactions often last several thousand to over one million times faster than non-enzymatic reactions (Pelczar and chan, 1986).

This study aims to isolate the fungal laccase from oil palm empty fruit bunches (OPEFB) and optimation of the temperature and pH during mycelium growth to study the activity of fungal laccase were obtained from isolation. Addition of fungal laccase to corn cob and rice straw waste were studied to know the effect of fungal laccase adding to break down the hemicellulose and cellulose bond on the lignocellulose waste.

MATERIALS AND METHODS

Selection of Isolate

Fungi was isolated from OPEFB located at pal oil farm of Riau, Indonesia. The growing colonies are planted in black liquor solid medium by taking fungi spore using a sterile ose. The culture was then placed on a selective black liquor medium in a petri dish, incubated at 37°C for 5 days. The halo (clear area) observed around the colony and one isolate with the largest halo and also showed the highest laccase activity.

Laccase profile during mycelium growth of fungi from OPEFB

The growth curve was carried out by dry cell weight measurement. A selected fungus grown on Glucose Malt Yeast (GMY) medium. The inoculum was incubated at room temperature, shaken at 150 rpm for 10 days. The mycelium mass was measured everyday. The incubated GMY media was centrifuged at 3300 rpm, 4 °C for 2 min.

Determination of laccase activity

The Enzyme assay was carried out using a Bourbonnais and Paice (1990) method. Laccase activity was determined by adding 40 µl of enzyme solution to 1160 µl ABTS (0,4 mM) dissolved in sodium-acetic buffer (pH 4.5) (Kalyani, *et al.*, 2008). Then mixtures were shaken until homogenous. Subsequently, the absorption of radical cation was measured at λ = 420 nm (ε_{mM} = 36 $mM^{-1}.cm^{-1}$) during five minutes by spectrophotometer UV-Vis (Bar, 2001). One unit of laccase activity was defined as the amount of enzyme to release 1 µmol ABTS per minute. The protein concentration was performed by using the Bradford method (Bradford, 1976).

Characterization of Laccase

The laccase is an extracellular enzyme. The laccase is secreted by fungus in its production medium. To obtain the laccase enzyme, growth media is separated from the fungus cells by filtering. Furthermore, the growth medium was centrifuged to obtain the supernatant used in the characterization of lacase. In addition, centrifugation was done at 3300 rpm at 4 °C for 20 minutes. After centrifugation, the supernatant is separated from the precipitate. This supernatant is a lacase that will be used in further analysis.

The optimum pH of laccase was measured in the pH range between 4.0 and 8.0, and the optimum temperature was measured at diverse temperatures from 20 to 80 °C for 10 h.

Ammonium sulphate precipitation

Proteins were sequentially precipitated from 100 mL of this crude extract by stepwise addition of solid ammonium sulfate with stirring at a certain degree of saturation (Bollag *et al.*, 1996), followed by incubation on ice and stirred slowly for at least 30 min and centrifugation at 6000 rpm at 4 °C for 20 minutes. The pellet obtained after each centrifugation was resuspended in 0.1 M phosphate-citric

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buffer pH 7. Those steps above were done for 40-100% of ammonium sulfate saturation. respectively.

Dialysis

The cellophane tube containing the laccase is stirred and immersed in a 0.05 M phosphate buffer solution of pH optimum. This dialysis process continues until the enzyme solution free from the ammonium sulphate salt. The dialysis process is declared complete when the enzyme solution is free of ammonium sulphate characterized by the absence of a brown precipitate when the buffer solution is dropped with Nessler reagents. After dialysis, the enzyme solution is removed from the cellophane tube and tested for its activity with the ABTS substrate to determine the purity level of the laccase.

Pretreatement of cereal agriculture waste using fungal laccase

Five grams sample of rice straw and corn cob added with 5 mL of laccase until the sample is immersed in the enzyme. The mixtures were incubated at 70 °C for 8 hours. The surface profile of corn cob and rice straw as part of cereal agriculture waste analyzed using Scanning Electron Microscope (SEM).

