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Airlangga University
University of Groningen
Koninklijke Nederlandse
Akademie van Wetenschappen

SEMINAR PROGRAM & ABSTRACTS

Enzymes :
Industrial and Medical Prospects

ASEAN Biochemistry Seminar

SURABAYA, February 6-7, 2006

17/7/06

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FOREWORD BY CHAIRPERSON

Assalamualaikum Wr.Wb.

Distinguished guests and participants,

On behalf of the Organizing Committee, I am deeply grateful to have to welcome all of you to the ASEAN Biochemistry Seminar and Workshop with the theme *Enzymes : Industrial and Medical Prospects.*

Industrial (White) Biotechnology is the application of microorganisms and biocatalysts for the sustainable production of chemicals, biopolymers, materials and fuels from renewable resources. The aims of white biotechnology are reducing the waste, energy input and raw material for improving of the environmental. Enzymes are proteins which act as biocatalysts. Enzymes are increasingly expected to play a key role in the field of white biotechnology. In medicine, enzymes are very useful in therapeutics, and new technology is based on amino acids depleting enzymes that are highly effective against diseases such as cancer and HIV.

In view of these facts, the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Airlangga University in collaboration with University of Groningen and Koninklijke Nederlandse Akademie van Wetenschappen (KNAW) is organizing this seminar and workshop. The seminar is from February 6^{th} – 7^{th} at Hotel Sahid and about 90 participants are registered. The workshop is from February 8^{th} – 10^{th} at department of Chemistry, Airlangga University and about 30 participants are registered.

I am also glad to invite all participants to the welcome party tonight , here at 7:00. When we'll all have a chance to meet each other and our distinguished speakers from University of Groningen Prof. Lubbert Dijkhuizen and Prof Bauke W Dijkstra. We'll also have a short ceremony for the signing of LoI between RuG and Unair, announce the formation of the Indonesian Protein Society as well as our best poster contest winner.

We'd also like to express our deep appreciation to the following guest speakers Prof. Bauke W. Dijkstra, Prof Lubbert Dijkhuizen, Slavko Kralj (University of Groningen), Dr Arief B Witarto (LIPI), Prof Ibrahim Omar (USM, Malaysia), Dr Muhamad Hamid (Pakistan), Dr Dessy Natalia (ITB, Bandung), Dr Soetijoso Soemitro (Unpad, Bandung).

Wassalamualaikum Wr.Wb.

Dr. Ni Nyoman Tri Puspaningsih
Chairperson

FOREWORD BY RECTOR OF AIRLANGGA UNIVERSITY

Dear participants, lectures, guests,

Welcome to Surabaya!

It is a great pleasure for me to address this "Asean Biochemistry Seminar and Workshop" held by cooperation between Airlangga University, University of Groningen and KNAW ,and I feel honoured to deliver this message to all participants. Thank you for being here with us, and share with our faculty members of the Faculty of Mathematics and Natural Sciences as well as members of the Medical School about your experiences, your expert knowledge and deliver your lectures and addresses. Indonesian scientists in biochemistry are also facing the globalization era, and they need more cantacts and communications with all of you over the nation and the world, so it is also our wish to further build a stronger and closer friendship, whilst to achieve scientic know-how in biochemistry sciences with you as well.

Biochemistry, as also happened with other specialities, are developing enormous challenges. At the same time, however, biochemists have a lot of opportunity to improve the quality of the lives of the man kind. Therefore, biochemist must strive to improve the quality of scientific achievements and also asked to render their knowledge to post graduate education. This time, this seminar has a special topic in "ENZYMES: Industrial and medical prospects", and I believe that the new technology must be critically evaluated and its rational use to medical community should be supported. The strong advocates of the relationship between phycician-scientist and its benefit to the patient- cure should always remain, and the professionalism should be maintained.

Once again, thank you for being supportive, thank you for coming and accept our invitation and hope that your stay here in Surabaya will be a memorable one, and have a pleasant time with us during stay.

Puruhito
MD, Dr.med., FCTS, FAMN
Professor of Thoracic and Cardiovascular Surgery
Rector, Airlangga University, Surabaya

FOREWORD BY DEAN OF FACULTY OF MATHEMATICS AND NATURAL SCIENCES, AIRLANGGA UNIVERSITY

Dear participant, lectures and guest
Ladies and Gentlemen

On behalf of Airlangga University, I would like to express my feelings of gratitude that our Internasional Scientific Meeting about 'ASEAN Biochemistry Seminar and Workshop' has been done successfully and has come to a happy-end today.

This seminar has been held through a collaboration beetwen Airlangga University and university of Groningen and KNAW, in Surabaya - East Java, Indonesia. To all participants of this Seminar a like to convey my deepest gratitude for all the contribution given to make this seminar a success from Monday until now, at this closing ceremony.

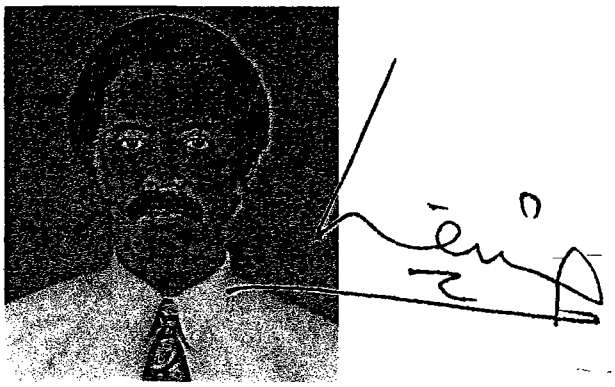
The discussion about enzymes is a very important point in Biochemistry, for all the research on Biomolecular and biotechnology is surely related to bioenzym. This seminar and workshop we have had is intended to enrich our knowledge and progress report about the enzyme report and biotechnology development.

We hope the result of this seminar and workshop will be suiatanable in the development of further research and methodology technique which develops very fast. The progress of science and technology in enzymes for industrial and medical prospects, must be for the benefit and welfare of the people , and also to fulfill the need of the technological industrial process as well as the natural resources which can be found in abundance in Indonesia. Because, mega-diversity in Indonesia has the prospect of exploration and exploitation in the bionatural resources which can be useful in many different kinds of natural research topic. This seminar and workshop is our activities to support the intention above.

To all the participants and lectures of this seminar, we wish you a happy and safe journey home, and once again. I sincerely thank you for your contribution and for all what you have done for this scientific meeting. In particular to Prof. Bauke W. Dijkstra, Prof. Lubbert Dijkhuizen, Dr. Muhmmad Hamid R, Prof H. Ibrahim Omar, Dr. Arief B. Witarto and all keynote speech, we convey our greatest gratitude for you scientific speech and participation in this seminar. May all these scientific activities be useful for the progress in science and technology.

On behalf of all participation of this seminar and workshop, I consider this seminar to be closed.

Good luck and success to all for you. Thank you.



Mr. LATIEF BURHAN
Dean of Faculty MIPA Airlangga University, Surabaya- Indonesia

SEMINAR SCHEDULE

Monday, February 6, 2006

Time	Program
07.30-08.00	Registration
08.00-08.30	Opening ceremony by Rector of Airlangga University
08.30-09.45	Plenary Session: ❖ **Prof.dr. Bauke W.Dijkstra.** Laboratory of Biophysical Chemistry, Rijksuniversiteit Groningen, Nijenborg 4, 9747 AG, Groningen, the Netherlands. Topic : **From rubber trees to human desease : The structure and function of chitinases. [PL-01]** ❖ **Prof. Yoes Priyatna.** TDC Airlangga University Topic : **Current Condition and Prospective Role of the TDC (Tropical Disease Center) Airlangga University in the Application of Health Science and Technology for Accelerating the Tropical Infectious Disease Elimination. [PL-02]** **Moderator** : Prof Sutjipto **Secretary** : Hery Suwito
09.45-10.00	Coffee break and poster exhibition
10.00- 12.15	Keynote speech : ❖ **Dr. Arief B. Witarto.** LIPI-Biotechnology, Jakarta, Indonesia. Topic : **Medical Protein Engineering in Indonesia. [KS-01]** ❖ **Slavko Kralj, PhD.** Microbiology & Centre for Carbohydrate Bioengineering (CCB), University of Groningen, the Netherlands. Topic : **Molecular and Biochemical Characterization of glucansucrases from Lactobacilli. [KS-02]** ❖ **Dr. Muhammad Hamid Rashid.** Principal Scientist, National Institute for Biotechnology and Genetic Engineering (NIBGE), P.O.Box 577, Jhang Road, Faisalabad, Pakistan. Topic : **Enzyme Engineering. [KS-03]** **Moderator** : Prof. Lubbert **Secretary** : Alfinda Novi Kristanti
12.15-13.30	Lunch and poster exhibition – Session I
13.30-15-30	Oral presentation – Session I
15-30-16.00	Coffee break and poster exhibition – Session II
16.00-17.00	Oral presentation – Session II
19.00-21.00	Welcome party

Tuesday, February 7, 2006

Time	Program
08.00-08.45	Plenary Session: ❖ **Prof.dr. Lubert Dijkhuizen.** Microbiology & Centre for Carbohydrate Bioengineering (CCB), University of Groningen, the Netherlands. Topic : **Fructans poly-and oligasaccharides of Lactobacillus reuteri 121 : Gene, enzyme and fructans. [PL-03]** **Moderator** : Prof Bauke **Secretary** : Ganden Suprianto
09.45-10.00	Coffee break and poster exhibition
10.00- 12.15	Keynote speech : ❖ **Prof. Hj. Ibrahim Che Omar.** Fermentation and Enzyme Technology Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia. Topic : **Production of xylanase and cellulose via solid state fermentation and its application in the enzymatic deinking of waste papers. [KS-04]** ❖ **Dessy Natalia, PhD.** Laboratory of Biochemistry, Department of Chemistry, Bandung Institute of Technology (ITB), Bandung Indonesia. Topic : **α-Amylase : from bacteria to yeast. [KS-05]** ❖ **Dr. Ni Nyoman Tri Puspaningsih.** Laboratory of organic and Biochemistry, Department of Chemistry, Airlangga University, Surabaya-Indonesia. Topic : **Hemicellulases : Complex enzymes and its industrial prospects. [KS-06]** **Moderator** : Slavko Karlj, PhD **Secretary** : Afaf Baktir
11.15-11.45	Closing ceremony Announcing of the best poster presenter
11.45-17.00	City tour (Purwodadi Botanical Garden, Art market, and Souvenir shop)

LIST OF ORAL PRESENTER

Oral Presenter	Code	Session*)	Room**)
Adrian Victor D'Ng	OP-31	I	Executive Lounge
Afaf Baktir	OP-33	II	Executive Lounge
Agung Budi Santoso	OP-25	I	Arjuno
Agustina Lulustyaningati N. A.	OP-12	II	Anjasmoro
Agustinus Robert Uria	OP-22	I	Executive Lounge
Aprilfana Laily Fitri	OP-15	I	Bromo
Ari Istiany	OP-03	II	Bromo
Bambang Kuswandi	OP-07	I	Bromo
Chusnul Hidayat	OP-04	I	Arjuno
Darmawi	OP-13	I	Bromo
Dewi Seswita Zilda	OP-21	I	Executive Lounge
Didik Pudji Restanto	OP-35	II	Executive Lounge
Djoko Agus Purwanto	OP-10	II	Arjuno
Dwi Winarni	OP-17	II	Bromo
Fajar Restuhadi	OP-20	II	Arjuno
Ganden Supriyanto	OP-29	I	Arjuno
Handoko Darmokoesoemo	OP-27	II	Arjuno
Ines Irene C. Atmosukarto	OP-38	I	Executive Lounge
Irma Ratna Kartika	OP-16	II	Bromo
Jayus	OP-23	I	Executive Lounge
Krishna Purnawan Candra	OP-08	I	Arjuno
Mahyudin Abdul Rachman	OP-06	I	Arjuno
Muktiningsih Nurjayadi	OP-14	I	Anjasmoro
Nanik Siti Aminah	OP-32	II	Anjasmoro
Ni'matuzahroh	OP-18	I	Anjasmoro
Nisa Rachmania Mubarik	OP-02	I	Bromo
Nuryono	OP-34	II	Anjasmoro
Purkan	OP-28	II	Bromo
Rita Sundari	OP-01	I	Bromo
Rudiana Agustini	OP-19	I	Bromo
Sofijan Hadi	OP-30	I	Anjasmoro
Sukardiman	OP-11	I	Anjasmoro
Supriyatin	OP-37	I	Anjasmoro
Win Darmanto	OP-24	I	Anjasmoro
Winda Haliza	OP-26	I	Executive Lounge
Y. Sri Wulan Manuhara	OP-09	I	Arjuno
Zulfikri	OP-05	II	Executive Lounge

*) Session I : 13.30 - 15.30
Session II : 16.00 - 17.00

**) Anjasmoro room at : 2nd floor
Arjuna room at : 3rd floor
Bromo room at : 2nd floor
Executive lounge at : 12th floor

LIST OF POSTER PRESENTER

Poster Presenter	Code	Session*)
Agung Astuti	PP-03	I
Anak Agung Istri Ratnadewi	PP-09	II
Anis Shobirin Meor Hussin	PP-05	I
Eriyusni N	PP-02	I
Fajar Restuhadi	PP-15	II
Haslinda Hasim	PP-06	II
Khomaini Hasan	PP-13	II
Krishna Purnawan Candra	PP-11	I
Mahyudin Abdul Rachman	PP-10	I
Mimi Sakinah Binti Abdul Munaim	PP-16	II
Mochamad Djunaedi	PP-17	I
Ni Made Dwi P	PP-19	I
Nik Marzuki Sidik	PP-04	I
Nike Triwahyuningsih	PP-01	II
One Asmarani	PP-20	II
Prima Endang Susilowati	PP-12	II
Rohani Salleh	PP-07	II
Rusdi	PP-18	II
Suryani	PP-24	I
Suyanto	PP-21	II
Suyanto	PP-22	I
Tati Herlina	PP-23	I
Yosi Nindita	PP-14	I
Zinatul Hayati	PP-08	II

*) Session I : Monday, Feb 6th [08.00 – 13.30]
Session II : Monday, Feb 6th [14.00 – 17.00] and Tuesday, Feb 7th [08.00 – 11.00]

ORAL PRESENTATION SCHEDULE

Session I (20 minutes/presenter)

Room	Time	Presenter	Code	Title
Bromo	13.30 – 14.30 Moderator : Slavko Kralj Secretary : Saenah	Bambang Kuswandi	OP-07	The Interplay of Enzyme, Indicator and Analyte in Optical Biosensor Layers
		Darmawi	OP-13	Protease Activity of Excreatory/ Secretory Released by Invasive Stage of *Ascaridia galli*
		Apriliana Laily Fitri	OP-15	Enhancer-Trap Technique to Screen the Genes That Expressed in The Drosophila Salivary Glands and The Effect of dead ringer (dri) Ectopic Expression in Drosophila Salivary Glands Development : As An Organogenesis Model
	14.30 – 15.30 Moderator : Hery Suwito Secretary : Saenah	Rita Sundari	OP-01	The Analysis of Enzyme by The Biosensor Methodology
		Nisa Rachmania Mubarik	OP-02	Isolation of Proteolytic Bacteria from Digestive Tract of Tilapias Strain GIFT (*Oreochromis niloticus* (Linnaeus) Trewavas) and Characterization of Its Extracellular Protease
		Rudiana Agustini	OP-19	The Isolation of proteases thermophile CG-10 isolate to produce coconut oil by enzymatic process
Executive Lounge	13.30 – 14.30 Moderato : Ganden S. Secretary : Dewi	Adrian Victor D'Ng	OP-31	A Journey To Manipulate Microbial Chitinase for Biotechnology Applications
		Jayus	OP-23	Is There Any Relationship Between Hyphal Growth Unit And Fungal Enzyme Production?
		Dewi Seswita Zilda	OP-21	Production And Characterization of Chitosanase Produced by JB4 Isolated From Terasi
	14.30 – 15.30 Moderator : Nanik S.A. Secretary : Dewi	Agustinus Robert Uria	OP-22	Properties of Chitinase Isolates From a bacterial Strain Associated With Marine Sponge
		Winda Haliza	OP-26	Characterization of Microbial Chitinase : a Review
		Ines Irene C. Atmosukarto	OP-38	Nitrile degrading activity of endophytic bacteria from the biological diversity of Indonesia

Session I continued (20 minutes/presenter)

Room	Time	Presenter	Code	Title
Anjasmoro	13.30 – 14.30 Moderator : Win Darmanto Secretary : Cindy	Muktiningsih Nurjayadi	OP-14	Mapping of *Salmonella typhi* proteins based on proteomic analysis Technology as an effort to discover Bioactive Protein
		Supriyatin	OP-37	The Effect of *Typhonium flagelliforme* Leaf Extract on The Inhibition of Melanomas Cell
		Sukardiman	OP-11	DNA Topoisomerase I And II Enzyme As Target to Discovery oF New Anticancer Drugs of Natural Product
	14.30 – 15.30 Moderator : Alfinda Novi K. Secretary : Cindy	Win Darmanto	OP-24	The Potency of Polysaccaride Krestine (PSK) As a Apoptosis Inhibitor And Protective Effect of Gamma Cobalt 60 Iradiation And 2-Methoxyethanol Induce Congential Malformation
		Ni'matuzahroh	OP-18	Prospect of *Bacillus substilis* 3KP biosurfactant as phytopthogenic antimicrobial agent
		Sofijan Hadi	OP-30	Glucoamylase gene of *Endomycopsis fibuligera* : isolation , partial nucleotide sequence and alignment analysis
Arjuno	13.30 – 14.30 Moderator : Ni Nyoman T.P. Secretary : Made	Mahyudin Abdul Rachman	OP-06	The Strategy for Efficient H2,2,3-Butanol Production and Involved Enzymes by *Enterobacter*
		Krishna Purnawan Candra	OP-08	Catabilism of 4-O-Acetylated Stalyc acid solubilization technique of membrane-bound enzyme : Sialidase from horse liver
		Chusnul Hidayat	OP-04	Development of sesame sprout lipase purification using immobilized metal affinity
	14.30 – 15.30 Moderator : Afaf Baktir Secretary : Made	Y. Sri Wulan Manuhara	OP-09	Expression of Soybean β-1,3-endoglucanase cDNA and Effect on Disease Tolerance in Transgenic Cabbage Plants
		Agung Budi Santoso	OP-25	Businnes Plan of Amall Scale Biotechnology Industry Enzymatic Fish Sauce Production
		Ganden Supriyanto	OP-29	Recent Developments of Sample Preparation Technique in Bioanalytical Chemistry

