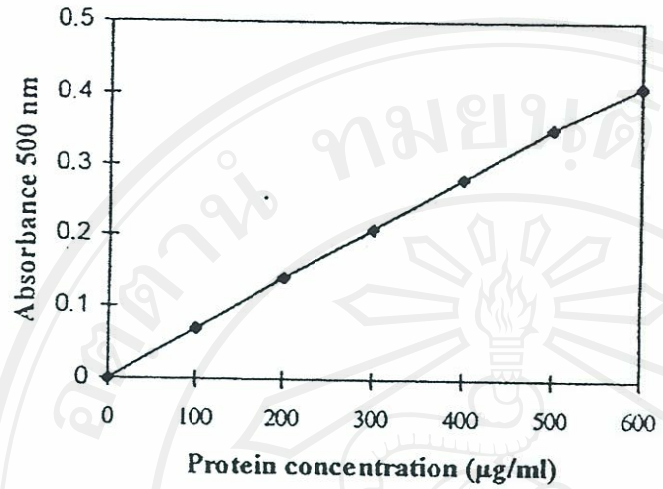
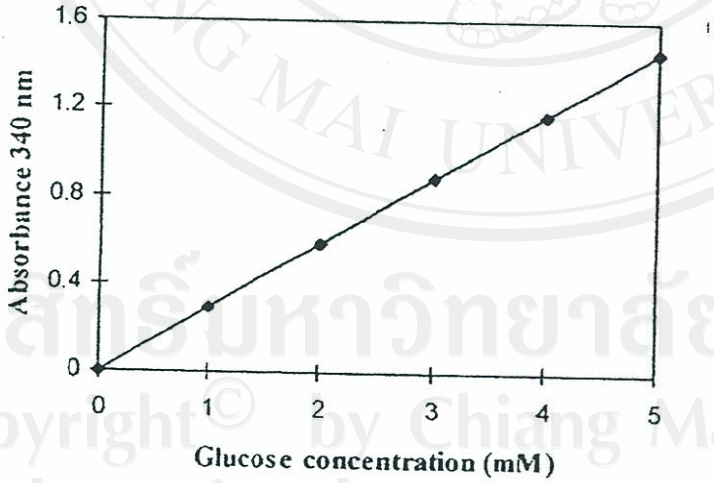
APPENDIX

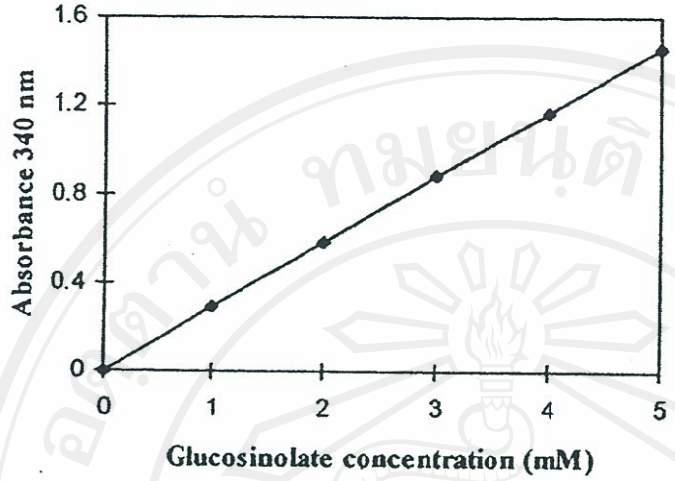
A-1 Calibration curve for the determination of protein by Lowry's method



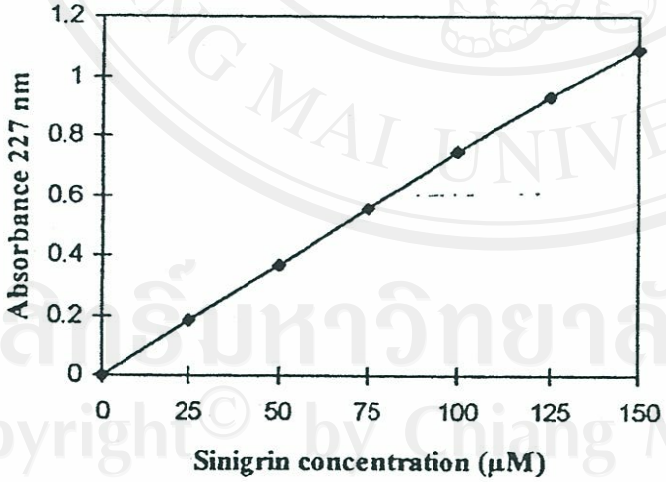
A-2 Calibration curve for the determination of glucose by couple-enzyme method