RESULTS AND DISCUSSION

Isolation of fungi from oil palm empty fruit bunch

Isolation of fungi from oil palm empty fruit bunch was done by spread plate method. Five grams of OPEFB was dissolved in sterilized water to minimaze the possibility of contaminants. The contaminats are other organisms that growing on OPEFB. Furthermore, sterilized water containing spores were grown on Potato Dextrose Agar (PDA) medium for 5 days at room temperature. After incubation, it molds to grow in PDA media with varying colours. The fungus growing in PDA medium with colour variations indicates that they heterogeneous (Fig. 1a). The picture shows that there are fungi that grow on PDA medium. Furthermore, each of these fungi is purified on slanted PDA medium. Purification was done by slanted PDA medium then inoculated each spore on slanted PDA medium. The pure fungus is characterized by a uniform color of fungus that grows on slanted PDA medium (Fig. 1b).

Selection of fungus isolate

The fungus of A, B, C, and D are selected for their abilities to degrade lignin. Fungi were grown in black liquor selective medium and observed for halo area. Selective media contains a source of carbon and minerals. Black liquor medium is made from a mixture of black liquor and salts. Black liquor serves as the main Carbon source (C), whereas the salts serve as a mineral source. Black liquor is the soluble solution of cooking chemicals in the pulping process, which contains many lignins. Black liquor still contains the chemical compounds of Sodium Hydroxide (NaOH) and Sodium Sulfide (Na_2S) which is alkaline (Wallberg *et al.*, 2003). On black liquor was added 1 N Hidrochloric acid (HCl) in order to become neutral pH and not interfere with fungus growth process.

Lignin is the only source of Carbon (C) in the selective medium. The halo area has been seen in lignin contained in selective media degraded by fungus. The halo area is a clear region of the medium around the fungus. The halo area is formed due to fungus secreting extracellular enzymes that can degrade lignin in selective medium ie laccase. The area of the halo is proportional to the laccase activity. Large halo areas exhibit a large laccase activity. From the results of this selection, it is known that all fungi have the laccase activity but the different of the large halo area were not significant. Its showed that the fungi have the same laccase activity.

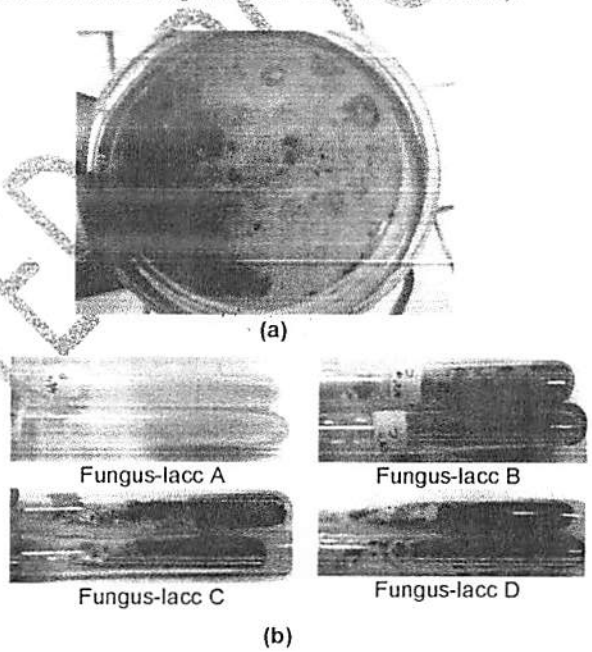

Figure 1: (a) Fungus colony from the isolation of OPEFB, **(b)** A pure colony of fungus isolation (Fungus-lacc A, B, C and D)

Laccase profile during mycelium growth of fungi from OPEFB

Growth curve of fungus was made for 14 days. After the dry weight of each of the stem cells is weighed, a grow curve is made by comparing the dry weight of the cell and the time of sampling.