Session II (20 minutes/presenter)

Room	Time	Presenter	Code	Title
Bromo	16.00- 17.00 Moderator : Ganden S. Secretary : Cindy	Dwi Winarni	OP-17	The Effect of Java Gingseng (*Talium paniculatum Gaerth*) Root Extract on testosteron level associated with testicular and hepatic protein
		Purkan	OP-28	Exploration amylolytic enzyme : Cloning gene encoding glucoamylase of *Endomycopsis fibuligera* ITB Rcc 64
		Irma Ratna Kartika	OP-16	Study on the reaction of tocopherol model compound 2,2,7,8-tetramethyl-6-chromanol
		Ari Istiany	OP-03	Sun Exposure's Effect to the Parathyroid Hormone Rate
Executive Lounge	16.00 – 17.00 Moderator : Slavko Kralj Secretary : Saenah	Afaf Baktir	OP-33	Amplification, Sequencing and Expression of a Dextranase gene from *Arthrobacter* sp B7
		Didik Pudji Restanto	OP-35	Cloning cDNA fragment of sucrose transporter (SoSUT) in sugarcane (*Saccharum officinarum*) leaf
		Zulfikri	OP-05	Comparison of beta cyclodextrin production between gelatinized and ungelatinized sago starch with cyyclodeztrin glucanotransferase (CGTase)
Anjasmoro	16.00- 17.00 Moderator : Nanik S. A. Secretary : Dewi	Nanik Siti Aminah	OP-32	Apigenin-(3'-O-7'')-quercetin-3''-methyl ether, a dimer flavonoid compound as inhibitor xanthine oxidase from *Cassia spectabilis*
		Agustina Lulustyaningati N.A.	OP-12	Phylogenetic analysis of bacterial communities in Pancuran 7 Baturraden hot spring
		Nuryono	OP-34	Encapsulation of lactate dehydrogenase (LDH) in rice hull ash derived silica
Arjuno	16.00 – 17.00 Moderator : Slavko Kralj Secretary : Saenah	Handoko Darmokoesoemo	OP-27	Study on physical-chemical mechanism of iron removal from the ferric complexe of azotobactine δ (siderophore isolated from *Acetobacter vinelandii* strain D bacteria) in vitro way
		Fajar Restuhadi	OP-20	From co-expression to regulation : Bioinformaticts approoach for studying gene expression topology mapping of yeast;s genes involved in axial and bipolar budding patterns
		Djoko Agus Purwanto	OP-10	Enhancement of DNA repair enzyme O^6-alkylguanine-DNA alkyltransferase by (-)-epigalloocatehin gallatee from green tea

POSTER PRESENTATION SCHEDULE

Session I :
Monday, February 6 [08.00 – 13.30]

Presenter	Code	Title
Agung Astuti	PP-03	The engineering of actinidin-encoding protein through specific amplification of full-length actinidin cDNA
Eriyusni N.	PP-02	Studies on protein levels and calcium phosphate ($CaHPO_4$) incorporated into the diets on consumption, growth and carcass composition in *Tilapia* spp.
Anis Shobirin Meor Hussin	PP-05	Economical production of bacterial phytases isolated from Malaysian *Zea mays* plantation
Krishna Purnawan Candra	PP-11	Solubilization technique of membrane-bound enzyme : Sialidase from horse liver
Mahyudin Abdul Rachman	PP-10	The strategy for efficient hydrogen (H_2), 2,2-butandiol production and involved enzyme by *Enterobacter aerogenes*
Suryani	PP-24	Cloning, sequencing, and expression of the gene encoding the *Clostridium stercorarium* α-galactosidase AGA36A in *Escherichia coli*
Tati Herlina	PP-23	The inhibition of paralytic alkaloids from the seed of *Erythrina fusca* Lour (Leguminosae) of ATPase activity
Nik Marzuki Sidik	PP-04	Cellobiohydrolase (CBH) gene at Aspergillus feureus SUK-1 a complete open reading frame (ORF) and expression
Mochamad Djunaedi	PP-17	Side effect of 5-hydroxytriptamine, receptor antagonist in the prophylactic treatment of emesis induced by cisplatin and taxotere chemotheraphy : A case report
Suyanto	PP-22	Isolation of Vicanicin from Thallus of Lichen *Ramalina javanica* Nyl and anticancer test against leukemia cell
Yosi Nindita	PP-14	Construction of *alp1* using Site-Directed Mutagenesis
Ni Made Dwi P.	PP-19	Exploration of Xylanolytic Enzymes from Pacet

Session II :
Monday, February 6th [14.00 – 17.00] and Tuesday, February 7th [08.00 – 11.00]

Presenter	Code	Title
A.A. Istri Ratnadewi	PP-09	Effect of additive compounds on the activity and degradation patterns of catalase in apple flesh tissue
Rohani Salleh	PP-07	Thermophilic bacterial cellulases from Malaysian soil and hot springs *
Haslinda Hasim	PP-06	Thermophilic bacterial pectinase from Malaysian soil and hot springs for potential industrial application *
Prima Endang Susilowati	PP-12	Site directed mutagenesis of *SUP45* gene for interaction mechanism study of eRF1 – eRF3 yeast protein
Mimi Sakinah Bt. A. M.	PP-16	A pilot scale study on the performance of an enzymatic membrane reactor (EMR) during cyclodextrins production *
Zinatul Hayati	PP-08	Screening of Group B *Streptococci* Hyaluronidase use Plate Agar Hyaluronidase Technique
Nike Triwahyuningsih	PP-01	Bioassay of nitrate reductase activity of the red algae (*Rhodophytes*)
Fajar Restuhadi	PP-15	Topology mapping of yeast's genes involved in axial and bipolar budding patterns
Rusdi	PP-18	Effect of vitamin E on Na^+-K^+ ATPase activity in cell membrane of syncyotrophoblast placenta in women with pre-eclampsia
Suyanto	PP-21	Isolation of parietin from Thallus of Lichen *Ramalina javanica* Nyl and anticancer test against leukemia cell *
Khomaini Hasan	PP-13	Improvement the $(\alpha/\beta)_8$ barrel-rigidity of *Saccharomycopsis fibuligera*
One Asmarani	PP-20	Hydrolysis of Commercial Xylans by Xylanolytic Enzymes expressed in *E. Coli* DH5α (pTP510) *

A PILOT SCALE STUDY ON THE PERFORMANCE OF AN ENZYMATIC MEMBRANE REACTOR (EMR) DURING CYCLODEXTRINS PRODUCTION

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PP-16

ABSTRACT

Cyclodextrins (CDs) iss produced from starches via cyclization or intramolecular transglycosylation of the cyclodextrin glucosyltransferase (CGTase). This specialty chemicals have a wide range of applications espeacially in food, pharmaceutical, chemical industries, agricultural and environmental engineering. As the world demand for CDs is increasing, the pilot scale Enzymatic Membrane Reactor (EMR) was therefore fabricated in this study to produce a high volume of CDs. The 8 liters EMR comprises an enzymatic vessel and membrane modules. This EMR can produce and separate CDs from the reaction mixture continuously and simultaneously. The reaction mixture which contained unreacted starch and CGTase is recycled back to the enzymatic vessel. However, application of membrane technology in CDs separation is still limited due to membrane fouling which was found to reduce the membrane performance. Subsequently the main objective of this study is to investigate the types of membrane cleaning in CDs separation which can increase the effectiveness of membrane separation by regaining better flux recovery and produce high permeate flux. A series of membrane cleaning procedure was applied to remove the foulant from the membrane surface and within the membrane pore matrix. The experimental results showed that the alkaline cleaning was the most effective methods which can recover about 95% of the initial flux during CDs separation.

SIDE EFFECT OF 5-HYDROXYTRIPTAMINE$_3$ RECEPTOR ANTAGONIST IN THE PROPHYLACTIC TREATMENT OF EMESIS INDUCED BY CISPLATIN AND TAXOTERE CHEMOTHERAPY : A CASE REPORT

MOCHAMAD DJUNAEDI[1], ZURAIDAH BT MOHD YUSSOF[2], SAAD OTHMAN[2], JAYENDRAN[3]

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3. Department of Oncology, Hospital Pulau Pinang, 10450, Pulau Pinang, Malaysia

PP-17

ABSTRACT

The 5-hydroxytryptamine$_3$ receptor (5-HT$_3$ receptor) antagonists recently has been developed as a class of new anti-emetic agent. These drugs were initially found to prevent vomiting due to Cisplatin, a chemotherapeutic drug with strong emetic effect, but subsequent clinical studies have shown that 5-HT$_3$ receptor antagonist are also effective in controlling vomiting due to other anti cancer drugs and radiotherapy.

A 45 years old malay female was admitted for first cycle of Cisplatin and Docetaxel chemotherapy treatment for her right side breast cancer. She had diagnosed to have right site of medullary breast cancer.

Previously she had been surgically treated of her breast cancer followed by radiation three years ago. Adriamycin and Cyclophosphamide Chemotherapy regime had been given over 4 cycles. A next year follow-up investigation, included surgical biopsy didn't show neither any residual tumour nor lymph nodes involvement, Chest X ray, abdomen Ultrasonography and Mammography showed no evident of metastasis. Fine Niddle Aspiration Cytologic also didn't find diagnostic material. Unfortunately, she currently complained of pain at her chest and back. Furhter, bone scan had investigated increase in uptake of tracer or infiltration to the 6th rib of sternum. CT-thorax showed there is soft tissue mass at the right anterior chest wall involving the pectoralis major muscle. This is the latest diagnosed as a recurrent cancer with early metastasis.

On this regime, cisplatin was given 110 mg in 1 L normal saline for 3 hours and Taxotere 110 g in 500 ml for 1 hour. Intravenous 5-HT3 receptor antagonist (Granisetron^{R}) 3 mg with IV Dexamethason 8 mg and Dexamethason tablet 4 mg QID were given half hour prior the chemotherapy. Other medications given before chemo included Voltaren tablet 50 mg tds, Lactulose syrup 20 ml ON and Dihydrocodein 30 mg tds. During received this chemotherapy, generally patient was comfortable and did not complaint nausea and vomiting until complete cycle, but one day after this cycle patient complained of constipation and headache.

Cisplatin and Taxotere chemotherapy are effective in breast cancer but however, the side effect of this treatment include potentially debilitating nausea and vomiting. Successful anti emetic therapy can enable patients receiving chemotherapy to maintain and improve their quality of life

ENZYME ENGINEERING

Muhammad Hamid Rashid*, Abdul Jabbar and Ahmad Mukhtar Khalid

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KS-03

ABSTRACT

Enzyme engineering is the use of genetic and chemical techniques to alter the structure and function of a protein, thus producing a novel product with specific desired properties. The oldest and simplest way to engineer proteins is to treat them with a reagent that will react with the exposed functional groups on the surface of a protein. Therefore, carboxyl groups of CMCase from *Arachniotus citrinus* were chemically modified by coupling trimethylamine in the presence of carbodiimide (EDC) to evaluate the effect of hydrophobization on stability-function relationship. CMCases were modified for 1 and 10 min of trimethylamine coupling (TMAC-1 & TMAC-10). We found increase in hydrophobicity did not improve thermophilicity. Temp optima of both modified forms remained unchanged i.e. 55°C. The energy of activation for CMC hydrolysis of TMAC-1 was decreased, whereas that of TMAC-10 was increased as compared to control. The pH optimum range of TMAC-1 and TMAC-10 was decreased to 2.5-4 as compared to control having a range of 4-6. Turn over (K_{cat}) for TMAC-1 and TMAC-10 was lower than native, while K_m for CMC of both modified forms increased as compared to control. ΔG^* for both modified forms was higher than native, while ΔS^* of control was higher as compared to modified enzymes. The Gibbs energy for transition state formation ($\Delta G^*_{E\text{-}T}$) by modified CMCases was higher than native. Irreversible thermostability of both modified forms was higher than native. In the light of kinetic and thermodynamic parameters we concluded increase in surface hydrophobicity, due to trimethylamine coupling, resulted into thermostabilisation of CMCases, while, it had detrimental effect on function

PRODUCTION OF CELLULASE AND XYLANASE *VIA* SOLID STATE FERMENTATION AND ITS APPLICATION IN THE ENZYMATIC DEINKING OF LASER PRINTED WASTE PAPERS.

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KS-04

ABSTRACT

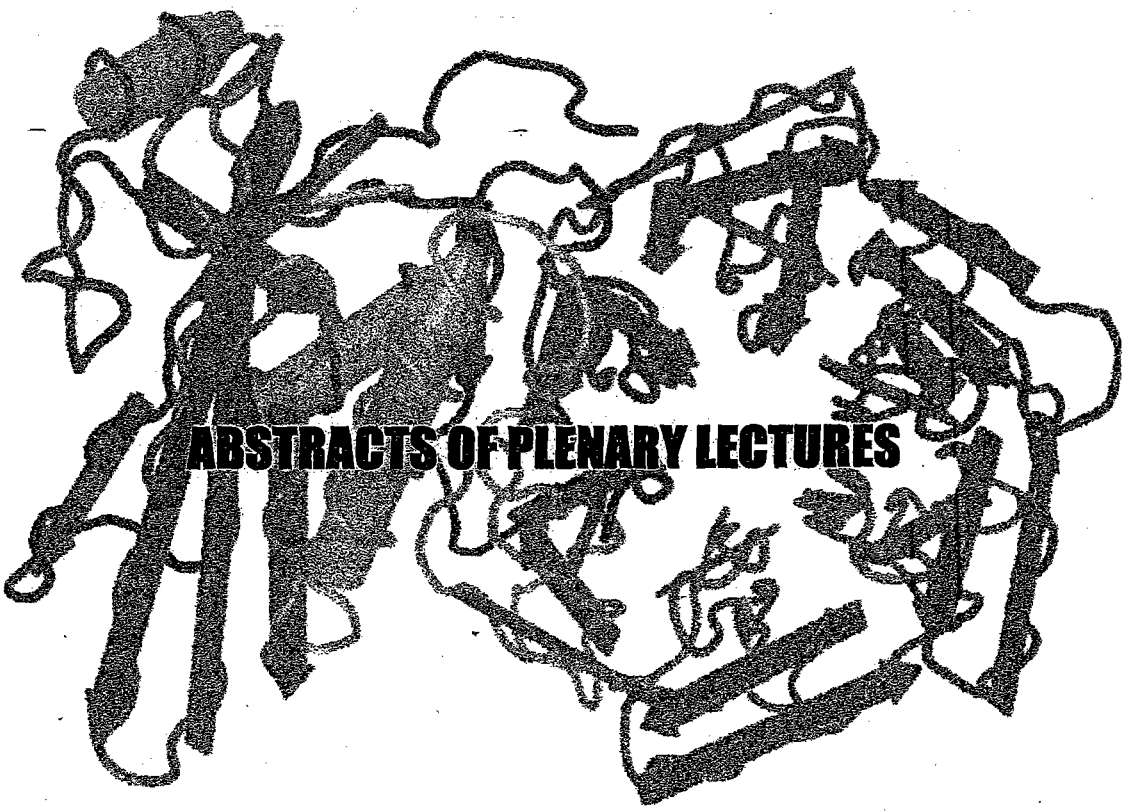
The work deals with the production of cellulase and xylanase by local isolates *via* solid state fermentation (SSF) processes for the application in the enzymatic deinking of laser printed wastepapers. The production of cellulase and xylanase was carried out using *Aspergillus niger* USM AI 1 and *Trichoderma* sp. FETL c3-2. The production of cellulase and xylanase by *Trichoderma* sp. used sugar cane baggase (SC):palm kernel cake (PKC) as substrates. The optimized condition for cellulase production consist of 5 g SC: PKC, moisture content of 75% (v/v), pH of the moistening agent of 7.0, at 30°C and inoculum size of 1 x 10^8 spores/ml. Dextrin at 4% and yeast extract at 6% (w/w) acted as supplementary carbon and nitrogen sources with cellulose at 0.2% (w/w) as inducer. The maximum FPAse and CMCase production of 3.3 U/g substrate and 18.05 U/g substrate, respectively were obtained after 4 days fermentation. The cellulase production was growth dependent.

Xylanase production was maximum under the optimized conditions consisting of SC:PKC 90:10 (%,w/w), moisture content 75%, pH of moistening agent of pH 7.0, at 30°C and inoculum size of 1 x 10^8 spores/ml. Dextrin at 4% (w/w) and tryptone at 6% (w/w) were added as additional carbon and nitrogen sources with 0.2% cellulose as inducer. Under the optimized conditions, the xylanase production by *Trichoderma* sp. was 75.0 U/mg glucosamine after 4 days at 30°C. The production of the enzyme increased by 180% while the growth by about 40%.

Xylanase production *via* SSF by *Aspergillus niger* USM AI 1 was also carried out using palm kernel cake as substrate. The modification of the physical parameters of the SSF system indicated a production level of 23.97 U/g PKC at the moisture ratio of 1:0.75 of PKC : moistening agent with the inoculum size of 1x 10^4 spores/ml at ambient temperature (28±3°C). The supplementation of additional carbon and nitrogen sources in the PKC medium could enhance the enzyme productivity. The presence of $NaNO_3$ at 0.075% (w/w) as additional nitrogen sources further enhance xylanase production to 33.99 U/g PKC.

The effect of substrate amount, moisture content and temperature on the production of cellulase and xylanase by *Trichoderma* sp. FETL c3-2 and *A niger* USM AI 1 was examined in the tray system (20 x 30 x 5cm) using SC:PKC as substrates in SSF system. The maximum xylanase activity was observed when *A. niger* USM AI 1 was grown at 65% moisture content, of 50 g substrate at 30°C for 4 days with the activity of 77 U/g PKC. As for the cellulase, the maximum activity obtained when 50 g of PKC, 75% moisture content and 30°C was 0.57 FPU/g PKC. Enzymes production varies significantly with the change in cultivation conditions in the tray system suggesting the positive influences on enzyme production. Large substrate amount affected the fungal penetration ability into the substrates, while high moisture content caused low oxygen transfer and decreased porosity of the substrates. High cultivation temperature affected fungal growth as a result of heat accumulation within the substrates.