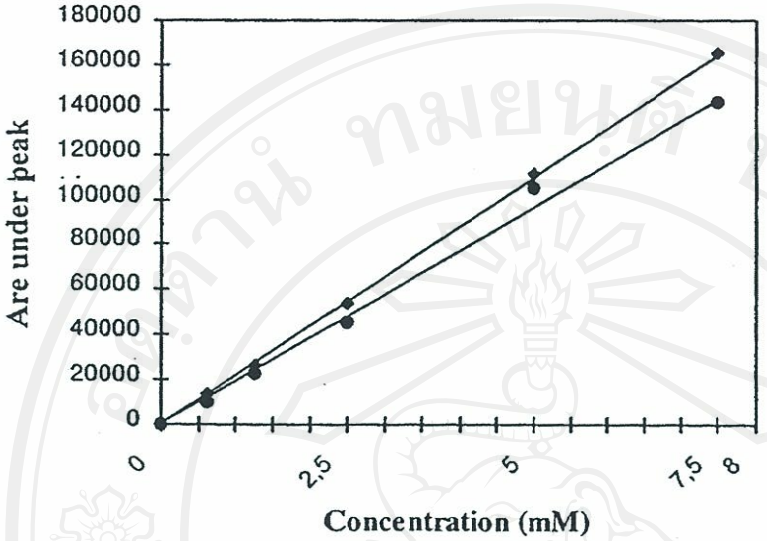
A-3 Calibration curve for the determination of total glucosinolates by coupled-enzyme method



A-4 Calibration curve for the determination of sinigrin by spectrophotometric method



A-5 Calibration curve for the determination of allylcyanide (●) and allylisothiocyanate (■) by a Hewlett-Packard 5890 series II gas chromatograph (Chrompack column)



A-6 Supporting papers

1. Bordin Butrindr, Hataichanook Niamsup, Lalida Shank and Nuansri Rakariyatham (2004). Myrosinase over producing mutants of *Aspergillus* sp. NR463. *Annals of Microbiology*., 54(4): 493-501.

2. Nuansri Rakariyatham, Bordin Butrindr, Hataichanoke Niamsup and Lalida Shank (2005). Screening of filamentous fungi for production of myrosinase. *Braz. J. Microbiol*., *Accepted.*

3. Nuansri Rakariyatham, Bordin Butrindr, Hataichanoke Niamsup and Lalida Shank (2005). Improvement of myrosinase activity from *Aspergillus* sp. NR4617 by chemical mutagenesis. *Electronic Journal of Biotechnology., Accepted.*.

Annals of Microbiology, **54 (4)**, 493-501 (2004)

Myrosinase overproducing mutants of *Aspergillus* sp. NR463

B. BUTRINDR, H. NIAMSUP, L. SHANK, N. RAKARIYATHAM*

Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Received 30 June 2004 / Accepted 30 September 2004

Abstract - A myrosinase (thioglucoside glucohydrolase or thioglucosidase, EC 3.2.3.1) producing fungus, *Aspergillus* sp. NR463, was newly isolated from decayed soil sample obtained in Lamphun province, Thailand and was subjected to mutagenesis and selection for myrosinase production on low cost medium prepared from mustard seed cake (*Brassica juncea*). Studies of production and stability of the enzyme showed that UV mutagenesis induced myrosinase overproduction. *Aspergillus* sp. NR463U4 produced myrosinase 2.35 U ml^{-1} at 36 h of the cultivation. The stability studies revealed that myrosinase from the mutant strains retained activity for 3.5 times longer than wild-type at 30 °C. Analysis of the glucosinolate-degradation products by myrosinase of wild-type and all mutant strains gave similar results showing that allylisothiocyanate was present as a main product while allylcyanide was not detected. Mutant strains consumed glucosinolate in the high concentration (10 mM) and completely degraded in 36 h. *Aspergillus* sp. NR463U4 maintained constant myrosinase production judging by the production stability comparing with the wild-type for 8 months. High production and prolonged stability of myrosinase demonstrated that this mutant could be a new found candidate for industrial application.

Key words: allylisothiocyanate, *Aspergillus* sp., β-thioglucosidase, myrosinase, UV mutagenesis.

INTRODUCTION

Myrosinase (EC 3.2.3.1) is a common name for the β-thioglucosidase enzyme responsible for the hydrolysis of glucosinolates, a group of sulphur-containing glycosides present in all members of the *Cruciferae*, including the brassica vegetables. Glucosinolate consists of a common glycone moiety and a variable aglycone side-chain derived from amino acids. Upon tissue disruption, glucosinolates are rapidly hydrolysed by myrosinase to unstable intermediates that, as dictated by chemical conditions, spontaneously rearrange to isothiocyanates, thiocyanates, or nitriles (Bones and Rossiter, 1996). The enzymatic catalysis of glucosinolates has previously been studied for its antinutritional effects in animal feed, although in recent years these compounds have been considered

* Corresponding Author. Fax: (66) 53 892277; E-mail: nuansri1@yahoo.com

msn Hotmail®

royter99@hotmail.com Printed: Thursday, January 5, 2006 9:25 AM

From : BJM <bjm@sbmicrobiologia.org.br>
Sent : Monday, November 7, 2005 7:50 PM
To : "bordin butrindr" <royter99@hotmail.com>
Subject : RES: Ask for the progress of the paper

Dear Dr. Dr. Nuansri Rakariyatham,

We are pleased to inform that your paper **"SCREENING OF FILAMENTOUS FUNGI FOR PRODUCTION OF MYROSINASE"**, was approved for publication. As soon we are sending the proof.