The result show different growths for each fungus. Based on the growth curve, can be determined rapid growth phase (log phase) where cells divided rapidly and constantly. The next phase, stationary phase, the number of stem population is almost unchanged. The absence of changes in the stationary phase is due to the decreased

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nutrients while the microorganisms continue to divide (Schlegel and Schmidt, 1994). After the stationary phase, there is a decrease in the dry weight of the cell. The decrease in dry weight of the cell showed that the cell had a lysis that resulted in death. This phase is called the death phase. In this phase, the growth rate is lower than the speed of death.

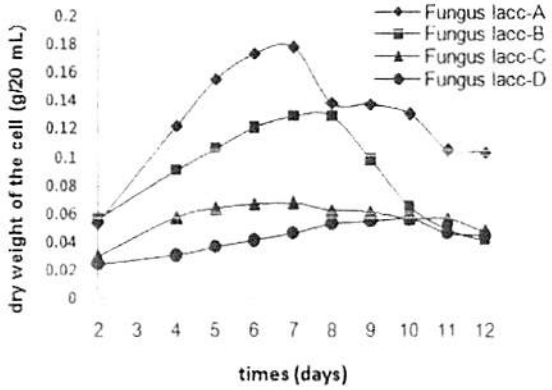

Figure 2. Laccase profile during mycelium growth of Lacc-A, B, C and D

The speed of fungus growth decreases because the nutrients contained in the medium have been exhausted. The log phase of fungus lacc-A urdergous the log phase of fungus lacc-B on the 2nd day until the 6th day. Stationary phase on day 7 and day 8. Further, experience death phase after day 8. Fungus lacc-C experienced log phase on the 2nd day until the fourth day. Stationary phase on day 5 and day 7. Then experience death phase after day 7. Fungus lacc-D has the longest lag phase ranges from day 2 to day 8. Stationary phase on day 8 and day 10. After the 10th day, fungus lacc-D has a phase of death (Fig. 2).

Based on the growth curve, fungus lacc-A showed the higest activity of laccase and the change of log phase is significant that indicated the high cell division. Fungus lacc-D showed the longest lag phase range was assumed as the long adaptation of the fungus to medium.

Characterization of Fungal Laccase

Optimum temperature and stability

The effect of temperature on laccase activity was observed at temperature of 20-80 °C. Temperature is related to the enzyme's energy required to perform a reaction. If the temperature is too low then the enzyme does not have enough energy to perform the reaction, so the reaction can't run optimally. At the optimum temperature, the energy obtained is equal to the enzyme's energy required to react so that the reaction can go well (Champe and Harvey, 1994).

The highest activity of laccase of fungus lacc-A was obtained at 50 °C with activity 5.5 U/mL. As the temperature increased, activity begins to decreased and at 60 °C the enzyme activity decreases by 75.76% of the activity at the optimum temperature. The highest enzyme activity of fungus lacc-B was obtained at 40 °C with 2.99 U/mL activity. When the temperature is raised, activity begins to decrease at 80 °C to only 0.999 U/mL. The activity decreased by 66.67% from activity at optimum temperature. Fungus lacc-C has an optimum temperature of 40 °C with 3.77 U/mL. At 80 °C the enzyme activity is only 0.8333 U/mL. The activity decreased by 97.79% activity from the optimum temperature. The highest activity of laccase from fungus-lacc D was obtained at 50 °C with activity 4,4999 U/mL. At 80 °C the enzyme activity is 0.8333 U/mL. The activity decreased 81.48% of the activity at the optimum temperature (Fig. 4). The high optimum temperature of each fungus are very beneficial for the pulp and paper industry.