Enzymatic deinking on a laboratory scale using enzyme preparations consisting of cellulase and xylanase from laser printed wastepapers was performed. A maximum deinking efficiency of almost 73% was obtained using the optimized enzymatic hydrolysis conditions consisting of pulp consistency 1.0% with the pulping time of 3 min, temperature 50°C, pH 3.5, agitation rate 60 rpm, pulp concentration 4% (v/v), enzyme concentration of 2.5 U/g dried pulp and the enzyme ratio of 1:1. To further enhance the deinking efficiency, the flotation system must be optimized to enable effective detachment and dispersion of toner particles from the surfaces of the paper fibres. The results indicated that high deinking efficiency can be obtained under acidic condition in the presence of Tween 80 as the surfactant. Based on the optimized flotation system consisting of pH 6.0, Tween 80 of concentration 0.5% (w/w), air flow rate of 2.0 L/min and temperature of 45°C, an almost 100% deinking efficiency was obtained. Effective air flow rate is important in preventing redeposition or promote separation of ink or toner particles from the surface of the fibre network. When compared to the commercially prepared paper, the properties of the deinked paper were comparable suggesting that the effectiveness of the enzymatic deinking process. These properties include the drainage rate of 103.7 L/min, tensile strength 22.77 Nm/g, tear index 7.10 mN m^2/g and burst index of 4.71 kPa m^2/g. When compared to the control pulp, it was observed that the deinked paper showed either similar performance or better properties suggesting that the enzymatic deinking process was able to produce papers of comparable properties to that produced by chemical methods. However, lower tensile strength in the enzymatic hydrolysed papers is expected due to the enzymatic degradation of the fibres.



ABSTRACTS OF PLENARY LECTURES

FROM RUBBER TREES TO HUMAN DISEASE : Structural Investigations of Family 18 Chitinases and Chitinase-Like Lectins

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PL-01

ABSTRACT

Chitin is an abundant polymer of *N*-acetylglucosamine (NAG) residues. Consequently, many organisms ranging from bacteria to eukaryotes contain hydrolytic enzymes able to degrade chitin, either to make the NAG residues available as nutrients, or as a defense against chitin containing parasites such as fungi, protozoans and insects. Two different homology families of chitinases are known, that are not related in neither amino acid sequence or in three-dimensional structure. These families are glycoside hydrolase families 18 (GH18) and 19 (GH19).

The latex of the rubber tree, *Hevea brasiliensis*, contains a GH18 chitinase, called hevamine, that protects the plant against attack by fungi and bacteria. We solved its crystal structure, and then discovered that this enzyme does not work like a lysozyme, as had been expected, but that it uses the *N*-acetyl-group of the substrate to assist in cleaving the glycosidic bonds in chitin. The evidence for this comes from the way a specific inhibitor of GH18 chitinases, allosamidin, binds in the active site.

Even mammals possess enzymes with chitinolytic activity, although they do no make any chitin. One such enzyme is chitotriosidase, which is present in 95% of all humans, yet 5% do not have it. In particular, the levels of this enzyme were found to be elevated by up to 2 orders of magnitude in the plasma of patients suffering from Gaucher disease, a rare genetic disorder that is caused by a mutation in the glucocerebrosidase gene. The 2.1 Å crystal structure of the human chitotriosidase shows that the enzyme has the $(\beta/\alpha)_8$-barrel fold of GH18 enzymes. The active site is similar to that of hevamine, but due to extensions of the peptide chains the active site has a more groove-like character. Glu-140, the catalytic acid, is located in the centre of this groove. The open and accessible groove (which can contain up to nine sugar residues) suggests that chitotriosidase is an endochitinase. This would be consistent with its proposed role as a defense protein against chitin-containing pathogens such as fungi and its ability to directly degrade chitin from *C. albicans* cell walls.

Apart from the human chitotriosidase, two other family 18 chitinase-like mammalian proteins have been described recently. YM1 and HCgp-39 both show a high degree of sequence similarity to family 18 chitinases, but they lack two key catalytic residues. HCgp-39 (human cartilage glycoprotein 39) is expressed by synovial cells and macrophages during inflammation. It binds strongly to heparin and chitin. Its 2.0 Å crystal structure confirms that it contains the typical $(\beta/\alpha)_8$-barrel fold of glycoside hydrolase family 18 enzymes. Chitin fragments of diverse lengths could be bound in this groove, convincingly identifying nine subsites in the groove. Residues from the $\alpha+\beta$ domain contribute to the binding of the oligosaccharides. Like in chitinases, the HCgp-39 chitin oligosaccharides are distorted upon binding, with the GlcNAc at subsite –1 in a boat conformation, even while HC-gp39 has no catalytic activity.

CURRENT CONDITION AND PROSPECTIVE ROLE OF THE TDC (TROPICAL DISEASE CENTER) AIRLANGGA UNIVERSITY IN THE APPLICATION OF HEALTH SCIENCE AND TECHNOLOGY FOR ACCELERATING THE TROPICAL INFECTIOUS DISEASE ELIMINATION

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PL-02

ABSTRACT

Critical situation resulted from the main combined factors as poverty, uneducation, radically environmental change alleged such as developing country to confront their own health problems. The problems consisted of the high rate of maternal and infant mortality and morbidity also increasingly number of infection and tropical disease that seemed largely caused by such a type of new-reemerging infectious disease.

It was needless to say the existence of TDC Project as an answer to serve the challenge for struggling to overcome the problems. The presence of TDC Airlangga University in Surabaya was founded by National High Education of Culture and Education Department, Japan International Cooperation Agency (JICA) and Japan Society for the Promotion of Science (JSPS) accompanied with some government institution's participation from South East Asia's countries. However, the TDC institution's existence is more than just the answer and even to anticipate all the arising challenges against great health problem over their task including basic and applied research, training and enlightenment programs.

Attainment on target was exerted by accomplishing actual research and training programs accompanied by the thought of formidable list of future concepts. It was organized by TDC with their both soft and hard wares, including staffing, laboratory facilities, library etc. and base on the TDC policy that always to develop cooperation with other excellent center as joint project of any health program.

FRUCTAN POLY- AND OLIGOSACCHARIDES OF *Lactobacillus reuteri* 121: Isolation and Characterization of Genes, Enzymes and Fructan Products

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PL-03

ABSTRACT

Various bacteria convert sucrose into fructan polymers, mostly levans with Beta-2,6 linkages, and in a few cases inulins with Beta-2,1 linkages. The fructosyltransferase enzymes involved are members of glycoside hydrolase family 68 [http://afmb.cnrs-mrs.fr/CAZY/index.html]. Limited information is available about synthesis of fructans by lactic acid bacteria. Most studies have focused on oral streptococci involved in the process of dental caries. Also *Lactobacillus reuteri* 121, however, produces a levan (with Beta-2,6 linkages) of 150 kDa.

Using PCR with degenerate primers based on conserved regions of known fructosyltransferase genes, an *Lb. reuteri ftfA* gene was isolated encoding an inulosucrase. FTFA expression in *E. coli* yielded a recombinant enzyme that synthesized an inulin of more than 2 Mda.

The *Lb. reuteri* levansucrase (FTFB) enzyme subsequently was purified to homogeneity. Based on N-terminal and internal amino acid sequences, degenerate PCR primers were designed and used to clone the levansucrase (*ftfB*) gene. Expression of *ftfB* in *E. coli* yielded a recombinant enzyme that synthesized a levan from sucrose. Structural features determining mechanistic differences between levansucrase and inulosucrase are currently studied.

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ABSTRACTS OF KEYNOTE SPEECHES

MEDICAL PROTEIN ENGINEERING IN INDONESIA

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KS-01

ABSTRACT

Indonesia has started developing biotechnology since 1980's lead by the government efforts. However to date there has been no significant contribution of biotechnology to the society as indicated by the weakness of bioindustry in the country. Indonesia has to focus on specific application of biotechnology, instead of trying to master all possible fields. I believe that medical/health biotechnology is most promising application of biotechnology for the following reasons. Modern biotechnology was founded in 1970's to supply recombinant proteins for therapeutical use. Even until today, biotechnology products for therapeutics, diagnostics and vaccines are growing steadily. Thus, research on proteins could be said as the core of modern biotechnology, which continue, develop into bioindustry. Almost 10 years after the development of recombinant DNA technology, in 1980's emerged the field of protein engineering. Techniques, which cover full topics in protein engineering, could be described with examples of my works on development of PQQ glucose dehydrogenase as component of blood glucose meter. Firstly, protein engineering started from molecular biology. Engineering of proteins basically is process to change/mutate protein, which is done more efficiently by mutating DNA sequence of the protein. Therefore, at the beginning, there is a need to clone the gene either by PCR cloning or genome cloning. Recombinant DNA technology is applied to construct vector for easy manipulation of the gene. Mutation of DNA sequence could be approached by site-directed mutagenesis or random mutation either by error-prone PCR or DNA shuffling. Secondly are techniques of protein chemistry. These techniques include production of recombinant proteins in various choices of organisms ranging from bacteria, yeast, and animal cell lines to plants, and isolation of targeted proteins by purification and sometimes also requires refolding. Thirdly, characterization of purified proteins is done biochemically with focus on assay of activities (enzymatic activity, binding activity, etc) and biophysically, which considers on structural information of proteins (folding, stability, etc). Recently, bioinformatics has also contributed much on the advancement of protein engineering by supporting prediction and simulation on effects of mutation. Next, shall we apply all techniques of protein engineering in Indonesia or emphasized partly, especially if contribution to society (i.e. developing strong bioindustry) is taken into consideration? In my opinion, providing various products of recombinant protein-based biopharmaceuticals is the most important and urgent. Among various production platform, which I have experienced, the use of plants as host of protein production has multiple benefits to conduct in Indonesia. The use of plants as bioreactor of recombinant protein production is relatively new compared with already matured *E. coli*, yeast, and animal cell lines. Thus, the field is still full of "place to play" for newcomer. The techniques in plants are more complicated such as the need to consider of glycosylation. Thus mastering plant systems will may means of ability to works with other organisms. To add more clues, Indonesia is a tropical country with comparative advantage for growing plants. Among many possible choices of plants, I focus my work now on tobacco for additional reason of social benefit for traditional tobacco farmers as "sub-consumers" of the products.

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MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF GLUCANSUCRASES FROM LACTOBACILLI

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KS-02

ABSTRACT

Glucansucrases (GS) or glucosyltransferases (GTFs, EC 2.4.1.5) of lactic acid bacteria are large extracellular enzymes responsible for the synthesis of alpha-glucans from sucrose. Much research is performed on GSs from oral streptococci and *Leuconostoc* species, whereas little is known about GSs of lactobacilli.

A large collection of *Lactobacillus* strains was screened for the production of exopolysaccharides (VAN GEEL-SCHUTTEN et al., 1998). One of the positive strains, *Lactobacillus reuteri* 121, produced two different soluble homopolysaccharides during growth on sucrose, a fructan and glucan. Structural analysis of the polysaccharides produced by *L. reuteri* 121 revealed that the fructan is a linear levan with beta-(2,6) linked fructosyl units. The glucan possessed a unique highly branched structure with alpha-(1,4) and alpha-(1,6) linkages together with (4,6) branching points (a reuteran) (VAN GEEL-SCHUTTEN et al., 1999). Furthermore, different dextran- [alpha-(1,6)] and mutan [alpha-(1,3)] synthesizing lactobacilli have been identified (KRALJ et al., 2004a).

The *L. reuteri* 121 glucansucrase gene has been cloned, expressed in *Escherichia coli* and the GTFA enzyme was purified. Analysis of the glucans (reuterans) produced by the recombinant enzyme and of glucans isolated from supernatants of *L. reuteri* revealed that both glucans were virtually identical (KRALJ et al., 2002). The purified recombinant GTFA was molecularly and biochemically characterized, including the investigation of its acceptor reaction (KRALJ et al., 2004b).

The variations in linkage specificity observed between different glucansucrase enzymes is most likely based on differences in the acceptor binding sites. To investigate this in detail the (putative) acceptor binding sites of the reuteransucrase (GTFA) from *L. reuteri* 121 were subjected to site-directed mutagenesis. This resulted in marked changes in linkage specificity in the glucans and oligosaccharides synthesized by the mutant enzymes. Largest differences were obtained when mutations in different putative acceptor sites were combined (KRALJ et al., 2005).

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α-AMYLASE : From Bacteria to Yeast

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KS-05

ABSTRACT

α-Amylase is a starch converting enzyme which has been widely used in industries, such as foods, textiles, paper, and detergent. Starch is the second most polysaccaride on earth. The estimated annual global sales values of α-amylase by the end of 1990s is US$ 135 millions. α-amylase belongs to family 13 glycosyl hydrolase which cleaves α 1→4 glycosidic linkages present in the starch substrate. It is a multidomain protein with a core structure of $(\beta\alpha)_8$ barrel containing glutamic acid and two aspartic-acid requered for activity. Properties of enzymes hydrolysing starch vary and are more or less related to the growth envronment of the producing organism. Our group has been working with α-amylase from yeast and bacteria isolated from terrestrial abd non terrestrisl area in Indonesia, A gene encoding for amylase (*AFP1*) of *Saccaromycopsis fibuligera* R64 has been isolated and the predicted amino acid sequence is similar to a previously publishes sequence (Acc. No Y00683, except for the substitution of Asn153, Val159, and Asn189 to Asp, Ile, and Ser, respectively. The *ALP1* gene has been expressed under the control of *GAL10-PGKI* hibrid promoter in. Addition of 4.8% galactose in rich growth media induced *ALP1* expression and interestingly, the use of 1.0 soluble starch as an inducer gave4-fold increase of enzyme activity, a new disuphide bond is introduced into *ALP1*. an alp1 mutant contaiting two new codons forcysteine has been constructed. We have isolated a gene encoding for α-amylase (bla) from *Bacillus licheniformis* based on published sequence (Acc. No. AF438149). The bla gene will be cloned and expressed in the same expression syatem as *ALP1* gene. We have explored a potential application of thermostable amylase of B. Licheniformis in hydrolysing of sago Papua starch (*Metroxylon sagu* Rottbol). α-amylase has K_M of 2.19% (w/v) of sago Papua starch based on reducing sugar released and V_{max} of 625 units/ mg and maltopentose (DP-5) represented dominant hydrolysing products amongst other malto-oligosaccarides. Finally, to broaden our understanding on diversity of α-amylase, we are aiming at discovery of novel α-amylase which has new domain organization and substrate specificity (degrading starch granules). We have already had a collection of microbes from non terrestrial are which shows amylase activity.

HEMICELLULASES : Complex Enzymes and Its Industrial Prospects

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KS-06

ABSTRACT

Hemicelluloses, the most abundant renewable biomass polymer next to cellulose, represent about 20-35% of the lignocellulosic biomass. They are heterogenous polymers of pentoses (D-xylose, L-arabonise), hexoses (D-mannose, D-glucose, D-galactose), and sugar acids. 1,4-D-Xylan is the major component of hemicellulose. It forms the 1,4-β-D-xylopyranoside backbone structure of hemicellulose, to which acetyl, L-arabinofuranosyl, and 4-O-glucuronosyl side chain are attached. The frequency and composition of the branches depend on the source of the xylan. The total enzymatic degradation of xylan requires a multitude of enzymes, such as endo-β-1,4-xylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, ferulic acid esterase, and *p*-coumaric acid esterase.

Cloning, sequencing and expression of the genes encoding thermophilic-xylanolytic enzymes from *Bacillus thermoleovorans* IT-08, isolated from Gunung Pancar Hotspring, Bogor-West Java Indonesia have been accomplished. The genes encoding exo-xylanase (exo-xyn), α-L-arabinofuranosidase (abfa), and β-xylosidase (xyl) were successfully cloned into pTP510 and expressed in *Escherichia coli* DH5α. Analysis of the recombinant enzymes revealed activity against multiple substrates with the highest activity towards *p*-nitrophenyl-β-D-xylopyranoside (pNPX) ,*p*-nitrophenyl-α-L-arabinofuranoside (pNPA), and wheat xylan respectively. The sequencing result showed that the abfa and xyl genes indicated closely nucleotides amount , 1509 bp for abfa and 1536 for xyl, mean that both of those gene have closely molecular weight approximately 60 kDa. Recently, both abfa and xyl were successfully cloned and over-expressed separately in pET101/D-TOPO system, namely pET-abfa and pET-xyl2. The Abfa was belong to family GH51 and showed high similarity with Abfa from *Geobacillus stearothermophilus* T-6, although the Xyl indicated slight similarity with family GH43. Moreover, the crystallization experiment of Abfa and Xyl have also performed and preliminary X-Ray analysis of Abfa have done.

