

**EFFECTS OF PHENOLIC MONOMERS ON ENZYMATIC AND  
FERMENTATION ACTIVITIES OF THE RUMEN FUNGUS,  
*NEOCALLIMASTIX FRONTALIS***

**By**

**WAN ZUHAINIS SAAD**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

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**Chairman: Professor Norhani Abdullah, PhD**

**Faculty : Biotechnology and Biomolecular Sciences**

The present study was undertaken to investigate the effects of phenolic monomers on the enzymes and fermentation activities of a rumen fungus, *Neocallimastix frontalis*, and to evaluate the ability of the fungus to colonize and degrade guinea grass which contained large amount of phenolic monomers. From 115 isolates of *N. frontalis*, 15 isolates of *Piromyces mae* and 3 isolates of *Orpinomyces joyonii* obtained from rumens of cattle and buffalo, a representative isolate of each species was selected for further studies on its cellulolytic activity. *Neocallimastix frontalis* B15, *P. mae* B6 and *O. joyonii* C3 were selected based on their good growth in straw and ball-milled filter paper media, and on their ability to maintain zoosporogenesis and viability in the subcultures. <sup>14</sup>C-labelled bacterial (*A. xylinum*) cellulose was used to determine the cellulolytic activity of the three rumen fungal species and the effects of phenolic acids (p-coumaric and ferulic acids) on the activity. The results showed that *N. frontalis* B15 had the highest cellulolytic activity, and the phenolic acids had an inhibitory effect on the activity. Other *N. frontalis* strains isolated from buffalo (B9), cattle (C20) and goat were (G8) further characterized for their cellulolytic activities,

and a strain, *N. frontalis* B9, which showed the highest activity was chosen for subsequent studies.

The enzymes produced by *N. frontalis* B9 grown in filter paper or guinea grass media included, CMCase, FPase, xylanase,  $\beta$ -glucosidase and  $\beta$ -xylosidase. The maximum production of all the enzymes was at 72 h of incubation. The activities of the enzymes in filter paper media in descending order were: CMCase > xylanase > FPase >  $\beta$ -glucosidase, and in guinea grass media were: xylanase > carboxymethylcellulase > FPase >  $\beta$ -xylosidase >  $\beta$ -glucosidase. Phenolic monomers were found to inhibit the production of the enzymes and fermentation activity of *N. frontalis* B9 in varying degrees. Of the four phenolic monomers,  $p$ -coumaric and ferulic acids were the most inhibitory and vanillin the least. The fermentation end-products were also inhibited by the phenolic monomers. Observations using scanning electron microscopy showed that *N. frontalis* B9 in control cultures without phenolic monomers could extensively colonise and degrade various tissues of guinea grass, but treatments with the phenolic monomers significantly reduced the colonization and degradation of the grass fragments. Phenolic monomers, particularly  $p$ -coumaric acid and ferulic acid also inhibited the dry weight loss and reduction in the textural strength of the grass fragments by *N. frontalis* B9, which indicated a reduction in the degradation of the grass fragments. *Neocallimastix frontalis* B9 produced esterases that released ferulic and  $p$ -coumaric acids from their methyl esters and guinea grass. Production of feruloyl and  $p$ -coumaroyl esterases, coupled with the penetrative ability provided by fungal rhizoids, provided a mechanism for the breakdown and subsequent utilization of the phenolic ester-linked carbohydrates present in plant cell walls.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN MONOMER FENOLIK TERHADAP AKTIVITI ENZIM  
DAN PENGHADAMAN OLEH KULAT RUMEN,  
*NEOCALLIMASTIX FRONTALIS***

Oleh

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Kajian ini bertujuan untuk melihat kesan monomer fenolik terhadap aktiviti enzim dan fermentasi kulat rumen, *Neocallimastix frontalis* dan melihat keupayaan kulat ini menghadam rumput guinea yang mempunyai kandungan fenolik monomer yang tinggi. Daripada 115 strain *N. frontalis*, 15 strain *Piromyces mae* dan 3 strain *Orpinomyces joyonii* yang dipencil dari rumen lembu dan kerbau, tiga strain kulat telah dipilih sebagai mewakili setiap spesies kulat untuk mengkaji aktiviti selulolitik kulat tersebut. *Neocallimastix frontalis* B15, *P. mae* B6 dan *O. joyonii* C3 dipilih berdasarkan pertumbuhan yang baik di dalam media jerami padi dan kertas turas, serta keupayaan zoosporogenesis mereka. Penggunaan radiolabel <sup>14</sup>C ini bertujuan untuk menentukan aktiviti selulolitik serta kesan monomer fenolik terhadap kulat rumen tersebut. Keputusan kajian menunjukkan ketiga spesies kulat adalah selulolitik, dengan *N. frontalis* menunjukkan aktiviti selulolitik yang tertinggi. Aktiviti selulolitik *N. frontalis* dari 3 perumah iaitu, kerbau (B9), lembu (C20) dan

kambing (G8) tidak menunjukkan perbezaan yang signifikan tetapi aktiviti selulolitik *N. frontalis* (B9) dari kerbau adalah lebih tinggi dari strain yang lain, oleh itu strain tersebut telah dipilih untuk kajian seterusnya.

*Neocallimastix frontalis* B9 menghasilkan enzim berikut karboksimetilselulase, filterpaperase, xilanase,  $\beta$ -glukosidase dan  $\beta$ -xilosidase apabila menghadam selulosa media kertas turas dan rumput guinea. Perembesan enzim ini adalah maksimum pada 72 jam waktu pengeraman kulat. Keaktifan enzim ini dalam media kertas turas adalah karboksimetilselulase > xilanase > filterpaperase >  $\beta$ -glucosidase manakala bagi media rumen guinea adalah xilanase > karboksimetilselulase > filterpaperase, >  $\beta$ -xilosidase >  $\beta$ -glukosidase. Secara amnya, asid  $\rho$ -kumarik dan asid ferulik perencat yang kuat dan vanilin adalah perencat yang terendah.

Dari ujikaji yang telah dijalankan, didapati *N. frontalis* B9 dapat mengkolonikan tisu tumbuhan termasuk tisu berlignin dengan aktif dan baik bila tiada monomer fenolik. Walau bagaimanapun, daripada ujikaji terhadap peratus kehilangan berat kering dan kekuatan tekstur, monomer fenolik seperti asid  $\rho$ -kumarik dan asid ferulik didapati merencat degradasi rumput guinea. Ini menunjukkan  $\rho$ -kumarik dan asid ferulik adalah perencat yang lebih kuat dari  $\rho$ -hidroksibenzaldehyde dan vanilin. Kajian ini mendapati *N. frontalis* B9 mengeluarkan enzim-enzim esterase yang meleraikan ikatan asid  $\rho$ -kumarik dan ferulik dari bentuk metil-ester masing-masing. Keupayaan mengeluarkan enzim ini serta rizoid yang dapat menembusi tisu tumbuhan memberi satu mekanisme untuk penggunaan karbohidrat yang terikat ke fenolik ester yang hadir dalam tisu tumbuhan.

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*Learning is an ornament in prosperity,  
a refuge in adversity,  
and a provision in old age.*

Aristotle

I certify that an Examination Committee has met on 18<sup>th</sup> May 2006 to conduct the final examination of Wan Zuhainis Saad on her Doctor of Philosophy thesis entitled “Effects of Phenolic Monomers on Enzymatic and Fermentation Activities of the Rumen Fungus, *Neocallimastix frontalis*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

**WAN ZUHAINIS SAAD**

Date:

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