The fungus lacc-A was found stable at 50 °C until 8 h and retained 51.52%, fungus lacc-B at 40 °C until 6 h and retained 69.45%, fungus lacc-C at 50 °C until 6 h and retained 62.25%, and fungus lacc-D at 50 6 h °C until 54.59% (Fig. 5). In the other research showes the laccase from *A. flavus* found stable in a temperature range of 25-50 °C (Kumar *et al.* 2016)

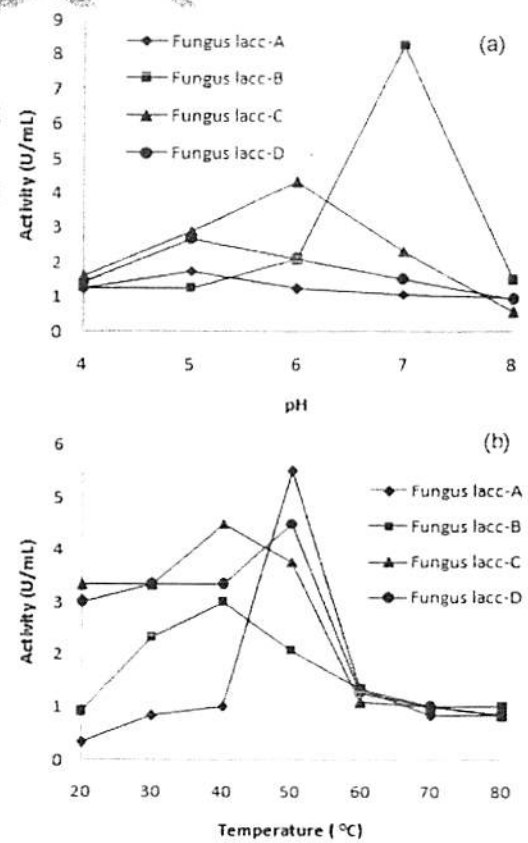

Figure 4: (a) The optimum pH and, **(b)**Temperature of fungus lacc-A, B, C and D

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pH optimum and stability

It was evident that the pH significantly effect activity from fungal laccase. The activity of fungus lacc-A has increased from pH 4 to 5 and achieved the highest activity of 1.7499 U/mL at pH 5.

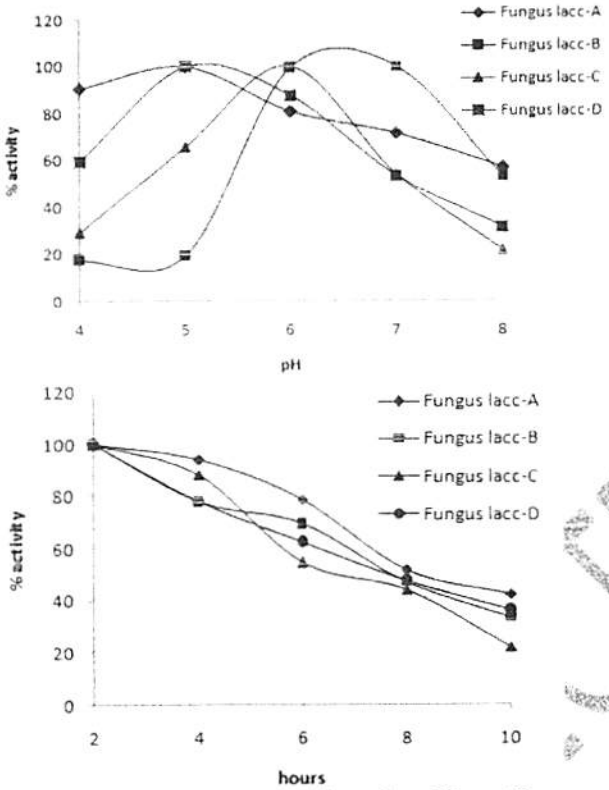

Figure 5: The stability pH and temperature of fungus lacc-A, B, C and D

The fungal laccase from fungus lacc-B has increased from pH 6 to 7 and reached the highest activity of 8.2499 U/mL at pH 7. The laccase activity of fungus lacc-C elevated activity of pH 4 to 6 and achieved the highest activity of 4.333 U/mL at pH 6. The laccase activity of D fungus has increased from pH 4 to 5 and reached the highest activity of 2.666 U/mL at pH 5. After passing each optimum pH, the laccase activity decreased.

The fungus lacc-A was stable between pH 4.0-8.0 and the laccase activity retained 57.14%, fungus lacc-B was stable between pH 6.0-8.0 and retained 52.52%, fungus lacc-C was stable between pH 5.0-7.0 and retained 65.38%, fungus lacc-D stable between 4.0-7.0 and retained 59,37% (Fig. 5).