ABSTRACTS OF ORAL PRESENTATIONS

THE USAGE OF ALCOOLOXIDASE FOR FORMALDEHYDE DETERMINATION IN FOOD PRESERVATIVE BY APPLYING BIOSENSOR TECHNIQUE

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OP-01

ABSTRACT

A novel biosensor technique with an ion selective electrode (ISE) can be utilized to detect formaldehyde in food preservative by applying alcooloxidase (AOX) to decompose the formaldehyde into H^+ ion, and then the H^+ ions are collected in a hydrogen ionophore. This preliminary investigation is to do with the fabrication of hydrogen biosensor, which is an ion selective electrode (ISE) for collecting H^+ ions produced by formaldehyde. The ISE consists of a solid state Ag/AgCl electrode as the working electrode, which is firstly layered by a hydrogen ionophore I in poly(n-butylacrilate) or PBA as supporting matrix to form the first solid membrane. A 3 minute photocuring technique is applied to polymerize n-butylacrilate employing a uv light together with the lyphophylic salt, i.e the sodium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate or NaTFPB, and the Ag/AgCl working electrode. The NaTFPB is used to increase H^+ ions attractions to penetrate the solid membrane. The second solid membrane is pasted to the first solid membrane, which contains the AOX enzyme immobilized in p-Hema. The Ag/AgCl working electrode, which is pasted with two membranes, is immersed in a formaldehyde solution. The reference electrode is a double junction Ag/AgCl electrode with 1.0 M tris-HCl pH 7.0 and 1.0 M lithium acetate in the gel bridge electrolyte. The electrical network is connected to Orion ion-meter to detect the potential difference between the working and the reference electrodes, which is linearly correlated to the logarithmic of H^+ ions concentration. The reaction mechanism is as follows: when the formaldehyde solution in contact with the AOX in p-Hema, H^+ ions are produced and then collected in the hydrogen ionophore in PBA. The NaTFPB makes the membrane more negative, and hence more H^+ ions attracted to the membrane, and then carried by the ionophore that caused an increase in electrical charge on the working electrode surface. Thus, the deflection in the ion-meter indirectly indicates the concentration of H^+ ions yielded by formaldehyde in the sample. The movement of H^+ ions in solid membrane follows the Hopping method. The hydrogen ionophore is highly selective to H^+ ions, this is really worthy in the employment of real samples such as food preservatives, biological materials, environmental samples that produced other cations, e.g. K^+, Li^+, NH_4^+, etc. The usage of NaTFPB is very useful since it causes more negative membrane that attracts more H^+ ions and repels the negative particles. Although this investigation is still in a qualitative design form, however, previous work using different matrix, i.e. the MB-28, which is a copolymer of methylmetacrilate and n-butylacrilate (2:8) instead of PBA, showed good Nernstian value of 50.7 mV/decade with pH linearity of 4.14 - 8.21 and formaldehyde range of 10^{-1} – 10^{-4} M in 0.1 mM tris-HCl buffer pH 7.0. Moreover, previous work employed 16.7% w/w AOX in p-Hema. This sensor fabrication applies a screen printed electrode instead of the former rod type. The chemicals quantity used in this investigation have been undertaken by considering previous work. This analytical technique is feasible for ASEAN developing country like Indonesia since it gets involved with low cost equipment.

ISOLATION OF PROTEOLYTIC BACTERIA FROM DIGESTIVE TRACT OF TILAPIAS STRAIN GIFT (*Oreochromis niloticus* (Linnaeus) Trewavas) AND CHARACTERIZATION OF ITS EXTRACELLULAR PROTEASE

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OP-02

ABSTRACT

The aims of this experiment was to isolate proteolytic bacteria from digestive tract of tilapias strain GIFT (Genetic Improvement of Farm Tilapias) and to study biochemical properties of the enzyme produced by the selected bacteria. Thirty one bacterial isolates that were incubated at room temperature (25-27 °C) revealed proteolytic activity in nutrient agar containing 0.5% skimmed milk. Isolate *Enterobacter* sp. strain NU5 and *Aeromonas* sp. strain NU8 were selected based on proteolytic index. Their proteolytic activity will compared with the fish pathogenic bacteria *Aeromonas hydrophila*. The optimal protease production of NU5 and NU8 was obtained after 24 hours of incubation at pH 7 and room temperature. The highest activity of NU5 and NU8 protease was observed at pH 7, 50 °C, and pH 7, 70 °C respectively. In the presence of 5mM EDTA, the crude enzyme activity decreased down to 24.6% and 14.1%, respectively, which indicated that the enzyme might be metalloprotease. Study of thermal stability of the enzyme showed that decreased 85% (NU5) and 94% (NU8) activity up to 30 minutes incubation time at optimum temperature. *Aeromonas hydrophila* produced lower protease activity than NU5 and NU8 at pH 8 and 70 °C, and the addition of 5 mM EDTA did not affect the protease activity. The activity was increased up to 500% after 30 minutes incubation at 70 °C.

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SUN EXPOSURE'S EFFECT TO THE PARATHYROID HORMONE RATE

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OP-03

ABSTRACT

The aim of this research is to analyze effect of sun exposure to the Parathyroid Hormone (PTH) rate. This experimental research involves 73 Malay post-menopausal women who lived in the rural community in the region of Sepang, Selangor, Malaysia. However, after giving the sun exposure treatment research subject decreased to 58 persons. The treatment has been done for 3 months, in which the subject has to expose to the sunlight between 10 am to 12 pm for 20 – 30 minutes, twice or three times a week. Age of the research subject is between 49 to 74 years old with the average of 62.1 ±□□□years. PTH is determined using *immunoraiometric assay* (IRMA) method. Questioner is handed out to obtain demography data of the subjects. Result of this research shows that average rate of PTH (pmol/l) before sun exposure treatment is 5.39 ± 2.39 with minimum rate is 2.1 and maximum rate is 13.2. In the meanwhile, average rate of PTH (pmol/l) after sun exposure treatment is 5.15 ± 1.83 with minimum is rate 2.4 and maximum rate is 9.6. Before treatment 87.7% women have a normal PTH and 12.3% is categorized as a hyper parathyroid. After treatment total research subjects that have normal PTH increased to 93.1% and the hyper parathyroid distressed decreased to 6.9%. There are some increments on the number of subject that has normal PTH rate after sun exposure treatment to 5.4% and some decrement of number to the hyper parathyroid distressed to 5.4%. Eventhough subject with normal PTH rate increased in number after treatment, based on t-test taken the PTH rate before and after sun exposure treatment is not significant ($p>0.05$).

DEVELOPMENT OF SESAME SPROUT LIPASE PURIFICATION USING IMMOBILIZED METAL AFFINITY

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OP-04

ABSTRACT

A high-density matrix was prepared by coating an alumina particle with agarose using an emulsion technique. Iminodiacetic acid was immobilized onto this matrix. Charging this matrix with copper created a useful chromatography matrix for purification of indigenous sesame sprout lipase. Butanediol diglycidyl ether (BDGE) was used as spacer arm. Factors such as, pH, concentration of BDGE, NaCl and imidazole were investigated. Based on both the adsorption capacity of matrix and the eluted adsorbed lipase, the optimum BDGE concentration and the adsorption pH were 40 % and 7, respectively. However, an increase in NaCl concentration in adsorption buffer from 0.5 to 1.5 M resulted in 2 times decrease in the ratio between adsorbed lipase and adsorbed total proteins. More interestingly, immobilization of Cu^{2+} on this matrix was highly effective in the purification lipase, since lipase could be easily eluted from matrix using low concentration of imidazole (10 mM). Enzyme recovery and purification factor were 80% and 9.4, respectively.

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COMPARISON OF BETA CYCLODEXTRIN PRODUCTION BETWEEN GELATINIZED AND UNGELATINIZED SAGO STARCH WITH CYCLODEXTRIN GLUCANOTRANSFERASE (CGTase)

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OP-05

ABSTRACT

Cyclodextrin is a cyclic and nonreducing oligosaccharide consisting of α-D-glucose linked with α-(1,4)-glycosidic bond. Cyclodextrin is very useful for food, cosmetic, pharmaceutical, agriculture and plastic industries. Beta (β)-cyclodextrin can be produced from several types of starch and treatments. The purpose of this study is to observe the effect of treatment to sago starch; gelatinized and ungelatinized in β-cyclodextrin production. The reaction temperature for both starches are based on the gelatinization temperature of sago starch. β-Cyclodextrin production using the ungelatinized sago starch was done at 65°C. Four parameters reaction which were pH and buffer; concentration of enzyme; concentration of substrate and reaction of time have been compared in β-cyclodextrin production. β-cyclodextrin production with gelatinized sago starch gave the maximum yield with conditions at pH 8 in Phosphate buffer, at 70°C, 2% (v/v) concentration of CGTase and 6% (w/v) substrate for 3 hours reaction. While using the ungelatinized sago starch found that the optimum conditions were pH 9 in glycine-NaOH buffer, 0.5% (v/v) CGTase concentration, 15% (w/v) sago starch for 4 hours of enzymatic reaction.

SELECTION AND CHARACTERIZATION OF *Bacillus subtilis* ON ENZYMES PRODUCTION FOR INDUSTRIAL DETERGENT

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OP-06

ABSTRACT

Detergent enzymes have been introduced in the Indonesian market since 1990. However, because of the washing habit using lower temperature, the efficacy of the detergent enzymes in strain removal has not significance The alkolophilic protease, α-amylase cellulase and lipase enzymes is used in general as protein hydrolyzing, α-1,4 glycoside hydrolyzing, β-1,4 glycoside hydrolyzing, hydrophobic triglyceride reducing, respectively.

The main target of the experiments is to look for the best-isolated Baccillus of BPPT collection for protease, α-amylase and cellulase production by using local-cheap substrates of 3 % molasses and 1 % of urea as carbon and nitrogen source, respectively. The experiments were carried out by using 3 *l* of biostat fermentor in 37 °C, 8, 225 rpm, 1 vvm of temperature, pH, agitation and aeration, respectively, in order to obtain the performance curve of selected microbe. Moreover, we have learned the characterization of selected enzymes which is related to pH, temperature, and the stability of enzymes production.

The CC 02 of *Bacillus subtilis* BPPT was selected among the microbes collection. Because of protease, α-amylase, and cellulase enzymes activity, the specific growth maximum of fermentation curve is 0.248 h^{-1}. The activity of protease, α-amylase and cellulase is 3,1; 1,02 and 0.94 U/ml.min, respectively. We have also obtained that the pH, temperature and stability of enzymes were 8, 50 °C and 90 minute, respectively.

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THE INTERPLAY OF ENZYME, INDICATOR AND ANALYTE IN OPTICAL BIOSENSOR LAYER

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OP-07

ABSTRACT

The interplay of enzyme and analyte (i.e. substrate and inhibitors) has been studied in the presence of bromothymol blue (BTB) as pH indicator on the sol-gel glasses of the optical biosensor layer. Here, the optical biosensor layer consist immobilized urease and BTB as an indicator on sol-gel glasses. The operation of the biosensor is based on the well-known urease enzyme-catalyzed hydrolysis of urea. The hydrolisis of urea by urease that release a pH change can be detected suitable using BTB at the wavelength 670 nm (at pH 7.0). With this arrangement, the optical biosensor layer can be used to detect urea in the linear range 0-1 μg/ml, with limit detection 0.1 μg/ml. The response time of 3 mins, with reproducible response and readily reversible. Furthermore, the optical biosensor can also be used for detection of heavy metal ions as inhibitors. The sensing process was based on urease inhibition by heavy metal ions as, where the net of pH change resulted by enzymatic reaction of urea toward ammonia was detected by BTB at 665 nm. Here, heavy metal ions tested were Ag(I) and Hg(II), since both metal ions show good inhibition towards urease. The optical biosensor shows linier response in the concentration range 5-100 ppb for Ag(I) and 10-500 ppb for Hg(II). The optical biosensor response has shown in good agreement with enzymatic assays in solution, when applied for determination of urea and heavy metal in water samples.

CATABOLISM OF 4-*O*-ACETYLATED SIALIC ACID

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OP-08

ABSTRACT

Term of sialic acids (*sia*) refers to *N*-acetylneuraminic acid (Neu5Ac) or *N*-glucolylneuraminic acid (Neu5Gc), a pyranose sugar with 9 carbon atoms, having negative charge due to the carboxyl group at C-atom 1 (**Schauer *et al.*, 1995**). It usually found as terminal in glycolipid and oligosaccharide. Nowadays more than 40 kinds of *sia* were found from organisms except plants (**Traving and Schauer, 1996**). Enzymes involve in modification of the *sia* are 8-*O*-methyltransferase as well as 4-and 7(9)-*O*-acetyltransferases (**Schauer, 1997**). Catabolism of 4-*O*-acetyl-*N*-acetylneuraminic acid ($Neu4,5Ac_2$), which blocked most of sialidase activity, was first elucidated as esterase was found in horse liver and demonstrated that it involved in the $Neu4,5Ac_2$ catabolism (**Schauer *et al.*, 1988**). It is interesting to explore more information from animal rich in $Neu4,5Ac_2$ like horse, concerning the catabolism of $Neu4,5Ac_2$ (**Tiralongo *et al.*, 2000**). Here we report that "different" sialidase found in two kinds of horse liver showing no activities on $Neu4,5Ac_2$.

Sialidases from two kinds of horse liver (A and B) found to be different in their properties. Sialidase from horse liver A was partially purified using S-Sepharose FF and *p*-aminophenyl oxamic acid agarose, following solubilization and "activation" with the yield of 6 % and purification factor of about 500. Sialidase from horse liver B was partially purified using Fractogel, *p*-Aminophenyl thio-β-D-galactopyranoside agarose, and chromatofocusing with PBE94, following solubilization with the yield of 0.2 % and purification factor of about 20. Sialidase isolated from horse liver A showing no activities of β-galactosidase and could be "activated" by incubating the homogenate at 37 °C in acidic condition. However, the sialidase isolated from horse liver B showing activities of acid β-galactosidase and could not be "activated".

Sialidase from horse liver A showed no activities of β-galactosidase, it seemed to be single enzyme of sialidase as reported in some different sources (**Michalski *et al.*, 1982; Miyagi *et al.*, 1990**). However, sialidase from horse liver B showed activities of β-galactosidase and carboxypeptidase A. This evidence proved that the sialidase from horse liver B is a complex enzyme with β-galactosidase and carboxypeptidase A as reported by **Hiraiwa *et al.*, 1996,** and **Pshezhetsky and Potier *et al.*, 1996.**

The two sialidases showed no activities on $Neu4,5Ac_2$ when subjected to bovine submandibular mucin and guinea pig serum as substrate. The free acetylneuraminic acid was detected by Fluorimetric HPLC (**Reuter and Schauer, 1994**). The HPLC data emphasized that the catabolism of $Neu4,5Ac_2$ involve esterase activities, the esterase was first modified the acetyl at C-4 allowing the sialidase to hydrolyze the normal sialic acid from sialoglycoconjugate (**Schauer *et al.*, 1988**)

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EXPRESSION OF SOYBEAN β-1,3-ENDOGLUCANASE cDNA AND EFFECT ON DISEASE TOLERANCE IN CABBAGE PLANTS

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OP-09

ABSTRACT

Cabbage (*Brassica oleracea* cv. *capitata* L.) is one of the main and important upland vegetables in Indonesia. However, growing cabbage is facing so many problems, especially fungal disease where there is no resistance gene found. Therefore, the development of cabbage resistant to fungal disease through the use of unconventional method is utmost important. *Agrobacterium*-mediated transformation of soybean β-1,3-endoglucanase cDNA, a fungal disease resistance gene, into cabbage genome has been done and got seven cabbage transformant. The expression of soybean β-1,3-endoglucanase cDNA in cabbage transformant was checked by dot blot hybridization of total RNA of recombinant planlet using amplified β-1,3-endoglucanase cDNA fragment as a probe. Disease tolerant of the recombinant cabbage was analyzed by inoculating the recombinant planlets using *Fusarium* sp. Analysis of soybean β-1,3-endoglucanase cDNA expression demonstrated that four out of seven recombinant cabbage planlets were able to express transformed gene, and three of them showed a complete resistance to *Fusarium* sp infection. However one of them showed a partial resistance as demonstrated by appearance of necrotic area that was smaller than untransformed cabbage.

ENHANCEMENT OF DNA REPAIR ENZYME O^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE BY (-)-EPIGALLOCATEHIN GALLATE FROM GREEN TEA

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OP-10

ABSTRACT

O^6-Alkylguanine-DNA alkyltransferase (AGT) is an important DNA repair enzyme that protects cells from killing and mutagenesis by alkylating agents. This protein can corrects DNA damage on O-6 position of guanine DNA. The purpose of this study was to determine effects of (-)-Epigallocatechin Gallate (EGCG) from green tea on increasing AGT level of hepatocyte primary rat liver cell culture for 2 days. AGT activity was identified by radioactivity method and counted by Liquid Scintillation Counter. After exposured by EGCG 8.3 ppm, 16.7 ppm, 33.3 ppm and 66.7 ppm, AGT activity was found 16.1, 23.5, 33.7 and 41.2 fmol/ug DNA, respectively. These result mean that alkyltransferase level increase 1.36, 1.95, 2.57 and 2.22 fold, respectively. Because of that fact, we conclude that EGCG plays important role in enhancing of AGT activity in mouse hepatocyte.

DNA TOPOISOMERASE I AND II ENZYME AS TARGET TO DISCOVERY OF NEW ANTICANCER DRUGS OF NATURAL PRODUCT

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OP-11

ABSTRACT

DNA topoisomerase alter the topological state of DNA, thereby carrying out function essential for several cellular processes including DNA replication, transcription, recombination, DNA repair, and chromosome segregation at mitosis . There are two types of DNA topoisomerase : type I enzymes changes the DNA linking number by transiently breaking one strand of duplex DNA, while type II enzymes transiently breaks both strand. In addition to their important fuction in DNA metabolism, both topoisomerase I and II have generated extensive clinical interest in cancer chemotherapy . In human, the level of DNA topoisomerase in various tumors was result of incresed, compared as normal cells. The increase in topoisomerase in various tumors was a result of increased transcription and translation of of DNA.

In the 1980's , DNA topoisomerase I and II enzyme were shown to be the principal target for a number of clinically important anticancer agents which includes plant-derivied topoisomerase I inhibitor , camptothecin and topoisomerase II inhibitor VP-16 (epipo-dophyllotoxin). All these drugs trap the enzyme in intermediary reversible complex with DNA, termed the " cleavable complex", which prevent the final rejoining step of the reaction and result in increased DNA strand cleavage and resulted replication fork arrest and cell death followed programmed cell death " apoptosis". So, topoisomerase I and II enzyme could used to screening of natural product to get the selective of new anticancer drugs.

The result of anticancer activity screening by DNA topoisomerase I and II enzyme as molecule target of many Indonesian herbal medicine which generally used as anticancer , shown the andrographolide from *Andrographis paniculata* Nees was inhibited topoisomerase II, alkaloid carpaina from *Carica papaya* was inhibited topoisomerase II and pinostrobin from *Kaempferia pandurata* was inhibited topoisomerase I. The study of expression of protein molecules of *p53, bax, bcl-2 , cytochrom c* and *caspase-3* that regulate the cells cycle and apoptosis mechanism of human breast cancer by andrographolide , alkaloid carpaina and pinostrobin is in progress on our laboratory.