Sincerely yours,

Nancy Yuri Kawakosi de Amo

Secretary

BJM - Brazilian Journal of Microbiology

Departamento de Microbiologia - ICB II - USP

Av. Prof. Lineu Prestes, 1374 - S.214 - Cidade Universitaria

05508-900 SÃO PAULO - SP

Site: www.sbmicrobiologia.org.br

Atenciosamente,

Nancy Yuri Kawakosi de Amo

Secretaria

BJM - Brazilian Journal of Microbiology

Departamento de Microbiologia - ICB II - USP

msn Hotmail®

royter99@hotmail.com Printed: Thursday, January 5, 2006 9:20 AM

From : Nuansri Rakariyatham <nuansri1@yahoo.com>
Sent : Wednesday, January 4, 2006 7:54 PM
To : royter99@hotmail.com
Subject : Fwd: Electronic Journal of Biotechnology

--- Biotec <biotec@ucv.cl> wrote:

> From: "Biotec" <biotec@ucv.cl>
> To: <nuansri1@yahoo.com>
> Subject: Electronic Journal of Biotechnology
> Date: Thu, 29 Dec 2005 12:29:20 -0300
>
> November 18, 2005
>
> Dr Nuansri Rakariyatham
> Department of Chemistry
> Faculty of Science
> Chiang Mai University
> Chiang Mai
> 50200, Thailand
>
> Dear Dr Rakariyatham,
>
> It is a pleasure to inform that the article entitled
> "Improvement of myrosinase activity of Aspergillus
> sp. NR4617 by chemical mutagenesis"
> (EJBiotech/NRS/285) authored by Nuansri
> Rakariyatham, Bordin Autr-Indr, Natalchanoke
> Nlamsup, and Lalida Shank, has been accepted to be
> published in Electronic Journal of Biotechnology.
>
> The manuscript will be corrected only in formal
> aspects according to Electronic Journal of
> Biotechnologys' manual of style and will be
> announced at our Homepage.
>
> We would greatly appreciate receiving the BIP
> (Biotechnology in Public) version of the manuscript.
> The BIP is a shorter version of the article, written
> in a language appropriate for a broader public, no
> longer than 4 pages.
>
> We also need the Copyright Transfer Agreement
> corresponding to your manuscript, which you can find
> at http://www.ejbiotechnology.info/copyri.html.
> Please send it to the fax 56 32 27 31 83. All
> authors should send a copy of this document.
>
> Please select one or two areas for the manuscript:
>
> Animal Biotechnology
> Bioethics
> Biofilms
> Biopolicies of International Cooperation
> Biosafety
> Biotechnology Industry
> Biotechnology Teaching
> Biotechnology of Human Disorders
> Environmental Biotechnology
> Marine Biotechnology
> Microbial Biotechnology
> Molecular Biology and Genetics
> Plant Biotechnology
> Process Biotechnology
>

CURRICULUM VITAE

Name	Mr. Bordin Butr-Indr
Date of Birth	November 1, 1975
Education	
1993	B. Sc. (Medical Technology), Faculty of Associated Medical Science, Chiang Mai University
1997	M. Sc. (Biotechnology), Faculty of Science, Chiang Mai University
2000	Ph. D. (Biotechnology), Graduate School, Chiang Mai University

Presentation

1. Bordin Butr-Indr and Nuansri Rakariyatham, 2000. Hydrolysis of Mung Bean Flour by Proteases, the 12th Annual Meeting of the Thai society for Biotechnology, 1-3, Nov. The Felix Hotel, Kanchanaburi, Thailand.

2. Bordin Butr-Indr and Nuansri Rakariyatham, 2001. Effect of shaking on the mycerial growth and myrosinase production by liquid culture of *Aspergillus* sp. Bio-Thailand 2001, 7-10, Nov. Queen Sirikit National Convention Center, Bangkok, Thailand.

Publication

1. Bordin Butrindr, Hataichanook Niamsup, Lalida Shank and Nuansri Rakariyatham (2004). Myrosinase over producing mutants of *Aspergillus* sp. NR463. *Annals of Microbiology.*, 54(4): 493-501.

2. Nuansri Rakariyatham, Bordin Butrindr, Hataichanoke Niamsup and Lalida Shank (2005). Screening of filamentous fungi for production of myrosinase. *Braz. J. Microbiol., Accepted.*

3. Nuansri Rakariyatham, Bordin Butrindr, Hataichanoke Niamsup and Lalida Shank (2005). Improvement of myrosinase activity from *Aspergillus* sp. NR4617 by chemical mutagenesis. *Electronic Journal of Biotechnology., Accepted.*