This decrease in activity is probably caused by conformational changes between enzymes and substrates due to pH changes. Based on the above curve, it is known that all fungi have a good activities on 5 to 7 pH, its compatible to paper industry. The pH of the writing and printing papers varies from 5.0 to 7.5 and hence xylanases working in acidic pH are more effective for deinking of old newspaper pulp (Dutt et al., 2013). The result are similar to the laccase from *T. orientalis* (Zheng *et al.*, 2017) and *A. flavus* (Kumar *et al.* 2016). It was well known that fungal laccase catalyze the ABTS substrate at optimum pH around 5.0 and lose their activities at pH over 7.0 (Zheng, *et al.*, 2017; Kumar *et al.*, 2016; Castano, *et al.*, 2015).

Concentration of laccase with Ammonium sulphate

The precipitation of the crude extract of laccase with ammonium sulphate was done to separate the other proteins contained in the crude extract of the enzyme. Thus, the laccase that precipitates with ammonium sulphate salt will be detected and has a higher specific activity compared with the crude extract.

The addition of ammonium sulfate salt into the protein solution will attract water molecules that initially protect the surface of protein molecules, consequently each protein settles at the optimum saturation of ammonium sulphate The presence of ammonium sulphate serves to attract water trapped in the hydrophobic area, thus allowing the aggregation and precipitation of enzyme molecules. Specific laccase activity test results from fungus lacc-A amounted to 0.655 U/mL, fungus lacc-B of 0.918 U/mL, fungus lacc-C of 1,259 U/mL, and fungus lacc-D 1.336 U/mL. The next stage is dialysis with the aim of removing the ammonium sulphate salt that precipitates with the protein, the enzyme. In the process of dialysis, there is a process of separation of proteins from molecules that have smaller sizes.

The ammonium sulfate precipitated optimization enzyme was dissolved in the optimum pH buffer of each shell, then the cellophane tube was immersed in a buffer solution having a lower concentration than the buffer solution in the tube. This is so that the ammonium sulphate salt can go out to the lower concentration of buffer solution. The specific activity of laccase from dialysis is increasing because the enzyme solution is free from ammonium sulphate salts and other proteins dissolved in the supernatant, so that the specific activity of the laccase enzyme is higher. Specific laccase enzyme activity test results from fungus lacc-A were 1.083 U/mL, fungus lacc-B was 1.372 U/mL, fungus lacc-C was 1.527 U/mL, and fungus lacc-D was 2.218 U/mL. The degree of purity of laccase resulted from precipitation of ammonium sulphate salt for fungus lacc-A is 1,547 times, fungus lacc-B fungus is 1.769 times, C fungus is 1.996, and fungus lacc-D fungus is 3.044 times from its crude extract.

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Table 1: Purification of laccase from empty fruit bunch of palm oil fungi

Fungus	Step of Concentration	Total Activity (U/mL)	Protein conc. (mg/mL)	Specificy Activity (U/mg)	Yield (%)	Purity Level
Fungus lacc-A	Crude extract	1,4	1,998	0,7	100	1
	$(NH4)_2SO_4$	0,7	1,068	0,655	50	0,935
	Dialysis	0,462	0,426	1,083	33	1,547
Fungus Lacc-B	Crude extract	1,54	1,986	0,775	100	1
	$(NH4)_2SO_4$	0,728	0,792	0,918	47,272	1,185
	Dialysis	0,42	0,306	1,372	27,272	1,769
Fungus Lacc-C	Crude extract	1,33	1,738	0,765	100	1
	$(NH4)_2SO_4$	0,868	0,689	1,259	65,263	1,645
	Dialysis	0,462	0,302	1,527	34,737	1,996
Fungus Lacc-D	Crude extract	1,26	1,729	0,729	100	1
	$(NH4)_2SO_4$	0,77	0,576	1,336	61,1	1,833
	Dialysis	0,392	0,176	2,218	31,1	3,044

Scanning Electron Microscope of cereal agricultural waste

Delignification of the lignin contain from natural lignocellulose waste its use to broken down the lignin and hemicellulose which surround cellulose. After lignin removed, hemicellulose is degraded, and the crystalline structure of cellulose is changed to improve the availability and release of cellulose (Chen *et al.*, 2017).