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PHYLOGENETIC ANALYSIS OF BACTERIAL COMMUNITIES IN PANCURAN 7 BATURRADEN HOT SPRING

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OP-12

ABSTRACT

The phylogenetic diversity of the bacterial communities supported by culturing and capturing trough 0.2 m-pore-size filter was studied. The Pancuran 7 hot-spring has temperature 52°C and pH 7. Community fingerprint analysis by denaturing gradient gel electrophoresis (DGGE) of the PCR-amplified highly variable V9 region of the 16S rRNA gene from the domain *Bacteria* has performed. Five distinct DGGE bands have been chosen to be analyzed their phylogenetic relationship towards another bacteria. The 16S rDNA sequence fragment analysis of three DGGE bands revealed a high relationship with *Bacillus* group, two of them have a high similarity with *Anoxybacillus sp.* and one of the single colony that grown at ½ LB medium closely related to *Geobacillus lituanicus.*

PROTEASE ACTIVITY OF EXCRETORY/SECRETORY RELEASED BY INVASIVE STAGE OF *Ascaridia galli*

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OP-13

ABSTRACT

Excretory/secretory (ES) released by *Ascaridia galli* can modulate immune response mechanism and cause inflammatory in the small intestine of poultry. The mechanisms that underlie tissue damage and invasion by the invasive stages (L_3) of the parasitic nematode *A. galli* are poorly understood, but involvement of as yet uncharacterized protease has been suggested. Here, we employed casein assays to examine protease activity in ES product released by L_3 of *A. galli*. Nature female adult worm were obtained from lumen of village chickens in a comercial abattoir in Bogor. The eggs obtained from uteri female adult worms were incubated in sterile aquadestilata at room temperature for 21- 30 days developed embrionated eggs (L_2). *A. galli* L_3 were recovered from intestines of 100 heads chickens 7 days after each oesophagus inoculation with 6000 L_2. L_3 recovered in this manner were cultured (5 – 10 ml^{-1}) in flasks containing RPMI 1640 media, pH 6.8, without phenol red and suplemented with 100 units ml^{-1} penicillin G, 100 ìg ml^{-1} streptomycin, 5 ìg ml^{-1} gentamycin, and 0.25 ìg ml^{-1} kanamycin. Cultures were incubated at 37^{0}C in 5% CO_2 and ES product of L_3 released in culture was collected after 3 days. The protease activity was assayed against casein. The amount of degradation was determined from the absorbance at 578 nm. One unit of enzyme activity was defined as the amount of enzyme necessary to elaborate 1 ìg of tyrosine from casein in 1 ml of reaction volume per min. The result showed that enzyme activity and protease specific activity of ES product released by L_3 of *A. galli* were 0.5652 x 10^{-2} IU/ml and 0.575 x 10^{-3} IU/mg respectively. The result indicate that the infection process of a number of organisms, including some nematodes, depends on protease. *A. galli* may likewise utilize protease to facilitate larval invasion, migration within the host tissue, and modulation of host immune response mechanisms.

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MAPPING OF *SALMONELLA TYPHI* PROTEINS BASED ON PROTEOMIC ANALYSISTECHNOLOGY AS AN EFFORT TO DISCOVER BIOACTIVE PROTEIN

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OP-14

ABSTRACT

This investigation is aimed to create a two-dimensional protein map of *Salmonella typhi,* which is necessary for improving the understanding about molecular mechanisms of typhoid disease. For the purpose, the proteomics techniques were performed in five successive steps: isolation of *S. typhi* crude extract proteins, one-dimensional [1-D] electrophoresis, two-dimensional [2-D] electrophoresis, 2-D electrophoregram analysis using the TopSpot software, and homology comparison with the SwissProt database. The results, which were obtained from more than 4 replicates, revealed that the pattern of *S. typhi* spot proteins are oriented to the pH base area. Based their on pI and Mr, it coud be mapped more than 242 spot proteins. Four of those spot proteins, there are spot 1 [pI 6,7, Mr 46,8 kDa], spot 17 [pI 6,9, Mr 42,4 kDa], spot 492 [pI 7,2, Mr 47,2 kDa] dan spot 518 [pI 6,9, Mr 56,2 kDa], could be determined as Reference Spot for *S. typhi*. Since homology comparison indicated that the similar 2-D protein map is not yet present in the SwissProt database, therefore the gained 2-D protein map of *S. typhi* is a really contribution for the development of basic sciences and base for investigate protein bioactive.

ENHANCER-TRAP TECHNIQUE TO SCREEN THE GENES THAT EXPRESSED IN THE *Drosophila* SALIVARY GLANDS.

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OP-15

ABSTRACT

One approach to study development is to obtain genetic variants that are detective in some crucial step. One of the methods is the insertion mutagenesis using P-element to generate new alleles of the genes. P-element vectors also have been used to identify *cis-acting* sequence that confer tissues-specific expression on reporter genes like β-galactosidase (*lac Z*) fused to the weak promoter of the P-element transposase gene. There are 14 enhancer-trap lines that expressed β-galactosidase in the salivary gland of Drosophila embryos. The expression of β-galactosidase showed in the different stage and part of salivary gland embryo. It was consequence, by plasmid resque they showed the different genes that act in each lines.

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STUDY ON THE REACTION OF γ-TOCOPHEROL MODEL COMPOUND, 2,2,7,8-TETRAMETHYL-6-CHROMANOL

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OP-16

ABSTRACT

Endogenous oxidants, by-products of normal metabolism, protect humans from infection, but cause extensive damage to DNA, protein, lipid and mutation. This damage is contributes to aging, degenerative diseases of aging such as cardiovascular disease, and the carcinogenic process. Antioxidants have generated interest as anticarcinogens and as defenses against degenerative diseases.

Peroxynitrite is one of the endogenous oxidants, which is a strong oxidant and nitrating species. Peroxynitrite anion is stable but HOONO decomposes rapidly after protonation to form the reactive hydroxyl radical ($OH^{\cdot}$) and nitrogen dioxide free radical ($NO_2^{\cdot}$). The investigation of γ-tocopherol as an antioxidant to protect against peroxynitrite-induced lipid oxidation has been performed. By using the model compound of γ-tocopherol, 2,2,7,8-tetramethyl-6-chromanol (TMC) and ethanol solution as useful lipid-phase model, $NO_2^{\cdot}$ generated from $NO_2^{\cdot}$ during its reaction with nitrous acid, is capable of nitrating TMC. $NO_2^{\cdot}$ may add to the C_5 position as a nitrogen-centered radical ($NO_2^{\cdot}$), forming a nitro derivative which is relatively stable.

THE EFFECT OF JAVA GINSENG (*Talinum paniculatum* Gaertn) ROOT EXTRACT ON TESTOSTERONE LEVEL ASSOCIATED WITH TESTICULAR AND HEPATIC PROTEIN

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OP-17

ABSTRACT

The objective of this research was to evaluate the effect of Java ginseng (*Talinum paniculatum* Gaertn) root extract on testosterone level in normal and estrogen-treated mice that had low level of testosterone and on profile of protein expression in testis and hepar. Male mice (Mus musculus sp strain BALB-C) aged 9-10 weeks, weighed 25-35 g were divided into 2 groups. The first group was as control and second group was given with Java ginseng root extract (equal with 3,5 mg dried root/100 g bw/day). Both were divided to three groups: Subgroup 1 wasn't given estrogen, subgroup 2 was given with estrogen (1 µg ethynilestradiol /100 g bw/day) along 18 days and subgroup 3 was given with estrogen during 27 days. At the end of treatment (days 27th), blood serum, the extract of hepar and testis were collected. Testosterone level of serum was measured by RIA (radioimmunoassay) Extract of hepar and testis in PBS (phosphate buffer saline) were lyophilized then separated by 12,5% SDS-PAGE Laemnli method. The profile of testicular and protein expression were observed using densitometry on protein bands. The result suggested that Java ginseng root extract could increase testosterone level in normal mice but not in estrogen-treated mice. Testicular proteins with MW 23, 63 and 36 kDa were influenced by Java ginseng root while hepatic proteins with MW 155 and 90 kDa.

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PROSPECT OF *Bacillus subtilis* 3KP BIOSURFACTANT AS PHYTOPATHOGENIC ANTIMICROBIAL AGENT

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OP-18

ABSTRACT

Surface active compound produced by microorganism called biosurfactant was potential to be employed for industrial processes and environmental protection. Biosurfactant prospect from *Bacillus subtilis* 3KP bacteria, isolated from Donan Rivers Indonesia, as antimicrobial agent was tested against two pyhtopathogenic microbia (*Xanthomonas campestris and Fusarium solanii*). Biosurfactant product was obtained by cultivating *Bacillus subtilis* 3KP in mineral medium added with molasses (2%). Biosurfactant product from *Bacillus subtilis* 3KP was characterized as a lipopeptide compound. Antimicrobial activity of biosurfactant product was conducted in different concentrations (0, 100, 400, 700, 1000, 4000, 7000, and 10.000) ppm. Microbial inhibition potency of biosurfactant was measured by inhibition zone diameter and (Minimum Inhibitory Concentration) MIC value using diffusion and tube dilution methods. Data was analyzed statistically using one-ways variance analyze (ANOVA) and LSD (Leat Significance Difference) test. The results showed that *Bacillus subtilis* 3KP biosurfactant could inhibit *Xanthomonas campestris and Fusarium solanii* growth. The MIC values of *Bacillus subtilis* 3KP biosurfactant against *Xanthomonas campestris* was 1000 ppm and *Fusarium solanii* was 4.000 ppm.

THE UTILIZATION OF PROTEASES THERMOPHILE CG-10 ISOLATE TO PRODUCE COCONUT OIL BY ENZYMATIC PROCESS

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OP-19

ABSTRACT

The thermophile lives in extreme enviroments, at temperature above 50°C. Enzyme which is produced active at a higher temperature than it live enviroment, so it is possible to use at industries that use high temperature for the production proccess. The thermophile has been isolated from hot water spring at Cangar Batu Malang called CG-10 isolate. The characteristic of CG-10 are: bolt colony, has a brown-white colour, stick form cels and gram positive. Identification with 16S-rRNA gen of their isolate shows 98,305% similarity with *Bacillus caldoxylolyticus*. This isolate can be secretion extracellular protease. The protease characteristic of CG-10 isolate, fractionated by ammonium sulfate 35% (w/v), centrifuged by 4000 rpm of speed for 15 minute, is: (1) has optimum temperature of 80°C; (2) has optimum pH 8, (3) the molecule weight of: 43,000-170,000 Dalton. Research has been done for the utilization of protease thermohile CG-10 isolate for produce coconut oil by enzymatic process. The research is laboratory experiment with raw materials coconuts hibrida which was bought in keputran market. The principle of producing of coconuts is destroy the emultion system by enzymatic process. Coconut cream was mixed by protease CG-10 isolate and incubated for 24, 48, 72, and 96 hours. The oils that was produced than was analized their rendement and quality (content of water, acid number, Iod number, and organoleptic quality for colour and odor). The results of this research are incubation time protease CG-10 isolate effect to the product coconut oil. Incubation for 72 hours produced the highest rendement, is 20,24%. Base on the content water, acid number, and Iod number, the incubation for 48 hours showed the best quality (content of water: 0,39 %, acid number: 0,75 mg KOH/g, Iod number: 9,35 g iod/100 g.). Organoleptic test showed: 40 % respondent like the colour oil and 66,67 % like the odor oil which was produce in this treatment.

FROM CO-EXPRESSION TO CO-REGULATION: Bioinformatics Approach for Studying Gene Expression

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OP-20

ABSTRACT

The availability of the global gene expression data have provided abundant evidence that sets of functionally related genes are coordinately induced or repressed in response to developmental or environmental changes, presumably via the action of sequence-specific DNA-binding transcription factors (TFs). This provides a mechanism to control specific aspects of physiology and it also enables the use of gene co-regulation to predict gene function, and underlies the fact that expression profiles can be used to classify samples.

Gene expression is largely controlled at the transcriptional level, with transcriptional regulatory elements are located primarily in the upstream promoter region of each gene. With the wide availability of genome-wide expression data, it is possible to identify upstream regulatory motifs commonly shared by co-regulated genes.

The common strategy to reveal the possibility in identifying co-regulated genes is by clustering analysis. The approach used in this paper to mine yeast expression data was started from clustering on the expression profiles followed by function categorisation and promoter analysis of the upstream region of the genes. By combination of over-represented oligonucleotide analysis and multiple-sequence alignment programs, it is possible to identify upstream regulatory motifs commonly shared by co-regulated genes. It is believed that good clustering is better than sophisticated motif-search algorithms. It would be highly desirable if one could combine motif and cluster analyses, as good clustering can facilitate motif identification, and, conversely, conserved motifs (or any other functional information related to the sequences) can help to improve clustering. However, the lack of quality upstream experimental data has made systematic global investigations very difficult.

Predictions for which DNA-binding protein might be interacting with the motif can be obtained by computational methods, such as finding which predicted DNA-binding proteins have the motif in their upstream region, and searching for a member of a known DNA-binding protein family. The biological significance of some of the motifs presented here should be verified experimentally, including determination of factors binding to these motifs.

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PRODUCTION AND CHARACTERIZATION OF CHITOSANASE PRODUCED BY JB4 ISOLATED FROM TERASI

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OP-21

ABSTRACT

Jb4 is selected isolate from terasi which produced chitosanase. Chitosanase are chitinolytic enzyme involved in chitosan-oligomer production, chitooligosaccharide which is water soluble and useful in various applications, including nutraceutical, medical, and pharmaceutical applications. The isolate was growth in minimal synthetic medium (MSM) with addition of 0.5% colloidal chitin at 37°C for 24 hours. Optimum pH of enzyme was at 7 and temperature was at 50oC. The effect of metal ion (as chloride salt) inhibitors showed that enzyme was inhibited by mg^{2+}, Fe^{3+}, Cu^{2+} and Co^{2+} but not by Ca^{2+}, Zn^{2+}, K^{+} and Na^{+}.

PROPERTIES OF CHITINASE ISOLATED FROM A BACTERIAL STRAIN ASSOCIATED WITH MARINE SPONGE

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OP-22

ABSTRACT

Chitinase is one of hydrolytic enzymes which have gained much scientific attention in recent years, mainly due to its promising application in many industries and agriculture, including production of biologically-active chitooligosaccharides, biocontrol of plant pathogenic fungi and insects, and preparation of protoplast. In our work, chitinase has been isolated from a bacterial strain associated with a marine sponge by cell-free supernatant (CFS) preparation, ammonium sulphate percipitation, and dialysis. Study on the properties of the isolated chitinase shown that the optimal pH for its chitinolytic activity was found to be 6.0. Subsequently, the optimal temperature for this enzyme was 60°C, which was relatively higher compared with the majority of chitinases of mesophilic microbial origin. The addition of Cu^{2+} ion as chloride salt gave inhibition by 20% on the activity. Other metal ions, (*e.g.* Mn^{2+}, Ca^{2+}, Ba^{2+}) gave a small decrease in the activity. An increased chitinase activity was observed on Zn^{2+} ion, which was almost two times higher than the control without any metal ion. The apparent V_{max} and K_m of the chitinase towards colloidal chitin were 0.2346 U/mg and 1.61 mg/ml, respectively.

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IS THERE ANY RELATIONSHIP BETWEEN HYPHAL GROWTH UNIT AND FUNGAL ENZYME PRODUCTION?

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OP-23

ABSTRACT

Filamentous fungi in submerged culture can grow in several different forms, varying from dispersed filaments to pellets, where each type of form may be associated with a specific enzyme formation. Fungal morphology, depending on the organism and culture conditions used, affects directly the physical growth environment, potentially leading to an impact on enzyme secretion. For instance, glucoamylase production by *Aspergillus niger* may be related to its morphology since significant differences of its hyphal elements were observed, which become shorter in response to increases in agitation speed corresponding to the increase in glucoamylase activity. Therefore an objective quantification of fungal morphology is required in any study on the role of culture conditions on enzyme production. One of the common parameters used for dispersed filamentous growth is the hyphal growth unit (HGU). Furthemore, branching frequency is known to affect fungal culture rheology and viscosity, which reportedly fell with a decrease in HGU *in Aspergillus oryzae*.

Since enzyme secretion in filamentous fungi has been thought by some workers to occur predominantly at the growing hyphal tips, there has been considerable speculation as to whether enzyme productivity might be improved by growing the fungus under conditions that encourage branching, and thus the generation of more growing tips. However, the evidence available to support this view is contradictory. For example, although a recombinant strain of *Aspergillus oryzae* producing a denser, more highly branched mycelium produced more α-amylase than the wild type, another *Aspergillus oryzae* strain with lower branching frequency gave even higher α-amylase production. Furthermore, the increasing number of hyphal tips per unit of biomass did not always result in an increase in extracellular specific activities of glucoamylase in *Aspergillus oryzae*. This study carry out to examine the relationship if any, that might exist between the production of both (1→3)-β- and (1→6)-β-glucanases and the morphology of *Acremonium* sp. IMI 383068.

THE POTENCY OF POLYSACCHARIDE KRESTINE (PSK) AS A APOPTOSIS INHIBITOR AND PROTECTIVE EFFECTS OF GAMMA COBALT ^{60} IRRADIATION AND 2-METHOXYETHANOL INDUCES CONGENITAL MALFORMATION

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OP-24

ABSTRACT

Polysaccharides Krestine (PSK) produced by *Coriolus versicolor* are effective immunopotentiators, as biological response modifiers and play an important role in the defense against oxidative injury. 2-Methoxyethanol (2-ME) or ethylene glycol monomethyl ether (EGME) is a glycol ether used as an organic solvent in many industrial and household product. 2-ME and gamma irradiation causes embryotoxic and teratogenic effects in rodents, rabbits, and nonhuman primates. This study was undertaken to evaluate the potency of PSK on inhibitions of persentage of cell death and incident of congenital malformations induces by 2-ME and gamma irradiation.

Pregnant mice were injected with a single intraperitoneal dose of 11 mmol/kg body weight of 2-ME at gestational days (GD) of 7th, 9th, and 15th, however gamma irradiation were exposed on GD of 13. One hour after 2-ME injection or gamma irradiation mice were given 150 mg/kg bw dose of PSK by gavage. Fetus were colleted one day after treated and on gestation day of 18 (one day before give birth) to observed persentage cell death and incident of fetus malformation. The results of these research shown that persentage of cell death were decreased after treated of PSK from 12.76 % to 2.51 % in the group of treated 2-ME on GD 9, and 14.65 % to 10.87 % in the group of exposed gamma irradiation on GD 13. The incident of external malformtion also decreased from 86.31 % to 70.71% in group of treated 2-ME, especially on GD 9, however there were no effects on GD 7 or 13. These study suggested that PSK causes the decrease of incident of cell death induces by both of 2-ME and gamma cobalt irradiation and decreased of incident of fetus malformation especially treated on GD 9.

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BUSINESS PLAN OF SMALL SCALE BIOTECHNOLOGY INDUSTRY; ENZYMATIC FISH SAUCE PRODUCTION

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OP-25

ABSTRACT

Research on Biotechnology could be applied directly for public demand. But it is not easy to build up and running company that adopts new technique in Biotechnology. Here we will explain the business plan and experiences in build up Biotechnology Industry. From several years' research on production enzymatic fish sauce using pineapple protease we make business proposal and get funding from LIPI in 2003. We introduce technology, funding and management skill to society and working together to build up fish sauce fabric in Jember Area. We success to build up and running one fish sauce fabric even capital and production volume is not too high. We have no problem in production process, but still problem on marketing and get financial support for expansion

CHARACTERIZATION OF MICROBIAL CHITINASE : a Review

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OP-26

ABSTRACT

Chitinases which catalyze the hydrolytic degradation of chitin are to be regarded as important for the extensive carbon and nitrogen recycle which occurs in nature. Chitinases occur widely in soil microorganisms and fungi, fulfilling a possible defense role in the latter. Chitinases may find important industrial application in the utilization of the enormous chitin substrates, available from sea-food-processing units, for the generation of site specific chitin oligomers required particularly in pharmaceutical industries. Hitinases having very diverse characteristics are known and maybe of value in basic studies related to their biological role and structural elucidation of natural chitin.

STUDY ON PHYSICAL-CHEMICAL MECHANISM OF IRON REMOVAL FROM THE FERRIC COMPLEX OF AZOTOBACTINE δ (SIDEROPHORE ISOLATED FROM AZOTOBACTER VINELANDII BACTERIA) IN VITRO WAY

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OP-27

ABSTRACT

The aim of this research is to study of iron removal mechanism that very strongly bonded in the ferric complex of azotobactine δ, through two mechanisms i.e.: mechanism of ligand exchange reaction between azotobactine δ in the form of it's ferric complex with EDTA ligand with using the aide of acid catalyst and reduction mechanism of the ferric complex of azotobactine δ to form the ferrous complex of azotobactine δ that has much more lower stability than the stability of complex Fe(III)-Azotobactine. With using two mechanisms i.e.: mechanism of ligand exchange reaction and reduction mechanism, expected that Fe(III) ion that very strongly bonded in the ferric complex of azotobactine δ was much easier to be released. The result of reaction kinetics of ligand exchange reaction, to give the values of k_{obs} (total ligand exchange reaction Constant) were 3.263×10^{-4} s^{-1} at pH was same with 3.644 and 7.293×10^{-5} s^{-1} at pH of 5.183, were interpreted in terms. Of three step mechanism, involving : (1) protonation of ferric complex of azotobactine δ and (2) subsequent bimolecular reaction with EDTA to form ternary complex, finally (3) dissociation of a ternary complex formed with EDTA to produce free ligand of azotobactine δ. The result of mechanism of electrochemical reduction to give value of oxidation-reduction, potential was : (38910) mvolt and value of formation stability constant of complex Fe(II)-azotobactine (log K stab) was same with 8.456.

EXPLORATION OF AMYLOLYTIC ENZYME :
Cloning of Gene Encoding Glucoamylase from *Endomycopsis fibuligera* ITB Rcc64

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OP-28

ABSTRACT

Endomycopsis fibuligera in ascomycetous yeast species is as one of the best producers of amylolytic enzymes. The capability of *Endomycopsis fibuligera* ITB Rcc64 to degrade starch is connected with the production of two types of amylases, α-amylase and glucoamylase. *GLO1* (± 1600 bp) as coding region of gene encoding glucoamylase of *Endomycopsis fibuligera* ITB Rcc64 has been isolated by PCR using RW and RV primers. Cloning of *GLO1* gene in *Escherichia coli* DH5α using pMost-BlueT vector have resulted pMost-BlueT recombinant. Digestion of the DNA recombinant with *Bgl*II resulted a DNA fragment ± 4487 bp, and resulted two DNA fragments ± 2887 bp dan ± 1600 bp when digested with *Nde*I. DNA fragments ± 2887 was be the size of pMost-BlueT, then DNA fragments ± 1600 bp was be the size of *GLO1* gene. When aligned with *GLU1* and *GLA1* gene, DNA fragment encoding N and C-end terminal of *GLO1* gene showed 100% and 99,7% nucleotide sequence identity with *GLU1* gene, but 97,1% and 98,7% with *GLA1* gene. Deduction of amino acid of sequence the N and C-end terminal of *GLO1* gene showed 100% identity with *GLU1*, but 96 and 97% with *GLA1*. A high score of homology showed that *GLO1* gene resulted on the research was gene encoding glucoamylase of *Endomycopsis fibuligera* ITB R.cc.64. It was suggested to state nucleotides sequence completely of *GLO1* gene to describe *GLO1* gene structure more detail. In addition to do cloning of *GLO1* gene using expression vector.

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RECENT DEVELOPMENTS OF SAMPLE PREPARATION TECHNIQUE IN BIOANALYTICAL CHEMISTRY

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OP-29

ABSTRACT

There has been unprecedented growth in measurement techniques over the last few decades. Despite the sophisticated arsenal of analytical tools, complete noninvasive measurements are still not possible in most cases. One or more pretreatment steps are necessary. These are referred to as sample preparation, whose goal is enrichment, cleanup, and signal enhancement. Sample preparation is important in all aspects of chemical, biological, material and surface analysis. This paper will review recent developments of sample preparation techniques which are widely used in bioanalytical chemistry.

GLUCOAMYLASE GENE OF *Endomycopsis fibuligera* ITB Rcc64 : Isolation, Partial Nucleotide Sequence and Alignment Analysis

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OP-30

ABSTRACT

Isolation, partial nucleotide sequence and alignment analysis of gene encoding glucoamylase enzyme of *Endomycopsis fibuligera* ITB Rcc64 have been done. DNA chromosome as template on amplyfication was isolated by wizard genomic DNA purification kit method and the characterization through determination of nucleotide sequence of frgament DNA which have amplified. Amplification was carried out by PCR process using spesific primer FW and RV. Amplication could yield a band of DNA in agarose gel and the size is ± 1600 pb. The DNA band was compatible with fragment of DNA target that is coding region of gene encoding glucoamylase of *Endomycopsis fibuligera.* Characterization of DNA fragment ± 1600 pb through determination of nucleotides sequence that encoding N and C teminal done by dideoxy Sanger method. Nucleotides sequence that determined have homology 90% to gene encoding glucoamylase *Saccharomycopsis fibuligera* HUT7212 (*GLU1*) and 89-91% to gene encoding glucoamylase *Saccharomycopsis fibuligera* KZ (*GLA1*). DNA fragment ± 1600 pb then was called as *GLO1* gene.

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A JOURNEY TO MANIPULATE MICROBIAL CHITINASE FOR BIOTECHNOLOGY APPLICATIONS

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OP-31

ABSTRACT

Chitinases, (EC 3.2.1.14) are enzymes, which degrade chitin to produce shorter chitooligosaccharides. The chitinase gene has a potential application in the development of transgenic plants with resistance to insects, which have an exoskeleton composed of Chitin. Bioprospecting for soil-based microorganisms expressing chitinase genes is currently a priority area in the quest for the development of insect resistant transgenic crops. In this study, soil-based chitinolytic microorganisms were screened from the mangrove areas in Sabah, Malaysia, using Chitinase Detection Agar (CHDA), pH6.5. Five isolates, BRI 1, BRI 2, BRI 8, BRI 13, and BRI 36 were considered potential candidates as they produced halos indicating the presence of chitinolytic activity. The halos around the bacterial colonies were measured after a five-day incubation period at 28°C. Morphological and genetic analyses showed that all the isolates were Actinobacteria except BRI 8, which is a Proteobacterium. The sizes of the chitin degradation zones of BRI 13 and BRI 36 increased from 8.0mm to 9.0mm and 8.0 to 8.5mm respectively when temperature was elevated from 28°C to 37°C while the sizes of halos of other strains decreased, indicating the stability of the chitinases derived from BRI 13 and BRI 36 at elevated temperatures. Partial fragments of the chitinase genes were successfully amplified using chitinase degenerate primers and subsequently used as probes to recover the full-length chitinase genes by Southern Hybridization technique. These chitinase genes will be characterized to find candidates suitable for down-stream applications.

APIGENIN-(3'-O-7'')-QUERCETIN-3''-METHYL ETHER, A DIMER FLAVONOID COMPOUND AS INHIBITOR XANTHINE OXIDASE FROM *CASSIA SPECTABILIS*

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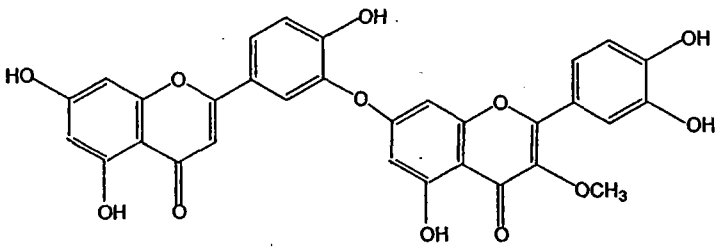
OP-32

ABSTRACT

A dimer flavonoid compound named apigenin-(3'-O-7'')-quercetin-3''-methyl ether was isolated from the acetone extract of the stem bark of *Cassia spectabilis* (Leguminosae). The structures of this compound was determined on the basis of spectroscopic data. The activity test of Apigenin-(3'-O-7'')-quercetin-3''-methyl ether as an inhibitor *xanthine oxidase* showed that this compound possesses inhibitor activity of *xanthine oxidase* (IC_{50} = 4,52 x 10^{-2} μM).



MOLECULAR CLONING AND NUCLEOTIDE SEQUENCING OF DEXTRANASE GENE FROM *Arthrobacter* sp B7 AND ITS EXPRESSION IN *E. coli*

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OP-33

ABSTRACT

DNA gene encoding the *Arthrobacter* sp B7 dextranase, namely *B7DEX* gene, was amplified by Inverse PCR and PCR. Primers for Inverse PCR was designed base on sequence of *B7DEX* fragment of 906 bp obtained in the previous work. Inverse PCR produced three specific amplicones in the down stream area of *B7DEX* gene, on with Inverse PCR gave additional sequence from 906 became 1710 nucleotides. PCR was done by reverse primer designed based on stop codon area of the 1710 nucleotides, and the forward primer was designed base on homology region in the start codon area of three dextranase sequences from database. PCR produced one specific amplicones of 1880 bp, and the complete nucleotide sequence was determined,. which showed high homology with three dextranase sequence from GH49 family of CAZY enzymes, the highest one is sequence with access number AB025195 showed 76 % homology. The 1880 bp amplicone was ligated with pMosBlueT vector yield recombinant namely pDexT. Transformant *E. coli* cells carrying pDexT produced dextranase activity in the cytoplasmic space.

ENCAPSULATION OF LACTATE DEHYDROGENASE (LDH) IN RICE HULL ASH DERIVED SILICA

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OP-34

ABSTRACT

In this research, Lactate dehydrogenase (LDH) enzyme was encapsulated in silica by sol-gel process using sodium silicate resulted from rice hull ash. Several parameters affecting the enzymatic activity of the encapsulated product were also studied; i.e. substrate and coenzyme concentration, medium acidity, thermal stability, and sodium content.

The encapsulation was carried out by mixing sodium silicate (Na_2SiO_3) solution and phosphate buffer pH 7 containing both LDH enzyme and coenzyme of nicotinamide adenine dinucleotide (NAD^+). The Na_2SiO_3 solution was prepared by destructing powdered rice husk ash with NaOH at 500 °C for 30 min and then was dissolved in distilled water. After 2-6 minute shaking, the mixture was then transferred into a 96-microwell plate and stored overnight. The activity of encapsulated enzyme was quantified by measuring absorption of NADH, as coenzyme hydrogenation product, at 340 nm with a microtitter spectrophotometer. For comparison similar experiment was also carried out for free enzyme.

In the present study, it was observed that the encapsulation of LDH in silica reduced the enzymatic activity in a range of 10 – 40 %. However, the thermal stability of the encapsulated enzyme increased. The increase of temperature up to 65 °C did not reduce the activity of the encapsulated enzyme. It was observed that after being used several times the activity of the encapsulated enzyme still remained approximately 30 % of the previous activity. In addition, a significant improvement on the activity of encapsulated LDH was also observed when the NAD^+ coenzyme and the LDH enzyme were encapsulated together.

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CLONING cDNA FRAGMENT OF SUCROSE TRANSPORTER (*SoSUT*) IN SUGARCANE (*Saccharum officinarum*) LEAF

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OP-35

ABSTRACT

Succrose Transporter (*SUT*) is carrier protein that useful for facilitating transport sucrose through the membrane. A transporter with homology to *SUT/SUC* family of plant sucrose transporter was isolated from a sugarcane (*Saccharum officinarum*) of leaf for 5 month old. Total RNA have been isolated by Phenol method (Sambrook., *et. al.*, 1989) with final concentration around 8.1 ug/ul. Primers have been designed from concervative region of cDNA of *Sacharum oficinarum* type 2A (*SoSUT 2A Accession number AY165599*), *Oriza sativa putative* (*OsSUT-1 putative Accession number AAP54842*) *Oriza sativa* (*OsSUT-1 mRNA Accession number XM 464773*). The sequences of nucleotide are *5' CAGATCUTCAACAGCGC 3';5' TGCCCTTTGTCT CCGGAACC 3'* for Forward (*F-SUT*) and Reverse (*R-SUT*) primers, respectively. The single band was found around 543 bp in length (*SoSUT-1 Accession number bankit 734628)* from RT PCR product and was sequenced by *sequencer* IBI-PRISM Big dey Terminator. cDNA fragment of gene *SoSUT-1* have homology around 89% , 87,3 % and 84,8 % with *ZmSUT-1, SoSUT 2A* batang and *OsSUT-1* mRNA, respectively. Restriction enzymes such as *AvaI* at the position 59 bp, *Ava II* at the position 353 bp, *Bgl II* at the position 381 bp and *Xho I* at the position 59 bp were found in the cDNA fragment *SoSUT*. By using *PSORT* analysis the fragment *SoSUT* of sugarcane leaf that may be located in the endoplasmic reticulum.

PURIFICATION AND CHARACTERIZATION OF PROTEASE ENZYME FROM RECOMBINANT *Bacillus subtilis* R1.

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OP-36

ABSTRACT

Recently, enzymes find extensive applications in many areas industry such as food, detergent, leather and biotechnology. Among of enzymes, protease is the most varying in used and highest in demand. Cloning genes of protease is applied to overproduction of the enzyme such as recombinant *Bacillus subtilis* R1. The *B. subtilis* R1 had previously been engineered from *B. subtilis* DB104, by introducing a protease gene at 3 kb DNA fragment originated from *B. pumilus* Y1. Purification and characterization is first important step to maximize the use of enzyme. The aim of this research is to purify and to characterize of protease from recombinant *Bacillus subtilis* R1.

Enzyme was used an extracellular protease from *B. subtilis* R1 which it produced in SFS S1 media and separated from bacterial cells by centrifuge at 12.000 rpm. Purification procedure was conducted by absorption with PEG6000 and further chromatography gel upon sephadex G100. Protein molecular weight was measured by SDS-PAGE and Zymogram analysis. Inhibitor effect was analyzed by specific inhibitor of PMSF and EDTA. Protease activity was analyzed by modification of Bergmayer method.

The crude extract analysis revealed that Recombinant B. *subtilisin* R1 has been identified to contain three protease enzymes at molecular weight of 28, 48 and 132 kDa. Among them, the 132 kDa showed the highest activity (fig. 1). Proteollytic activity of the purified enzyme is optimal in 11.0, and but stable in alkaline pH of 9-11 (fig. 2), and optimal temperature is 40 °C and the stable in room temperatur for 12 hours. Analysis by spesific inhibitor, indicated that the purified enzyme was inhibited completely by PMSF 1 mM and only 20 % by EDTA 5 mM. This indicated that the enzyme is a serine alkaline protease.