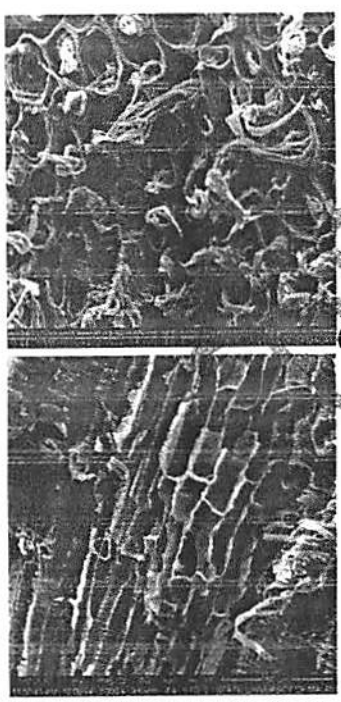

Figure 6: Cross section of corn cob enlarged 1000x (a) control (b) sample fungal laccase treatment

Delignification of corn cob and rice straw are corresponding superficial microstructural changes has been documented via scanning microscope as illustrated in Fig. 6 and 7. The surface of corn cobs and rice straw showed that there was a change of surface profile between control and sample that treated with fungal laccase.

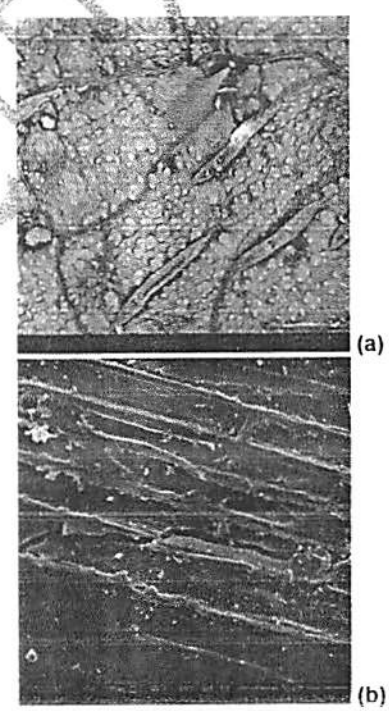

Figure 7: Cross section of rice straw enlarged 2000x (a) control (b) sample fungal laccase treatment

Profile of rice straw surface and corn cob after being treated by enzyme suffered damage such as hollow, damaged, and brittle. This damaged showed that the carbon linkage is broken by enzyme treatment. The

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distortion of the corn cobs and rice straw structure after laccase treatment can be credited to the lignin removal due to the disruption of cellulose-hemicellulose-lignin matrix structure. The result is coherence with previous study that using laccase from *P. djamor* to treatement of sugarcane tops for saccharification (Sherpa *et al.*, 2018) and Mukherjee *et al.* (2018) who worked on rice straw delignification using the chemical treatment.

CONCLUSIONS

Fungus Lacc-A, B, C and D successfully isolation from oil palm empty fruit bunch with the different growth time. Fungus lacc A, B and C were observed at 7th day optimum culture and fungus lacc-D at 11th day culture. The Optimum temperature of fungus-lacc A, B, C and D were 40 to 50°C. Further, optimum pH of fungus-lacc A, B, C and D were 5 to 7. The optimum pH of fungi that obtained shows the good activity of laccase that compatible to paper industry. Its assumed that the fungal laccase from OPEFB its potential to green biodeinking process. SEM results show that laccase from the fungi can be assumed to degrade lignin with apparent damage and hallow to the substrate lignocellulose. The result of this study raise the important possibility that laccase from oil palm empty fruit bunch fungi has tremendous potential to pulp and paper industrial.

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