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THE EFFECT OF TYPHONIUM FLAGELLIFORME LEAF EXTRACT ON THE INHIBITION OF MELANOMAS CELL

Supriyatin

Biology Department, UNJ

OP-37

NITRILE DEGRADING ACTIVITY OF ENDOPHYTIC BACTERIA FROM THE BIOLOGICAL DIVERSITY OF INDONESIA

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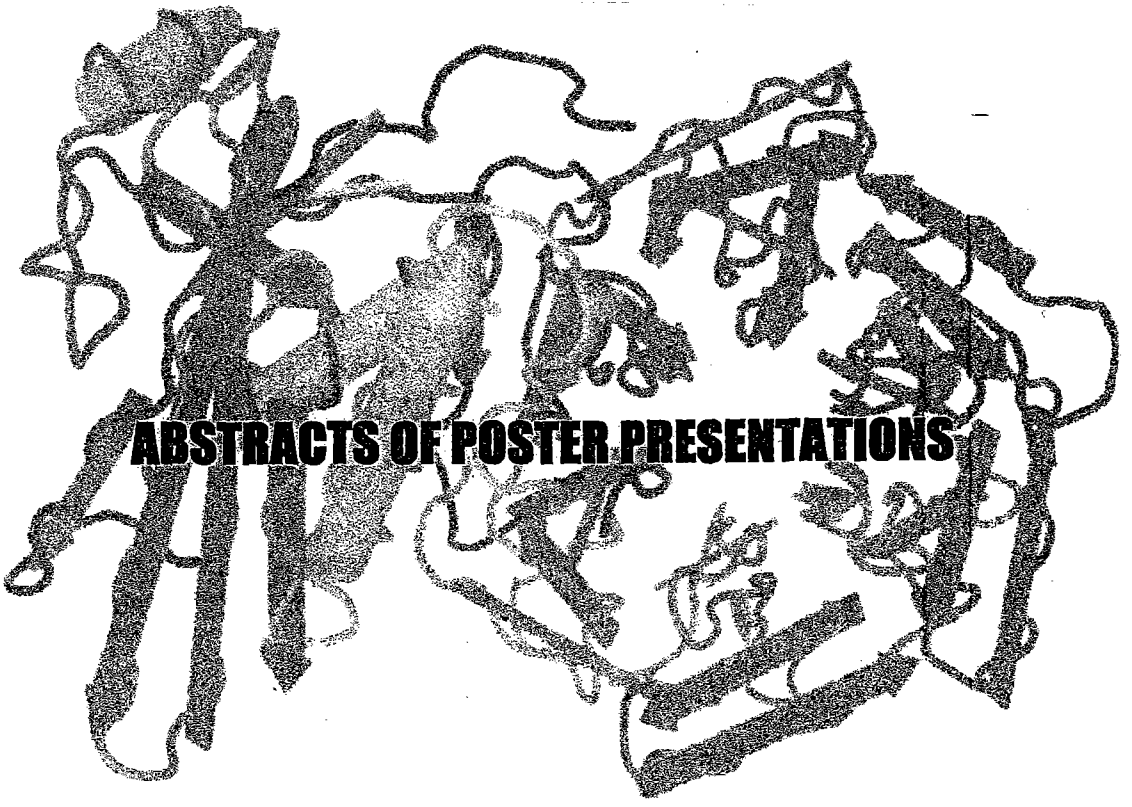
BP-38

ABSTRACT

Nitriles are an important group of compounds which appear in the environment via natural or industrial synthesis. Nitrile metabolising microorganisms usually convert nitriles via hydrolytic pathways : 1) conversion of the nitrile directly to corresponding carboxylic acid and ammonia by nitrilase, and/or 2) conversion of the nitrile by nitrile hydratase to an intermediate amide, which is then converted by an amidase to the corresponding carboxylic acid and ammonia. The potential biotechnological application of these enzymes to produce amides and/or acids from nitriles has a major industrial interest. The amount of papers in the literature describing reactions catalyzed by these enzymes and microorganisms as a method for obtaining enantiomerically pure compounds is increasing considerably. The usefullness of this biocatalysts in organic chemistry is now well documented and has greatly increased in the last decade. Uses for this biocatalyst include the inexpensive production of amides, enantioselective amide synthesis, and conversion of nitrile wastes to less toxic amides. Novel sources of these enzymes are thus of industrial interest. Higher plants are host to microorganisms, generally referred to as endophytic microbes (or endophytes) which colonies the internal tissue of living plants without causing over negative effects to their host. Rare and biologically active compounds have been isolated from these microbes ranging from anti-infective activity, antioxuidant, anti-cancer, insecticidal and others. Some of the compounds that have been discovered in endophytic microbes include taxol, cryptocin, cryptocandin, and ambuic acid among a few. The potential of this relative untapped source of microbial diversity as a source of microbe with nitrile degrading activity has yet to be evaluated.

In this study we have tested approximately 850 bacterial endophytes for their ability to utilize aliphatic nitriles such as acetonitrile as sources energy, carbon and nitrogen for their growth. The acetonitrile degrading system so far monitored by the production of carboxilic acid and ammonia by these bacterial endophytes suggest the presence of either pathways mentioned above. From thos endophytes 98 isolates have been identified that are able utilize acetonitrilbased on their growth pattern and metabolic product. This is to our jnowledge the first report of such activity for endophytic bacteria.



ABSTRACTS OF POSTER PRESENTATIONS

BIOASSAY ON NITRATE REDUCTASE ACTIVITY OF THE RED ALGAE (Rhodophytes)

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PP-01

ABSTRACT

Red algae (Rhodophytes) are kinds of algae that have red pigment – phycoerythrin. Industrial utilization is at present confined to extraction for phycocolloids and certain fine biochemicals. They have the potential to be used as a source of essential chemicals such as amino acid, essentially fatty acid, polysaccharides (fibres, agars, carrageenans, alginates), vitamins and nutrients (including essential microelements) with medicinal and industrial uses.

A research to study the nitrate reductase enzyme was done to optimize the bioassay condition to measure its activity. Thallus of three species of red algas were used as a source of nitrate reducatase in order to obtain the optimal bioassay condition. Those three species are *Gigartina harveyana*, *Agardhiella* sp. and *Gracilaria crassa*. Soaking techniques in assay media, pH of media, concentration of surfactant sodium dodecyl sulphate (SDS) and organic solvent n-propanol, concentration of $NaNO_3$, biomass weight, and duration of incubation in nitrate containing media were observed.

The results showed that nitrate activity rate were 1.905 ± 0.02 mM NO_2^-/g/hrs (*G. harveyana*), 1.975 ± 0.025 mM NO_2^-/g/hrs (*Agardhiella* sp.) and 0.853 ± 0.118 mM NO_2^-/g/hrs (*G. crassa*). The results showed that pH 7.5 applied with 0.3% SDS (surfactant) 0.3% instead of n-propanol (organic solvent) was the optimal condition for nitrate reducatase activity measurement. The nitrate reductase activity in the three red algae species were significantly influenced by light. Application of 0.3% SDS significantly enhanced the activity of lighted thallus.

Optimal concentration of $NaNO_3$ was 20 mM for *Gigartina*, and 40 mM for *Agardhiella* and *Gracilaria*. While the optimal soaked biomass weight was 400 mg for *Agardhiella* with 2 hours incubation, and 500 mg for *Gigartina* and *Gracilaria* with 3 hours incubation. The rate of enzyme activity seemly influence the growth of biomass fresh weight and Nitrogen content of thallus.

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STUDIES ON PROTEIN LEVELS AND CALCIUM-PHOSPHATE ($CaHPO_4$) INCORPORATED INTO THE DIETS ON CONSUMTION, GROWTH AND CARCASS COMPOSITION IN *Tilapia* spp.

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PP-02

ABSTRACT

Studies on levels of protein (PL) and calcium-phosphate ($CaHPO_4$) incorporated into the diets on consumption in *Tilapia* spp was investigated. The experimental were conducted for three replicate (R) and control (C) without calcium phosphate. At 24 weeks period on *Tilapia* spp the mean weight was 56.20 ± 1.41 for PL1, 76.05 ± 1.08 g for PL2, 53.80 ± 1.26 g for PL3 and 53.38 ± 1.26 for control (C). The level of protein diets was contained of 29% and 0.5% of calcium-phosphate for PL1, 30% and 1.0% of calcium-phosphate for PL2, 31% and 1.5% of calcium-phosphate for PL3, and control diet (C) was 31% crude protein without calcium phosphate. Interaction of growth was shown in diet PL2. Daily food consumption was variable. The mean of consumption, expressed either as g/g fish day^{-1} or g/fish day^{-1}, did not vary significantly between levels of protein incorporated calcium-phosphate except in diet PL2. The overall consumption was independent of the dietary protein contents and calcium-phosphate. Growth (percent daily weight gain (DWG)), food conversion ratio (FCR), protein efficiency ratio (PER) and net protein utilization (%NPU) ranged between 0.90 ($P_{29}L_{0.5}$) and 1.40 ($P_{30}L_{1.0}$) for daily weight gain, 2.61 ($P_{29}L_{0.5}$) and 3.42 ($P_{30}L_{0.5}$) for feed conversion ratio, 14.57 ($P_{29}L_{0.5}$) and 26.48 ($P_{30}L_{0.5}$) for protein efficiency ratio, and 26.48 ($P_{29}L_{0.5}$) and 43.41 ($P_{30}L_{0.5}$) for net protein utilization respectively. The best of body conformation and carcass consumption was higher 23 to shown in diet PL2 (($P_{30}L_{1.0}$)

THE ENGINEERING OF ACTINIDIN-ENCODING PROTEIN THROUGH SPECIFIC AMPLIFICATION OF FULL-LENGTH ACTINIDIN cDNA

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PP-03

ABSTRACT

Variants of actinidin-encoding DNA sequences have been created by specifically amplifying the full-length actinidin cDNA. The variants were developed in such a way to remove putative vacuole translocation signals present in the N-terminal and C-terminal extension-encoding sequences of actinidin precursor.

The first variant was created by amplifying the full-length actinidin-encoding DNA sequences starting (5' end) from the TN amino acids-encoding sequences in the N-terminal extension, to the 3' end which was located at an amino acid-encoding sequence just upstream the QR amino acid motif-encding sequences. This variant thus represents an actinidin-encoding sequences lacking both putative vacuolar translocation signals (QRTN or QR) present in the N- terminal and C-terminal extension.

The second variant was created by amplifying the full-length actinidin-encoding cDNA starting from a sequence just upstream the QRTN motif in the N-terminal extension, to the 3' end located at a sequence just upstream the QR motif in the C-terminal extension. The second variant thus represent an actinidin-encoding DNA sequences which includes the QRTN motif in the N-terminal extension but lacks the QR motif in the C-terminal extension.

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CELLOBIOHYDROLASE (CBH) GENE OF *Aspergillus terreus* SUK-1 : a Complete Open Reading Frame (ORF) and Expression

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PP-04

ABSTRACT

The species *Aspergillus terreus* SUK-1 isolated from oil palm waste was found to produce cellulose enzymes for the degradation of cellulose compounds. RLM-RACE methodology has been used in facilitating the isolation and cloning of full length cDNA, cDNA sequence was edited with BIOEDIT before been analysed with Blast platform and other bioinformatict tools for similarity search. The analyses showed high hits toward cellobiohydrolase (CBH) proteins and genes. The catalytic domain and cellulose binding domain also have been identified when scanned with Pfm and NCBI Concerved Domain Search. The CBH gene expression of *A. terreus* SUK-1 growing in Mendel's media with 10% Sigmacell was studied using RT-PCR methodology. Total RNA from the fungal was isolated 7 h after inoculation and for every 24 h until day 7. The expression pattern showed highest CBH expression after 24 h been inoculated, then the expression subsequently reduced until reaching stationary phase of growing on the fifth day. The gene was latter designated as *CBHat1* and the GenBank accession number AY864863.1

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ECONOMICAL PRODUCTION OF BACTERIAL PHYTASES ISOLATED FROM MALAYSIAN *Zea mays* PLANTATION

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PP-05

ABSTRACT

Phytases catalyze the release of phosphate from phytate, the predominant form of storage phosphate in cereal grains, oilseeds and legumes. Possible applications of phytases are in animal nutrition to increase mineral bioavaliability and to decrease phosphate pollution in the area of intensive life stock management, and in human health.

Zea mays is one of the cereals that contain phytate as the major phosphate storage compound. This fact serves as a very suitable rationale for the search of microorganism producing phytate-degrading enzymes in maize plantation. Over two hundred bacteria were isolated and screened for phytases from the halosphere, rhizosphere and endophyte of Malaysian maize plantation. The highest extracellular phytase activity was detected from bacteria that were isolated from the endophyte of the maize root.

Media costs are the limiting factor for good production and commercialization of bacterial enzymes. Malaysian plant biomass, although could be a good candidate for growth media, is not well utilized. We have developed a screening and production system using "rice waste" as growth media to obtain 30 bacterial isolates from Malaysian maize plantation that are capable of producing phytate degrading enzymes. To find the best conditions for enzyme production, different concentrations [w/v] of rice bran during different stages of cultivation were performed. The dephosphorylation of phosphate from rice bran phytate has shown regulatory effect on the secretion of bacterial phytases. The bacterial isolates have been characterized using molecular and biochemical method, and the enzymes have been partially characterized of their physical parameters. The gene from selected phytase producing bacteria will be partially cloned by PCR using phytase degenerated primers.

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THERMOPHILIC BACTERIAL PECTINASES FROM MALAYSIAN SOIL AND HOT SPRINGS FOR POTENTIAL INDUSTRIAL APPLICATIONS

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PP-06

ABSTRACT

Most enzymes used in industrial applications are extra-cellular enzymes derived from microorganisms. The production cost depends not only on the type of cellular extraction but also on the degree of purity required in the process. Among many enzymes, pectinases received particular attention since these biocatalysts are important for industrial processes including textile processing, food processing, paper industry and plant cell wall bioconversion.

In this paper, several pectinase-producing bacteria were isolated from Malaysian hot springs and Malaysian soil rhizosphere when samples were incubated at 37°C, 50°C, 60°C and 65°C. The production of these bacterial pectinases can be improved when different growth media containing various concentrations of pectic substrates were used. The bacterial pectinases (polygalacturonases and pectate lyases) were characterised for their thermostability and temperature optima for plant cell wall bioconversion and food processing. The total DNA from hot springs soil sample will be used to clone pectinases (polygalacturonases and pectate lyases) from uncultivated bacteria in *E. coli* hosts as metagenomic gene bank. Our sets of degenerated primers for polygalacturonases and pectate lyases from different genome sequences will be used for PCR cloning and molecular screening of pectinolytic enzymes.

THERMOPHILIC BACTERIAL CELLULASES FROM MALAYSIAN SOIL AND HOT SPRINGS.

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PP-07

ABSTRACT

One of the attractive sources of commercially important enzymes is bacteria. It is possible to clone bacterial enzymes that possess the necessary catalytic properties for industrial applications. Soil bacteria are a major part of mega-biodiversity, and Malaysia is one of the twelve countries identified as having mega-biodiversity. From this mega-biodiversity, it is very likely that new bacterial enzymes suitable for industrial applications will be identified. In this paper, several cellulase-producing bacteria were isolated from Malaysian hot springs and Malaysian soil rhizosphere when samples were incubated at 37°C, 50°C, 60°C and 65°C. The current sources of important cellulases are from fungi or strictly anaerobic bacteria, but this paper provides a new source for these important industrial enzymes: thermophilic aerobic and facultative anaerobic bacteria.

The production of these bacterial cellulases can be improved when different growth media containing various concentrations of cellulose and palm oil trunk were used. The latter contain abundant cellulolytic materials. The bacterial cellulases were characterised for thermostability and temperature optima for bioconversion of palm oil trunk into value added products. The genes encoding bacterial cellulases are much easier to be cloned and subsequently can be over-expressed in different hosts. The high numbers of highly diverse clones have enabled more chances for the molecular evolution and modelling of the enzymes for substrate and specific activity with high catalytic activity for palm oil trunk bioconversion.

SCREENING OF GROUP B STREPTOCOCCI HYALURONIDASE USE PLATE-AGAR HYALURONIDASE TECHNICUE

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PP-08

ABSTRACT

Group B Streptococcus (GBS) or *Streptococcus agalactiae* has been well known as a causing agent of pneumonia, septicemia and meningitis neonatal. Virulence factors of this bacteria which take the important role to the infection process such as hyaluronidase. It is an extracelluler product which is producted by GBS, being a bacteria virulence factor which able to destroy hyaluronic acid of connective tissue to easy the bacteria spreading, so that, this enzyme is called as "spreading factor". The aim of this research is to do the screaning GBS hyaluronidase isolated from the sufferer of obstetric complication by using fast and easy methode. That is plate-agar hyaluronidase methode. The result of this research shows that 10 GBS isolates which are screened, all of them show the positive reaction which is signed by the clear zone around the bacteria growth. It shows that GBS which is isolated by the obstetric complication sufferer is the invasive GBS bacteria.

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EFFECT OF ADDITIVE COMPOUNDS ON THE ACTIVITY AND DEGRADATION PATTERNS OF CATALASE IN APPLE FLESH TISSUE

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PP-09

ABSTRACT

The project is aimed to study effect of additive compounds on the activity and degradation patterns of catalase in apple flesh tissue. The enzyme was purified 66-fold with a total yield of 32.2%. The molecular weight was found to be on the range of 45 000 to 66 000 by SDS-PAGE, and it is that catalase is a monomer. The indicator of this study is catalase activity. Four additives were tested to evaluate their potential to improve of catalase activity. The results shows only 2.0 mM Na_2-EDTA give slightly improvement of catalase activity. The addition of 0.25 and 0.50% Triton X-100 resulted a decrease in activity of purified catalase 23.33 and 23.43%, respectively. Similar result was also observed on the addition of 1.0 and 2.0 mM DTT causing a reduction of catalase activity by 25.82 and 21.41%. Further, a significant decrease in the activity of catalase has been found on the addition of 0,10 and 0,25% SDS. The results has also showed that the addition of four additive compounds decreased the concentration of protein. Electrophoresis analysis shows a degradation of protein catalase that is indicated by a decrease in the activity of enzyme. Further, it is also supported by electrophoregram that shows a decomposition of tetrameric catalase structure, as well as a total protein catalase. The experiment and the results were discussed.

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THE STRATEGY FOR EFFICIENT HYDROGEN (H_2), 2,3-BUTANEDIOL PRODUCTION AND INVOLVED ENZYMES BY *Enterobacter aerogenes*

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PP-10

ABSTRACT

H_2 has the highest gravimetric energy density of any known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing carbon-based emissions that contribute to environmental pollution and climate change. Biological production of H_2 (bio-H_2), using (micro) organisms, is an exciting new area of technology development that offers the potential production of usable H_2 from a variety of renewable resources.
The wild type *E. aerogenes* AY-2 has been cultivated at 37 °C for 24 hrs in glucose- minimal medium, and the disruption process of *E.aerogenes ldh* gene have been also carried out. As the result indicated that the lactate yield and *ldh* decreased to 1/4. No more change on H_2 production yield but the 2,3-butandiol enhanced 2-fold compare to the wild type.

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SOLUBILIZATION TECHNIQUE OF MEMBRANE-BOUND ENZYME : Sialidase from Horse Liver

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PP-11

ABSTRACT

Purification of membrane-bound enzymes is faced difficulties because of its low stability and showing a small of yield. The common solubilization agent used for this purpose is detergent and protease, however high ionic charge of solution was also reported showing the same effect (**Schägger, 1998**). In this report we showed that high ionic charge buffer showing the best solubilization effect on sialidase (EC 3.2.1.18), a membrane-bound enzyme hydrolyzed sialic acid from glycolipid and oligosaccharide, among some different solubilization buffers used.
Two different buffers containing salt were determined to bring sialidase in 100,000 *g* supernatant, while some detergents like cholic acid, taurocholic acid and Triton X-100 were tried as solubilizing agents. A 500 µL homogenate in 1.5 mL eppendorf cap was added by water, 0.4 *M* acetate or phosphate buffer, 2 *M* sucrose, and 20 % detergent to bring an appropriate concentration for each. After mixing well, some of 4 *M* NaCl was added to bring at appropriate concentration. The mixture was then shaked gently at 4 °C, and was centrifuged using mini ultracentrifuge at 100,000 *g* for 15 min. The supernatant and the pellet were checked for protein and sialidase activity. In the acetate buffer system about 5 % of the sialidase activity could be solubilized, this was increased to 25 % with the addition of 0.15 *M* NaCl. Fifteen percent of the sialidase activity was recovered with the addition 1 % Triton X-100, and 40 % when 0.15 *M* NaCl and 1 % Triton X-100 were added. In the phosphate buffer system about 20 % of the sialidase activity was found in the supernatant, this was increased to 30 and 35 % with the addition of 0.15 *M* NaCl and 1 % Triton X-100, respectively. The sialidase activity found in the supernatant was increased of approximately 45 % with addition of both 0.15 *M* NaCl and 1 % Triton X-100. Cholic acid, taurocholic acid and Triton X-100 showed the same effect in solubilizing the sialidase. The amount of detergent required in solubilization of sialidase from membrane preparation could be reduce from 1 to 0.5 % with addition of 0.25 *M* sucrose in the solubilization buffer.
From the result above, phosphate buffer showed specific effect on sialidase solubilization from horse liver, and this solubilization effect increase when salt was added. But detergent showed no specific effect in solubilizing sialidase.

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SITE DIRECTED MUTAGENESIS OF *SUP45* GENE FOR INTERACTION MECHANISM STUDY OF eRF1- eRF3 PROTEINS

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PP-12

ABSTRACT

eRF1 and eRF3 protein, encoded by *SUP45* and *SUP35* gene respectively, play an important role as complex factor of termination translation in the termination phase of protein biosynthesis. The detailed mechanism of the interaction between the two proteins, however, is still unclear yet. By structure-function study of the proteins, through mutagenesis and biochemical analysis of the gene, the detailed mechanism of the interaction will be known. Previous studies indicated that residue tyrosine at the position of 410th eRF1 protein involved in its interaction to eRF3. In order to know the functional group of tyrosine 410th which is involved in the interaction, the others mutants: sup45-Y410F and of sup45-Y410A are constructed. Construction of the mutants was conducted by PCR based method using Megaprimer technich, using lenearized SUP45 gene at the pUKC1901 as template, and 3 kinds of primers and three phases of PCR per mutant. The condition of PCR are: denaturation: 94^{0}C (4,5 minutes), Annealing: 41^{0}C (2 minutes), Elongation 72^{0}C (2 minutes 30 seconds). First phase of PCR was performed in 1 cycle, the second phase 25 cycles and the third phase only elongation 10 minute, have succeeded to amplified two kinds of mutant amplicons (sup45-Y410F and sup45-Y410A) by 1,8 kb in length. Both of the mutant amplicons also had been isolated from agar and have the concentration of 30 ng / uL, and recently still being sequensed. Meanwhile the vector which is needed also had been isolated and have 11,3 ng/uL and 20 uL in concentration and volume respectively.

IMPROVEMENT THE $(\alpha/\beta)_8$ BARREL-RIGIDITY OF *Saccharomycopsis fibuligera* α-AMYLASE BY POLYETHYLENE GLYCOL MODIFICATION

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PP-13

ABSTRACT

The chemical modification of proteins has long been a useful approach to elucidating structure-function relationships and also extensively used to identify chemical groups on proteins which are involved in biological function. These applications include alterations to introduce new properties, such as improved stability, that take advantage of both broad and narrow ranges of selectivity (Lundblad and Bradshaw, 1997). $(\alpha/\beta)_8$ barrel-domain is a key-role domain in α-amylase structural stability. In this work, the effect of polyethylene glycol (PEG) modification of *Saccharomycopsis fibuligera* α-amylase to improve $(\alpha/\beta)_8$ barrel-domain rigidity was studied by comparing the trypsin proteolysis fragments of the α-amylase in the native and PEG-modified forms. The *S. fibuligera* α-amylase secreted to the media culture was separated from the cells by cold centrifugation. The supernatant was concentrated by ultrafiltration 10 kDa cut-off membrane. The concentrated enzyme was then purified on butyl-Toyopearl hydrophobic interaction, Sephadex G-25 size exclusion, and finally DEAE Toyopearl anion exchange columns chromatography. This purification scheme had successfully resulted in a pure enzyme. Chemical modification by activated-PEG was performed as described by Tsai *et al.* (1974). Proteolysis was conducted under denaturation condition in which the enzyme is in tertiary structure-unfolded conditions by using SDS as performed by Gilkes *et al.* (1989) with slight modification. Purification and proteolysis pattern of both native and modified enzymes were monitored by SDS-PAGE. The purification result showed that the specific activity of the enzyme was increasing from 721.95 U/mg to 15020.69 U/mg. The molecular mass of the purified α-amylase was ~54 kDa, PEG modification increased the mass to ~61 kDa. Structural analysis of both the native and modified enzymes under non-denaturation condition resulted in fragments with molecular weight ~39 kDa of A/B domain and ~10 kDa of C-domain (Hasan *et al.*, 2005). In SDS-denaturation condition, trypsin had digested completely the native α-amylase into small fragments, neither ~39 kDa nor ~10 kDa fragments were detected. In contrast, in PEG modified α-amylase, a fragment with a molecular weight ~35 kDa appeared with decreasing the intensity of ~61 kDa; it was suggested that ~35 kDa derived from further trypsin digestion of ~39 kDa fragment. The results showed that the chemical modification by PEG can improve the stability and rigidity of the $(\alpha/\beta)_8$ barrel-domain. We also concluded that PEG modification can prevent the α-amylase from trypsin proteolysis.

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CONSTRUCTION OF *alp1* USING SITE-DIRECTED MUTAGENESIS

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PP-14

ABSTRACT

α-Amylase (1,4-α-D-glucan glucanohydrolase) hydrolyses α-1,4-glycosidic linkage of starch. α-Amylase has broad application in industries such as food, paper, and textiles. Industrial enzymes should possess high degree of stability. Enzyme stability is determined by several factors, such as hydrophobic interaction, ionic interaction, hydrogen bond, and disulfide bond. The long term goal of this research is to enhance stability of recombinant *Saccharomycopsis fibuligera* α-amylase (ALP1) by introducing new disulfide bond. The short term of this research is to produce *alp1* mutant containing new codons which encode cysteine. Mutation of α-amylase *S. fibuligera* gene (*ALP1*) in YEp-*Secretex* expression vector was introduced by site directed mutagenesis method. Nucleotide sequence analysis showed that *alp1* mutants AGT_{314}(Ser)→TGC(Cys)/TCC_{415}(Ser)→TGC(Cys) and GAT_{433}(Asp)→TGC(Cys)/GCT_{464}(Ala)→TGC(Cys) had been obtained successfully.

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TOPOLOGY MAPPING OF YEAST'S GENES INVOLVED IN AXIAL AND BIPOLAR BUDDING PATTERNS

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PP-15

ABSTRACT

During the cell cycle division process, the cells are divided along specific cleavage planes. Polarised cell division is a fundamental process in which cells divide along specific cleavage planes, because it can mediate appropriate cell-cell contacts and partition cytoplasmic components asymmetrically between daughter cells.

Bud sites can be selected in either of two distinct spatial patterns, termed axial and bipolar. The axial pattern genes are AXL1, BUD10/AXL2, BUD3, BUD4, in which their products mark the mother-bud neck during one cell cycle as a site for budding in the next round of division. On the other hand, several genes are required for the bipolar budding pattern of diploid yeast cells but not for haploid axial budding. However, only two genes of this class, BUD8 and BUD9, have been described that shift the bipolar pattern to a whereas bud9 mutants bud with high frequency from the distal cell pole. Therefore, Bud8p and Bud9p have been proposed to act as bipolar landmarks that might recruit components of the common budding factors, e.g. Bud2p, Bud5p or Rsrlp/Budlp, to either of the two cell poles.

The Self-organising Map (SOM) is a type of neural network method, loosely based on how the eye works. SOM is a non-linear projection of high dimensional data to a lower dimensional space, typically the plane. The concept of "self-organised topological feature maps" was introduced by Kohonen as maps that preserve the topology of multidimensional representation within the new one- or two-dimensional array of "neurons" which can be associated as an array of clusters.

The topology of four genes of the BUD family found in the dataset (BUD3, BUD4, BUD8, BUD9) were successfully arranged in the map by the SOM according to their gene expressions pattern. Three genes (BUD3, BUD4, and BUD8) were placed in a close neighbourhood while BUD9 was spatially apart from these two genes neighbourhood in the U-matrix map. Consistently, SOM was able to visualise the spatial topography between the two genes, BUD8 and BUD9. Clearly, BUD8 is placed by SOM in adjacent neighbours of BUD3 and BUD4, while BUD9 is located at a further distance away. Several proteins, particularly Bud3p and Bud4p, presumably associated in a complex with others, assemble in the mother-bud neck to form the spatial landmark for the next round of axial budding. The spatial relationship in a close vicinity of neighbourhood between the BUD3/BUD4 with BUD8 is clearly visualised by the SOM, while positioning the BUD9 at other part of map away from these clusters.

EFFECT OF VITAMIN E ON Na^+-K^+ ATPase ACTIVITY IN CELL MEMBRANE OF SYNCYTIOTROPHOBLAST PLACENTA IN WOMEN WITH PRE-ECLAMPSIA

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PP-18

ABSTRACT

Pre-eclampsia is a major cause of maternal and fetal mortality and morbidity, particularly in developing countries. The membrane of syncytiotrophoblast involved in a wide range of function which maintains the mother-fetus exchange of nutrient, etc. Oxidative stress has been reported to be involved in the pathogenesis of pre-eclampsia. It is suggested that vitamin E supplement as antioxidant may overcome the oxidative stress. The aim of this research was to investigate the efficacy of vitamin E supplement in pregnant women with pre-eclampsia. The parameter used was Na^+-K^+ ATPase activity on membrane of syncytiotrophoblast.

This study used 18 pregnant women (6 women with normal pregnancy, and 12 pregnant women complicated by pre-eclampsia) with 30 weeks gestation, recruited from Budikemuliaan Maternity Hospital, Jakarta in September 2003 to February 2005. The pre-eclamptic women were divided in 2 groups. The first group received vitamin E 200 mg daily and the second group received placebo.

Immediately after the subjects giving birth, the placentas were cut (± 10 g) and placed in the sterile flask with tris-buffer saline solution (TSS), incubated in the ice box, and sent to the laboratory. The isolation of placental syncytiotrophoblast membrane was done by Smith, Rand, and Lodish Method with modification. Activity of Na^+-K^+ ATPase was measured spectrophotometrically (λ = 660 nm).

The activity of Na^+-K^+ ATPase ouabain sensitive in normal pregnancy was 0,28 ± 0,13 µmol Pi/mg prot/hour, in pre-eclmpatic women without vitamin E was 0,25 ± 0,14 µmol Pi/mg prot/hour, and in pre-eclamptic women supplemented with vitamin E was 0,27 ± 0,13 µmol Pi/mg prot/hour. The result showed that there was no difference of the Na^+-K^+ ATPase activity in syncytiotrophoblast cell membrane of women with normal pregnancy, women complicated by pre-eclampsia without vitamin E and with vitamin E.

EXPLORATION OF XYLANOLYTIC ENZYMES FROM PACET HOT SPRING, EAST JAVA

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PP-19

ABSTRACT

Xylan is the major component of hemicellulose in plant cell wall. Xylanase is complex enzymes that are use to degradate xylan into monosaccharide and olygosaccaride. The purpose of this research is to explore xylanase enzymes from bacteria which is isolated from Pacet hot spring, East Java. The result showed that there were 4 positive isolate that expressed xylanase activity. The selection method for xylanase activity was DNS method. The highest activity was indicated by isolat number 1 (12.28 U/ml). The activity was detected at 50^0C, pH 7.0.

HYDROLYSIS OF COMMERCIAL XYLANS BY XYLANOLYTIC ENZYME EXPRESED IN *E. coli* DH5α (pTP510)

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PP-20

ABSTRACT

The purposes of this research were to know the ability of recombinant xylanolytic enzymes from recombinant *E. coli* DH5α (pTP510) to hydrolyze several commercial xylan and analysis the reduction sugar product. Recombinant xylanolytic enzyme could hydrolyzed several commercial xylan (*oat-spelt xylan, birchwood, wheat, rye,* and *arabinan*) with xylanolytic activities were : *oat-spelt xylan* (173,33 U/mL), *birchwood* (92,22 U/mL), *wheat* (652,22 U/mL), *rye* (494 U/mL), and *arabinan* (340 U/mL). Xylanolytic enzyme assay used specific substrat *p*-nitofenil-β-D-xilopiranosida (pNP-x) showed xylosidase activity 1,5869 μ mol/menit. Hydrolysis product was analyzed by HPLC. The result showed that xylose, arabinose, and xylo-oligosaccaride was produced from *birchwood, wheat, rye,* and *arabinan,* meanwhile xylose and arabinose produced from *oat-spelt xylan.*

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ISOLATION OF PARIETIN FROM THALLUS OF LICHEN *Ramalina javanica* Nyl AND ANTI CANCER TEST AGAINTS EUKEMIA CELL L 1210

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PP-21

ABSTRACT

The isolation of parietin from acetone extract of thallus lichen *Ramalina javanica* Nyl was carried out. Its structure was determined based on spectroscopic evidences. Bioactivity test of acetone extract to *A. salina* Leach gives LC_{50} = 4.23 µg/mL, whereas anti-cancer activity test of ct acetone extract as well as parietin against leukemia cell L 1210 gave IC_{50} = 23.64 and 16.74 µg/mL.

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ISOLATION OF VICANICIN FROM THALLUS OF LICHEN *Ramalina javanica* Nyl AND ANTICANCER TEST AGAINTS LEUKEMIA CELL L 1210

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PP-22

ABSTRACT

The isolation of vicanicin from acetone extract of lichen thallus of *Ramalina javanica* Nyl. was carried out. Its structure was determined based on spectroscopic evidences. Bioactivity test of vicanicin to *Artemia salina* Leach gives LC_{50} = 2.24 µg/mL, whereas anticancer activity test against leukemia cell L 1210 gives IC_{50} = 19.25 µg/mL.

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THE INHIBITION OF PARALYTIC ALKALOIDS FROM THE SEED OF *Erythrina fusca* Lour (Leguminosae) OF ATPase ACTIVITY

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PP-23

ABSTRACT

Erythrina fusca Lour (Leguminosae) is plant used in Indonesian folk medicine. During the course of our contuining search for novel plant bioactive from Indonesian plants, the erythrina alkaloids from the seed of *E. fusca* showed paralytic activity against third instar larvae of silkworm (*Bombyx mori*) wih their ED_{50} values as 47, 37, and 28 µg/g diet, respectively. The purpose of this research is determine kinetic from ATPase and inhibition process of active compounds. The enzymatic assay of ATPase by using colorimetri method with ATP substrat and apyrase enzyme. The result of enzymatic assay showed inhibition effect of ATPase as value value $V_{max} = 4.8 \times 10^{-3}$ mM/minute, $K_M = 11.18 \times 10^{-2}$ mM. The active compounds from the seed of *E. fusca* showed competitive inhibition of ATPase as value $K_I = 2.38 \times 10^3$; 8.74×10^3 and 20.11×10^3 mM, respectively.

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CLONING, SEQUENCING, AND EXPRESSION OF THE GENE ENCODING THE *CLOSTRIDIUM STERCORARIUM* α-GALACTOSIDASE AGA36A IN *Escherichia coli*

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PP-24

ABSTRACT

α-galactosidase (a-Gal : EC 3.2 .1.22) hydrolyzes α-galactosidic linkages at non reducing end in galactose-containing oligosaccharides, galactolipids, and/or galactomannan. α-Gals have the potential for usage in various fields, e.g., in sugar beet industry, α-Gals was used to increase the sucrose yield by eliminating raffinose which prevents normal crystallization of beet sugar;α-Gals may be used to alleviate flatulance caused by carbohydrates such as raffinose and stachyose in beans, which can not be metabolized by monogastric animals including human. α-Gals can be used to improved the properties of guar gum, a type of galactomannan, as a gelation promoter and cogelator, by enzymatic released of galactose residues from guar gum.

This study was carried out in order to clone genes encoding thermophilic α-galactosidases and characterize their translated products. Chromosomal DNA of *C. stercorarium* was partially digested with *Sau3*AI and DNA fragments with molecular size of 5 - 10 kbp were recovered from the agarose gel. After 5'-protruding ends of the DNA fragment were partially filled with dG and dA by Klenow enzyme, the DNA fragments were ligated into the *Xho*I site of pBluescript II KS^+ that had been partially filled with dC and dT in advance and used to transform *E.coli* DH5α. Transformants were screened for α-galactosidase productivity on agar plates using 4-metylumbelliferyl-α-D-galactopyranoside as the substrate. As a result, two α-galactosidase-producing clones were isolated from about 5,000 transformants and the *aga36A* gene carried by pAN1 was chosen for further investigation.

The *aga36A* gene consists of 2,208 nucleotides encoding a protein of 736 amino acids with a predicted molecular weight of 84, 786. Comparison of the amino acid sequence of Aga36A with entries in the DDBJ database indicated that this enzyme could be classified in family 36 of the gylcoside hydrolases and Aga36A showed overall sequence similarity with some enzymes of family 36 such as *Geobacillus* (formerly *Bacillus*) *stearothermophilus* GalA (57%) and AgaN (52%) and 57% sequence identity with *B. halodurans* possible α-galactosidase.

The *aga36 gene* was subcloned into pBluescript II SK^- for gene expression. The recombinant Aga36A was purified from *E.coli* DH5α and characterized. The enzyme hydrolyzed raffinose and guar gum with specific activities of 3.0 U/mg and 0.46 U/mg for respective substrates.

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