
UNIVERSITI PUTRA MALAYSIA

# PRODUCTION AND CHARACTERISATI0N OF CELL·BOUND LIPASES SECRETED BY A NEWLY ISOLATED STRAIN OF Geotrichum candidum

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by

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**Specially for Bangdek, Aie, Kamal and Farah**

**They matter more above all else.....**

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| E.A | Enzyme activity |
| S.E.A | Specific enzyme activity |
| conc. | concentration |
| prot. | protein |
| rpm | revolution per minute |
| ppm | part per million |
| v/v | volume per volume |
| mol. wt. | molecular weight |
| TLC | Thin-layer chromatography |
| GLC | Gas liquid chromatography |
| min | minute |
| h | hour |
| C4 | butyrate/butyric acid |
| C6 | caproate/caproic acid |
| C8 | caprylate/caprylic acid |
| C10 | caprate/capraic acid |
| C12 | laurate/lauric acid |
| C14 | myristate/myristic acid |
| C16 | palmitate/palmitic acid |
| C18 | stearate/stearic acid |
| C18:1 | oleate/oleic acid |
| C18:2 | linoleate/linoleic acid |
| C18:3 | linolenate/linolenic acid |
| m.t | metric tonne |
| x g | times gravity |
| ppm | part per million |
| PVA | polyvinyl alcohol |
| mM | millimolar |
| umol | micromole |
| mmol | millimole |
| v/w | volume per weight |
| w/v | weight per volume |
| psi | pound per square inch |
| g | gram |
| mg | milligram |
| ml | milliliter |
| cm | centimeter |
| sp. | species |
| YM | yeast - malt extract |
| str. | strain |
| GYE | glucose - yeast extract |

Abstract of the Thesis Presented to the Senate of Universiti Pertanian Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy.

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Supervisor : Lee Kong Hung, PhD

Faculty : Food Science and Biotechnology

Indigenous lipolytic microorganisms were successfully isolated from soil samples collected from an oil palm plantation and were identified up to the generic level. Over seven hundred microbial colonies were screened and fifteen were found to be positive for the hydrolysis of triolein. Of these, three were yeast species, another three were strains of *Geotrichum candidum* and the rest were bacteria. Studies on the lipolysis of various natural oils on solid media and in liquid media by the yeasts and the *G. candidum* strains showed that the latter were the most potent lipolytic organisms. One of the yeasts was found to be weakly lipolytic. These organisms shared two common features : they were not able to hydrolyse tributyrin and they hydrolysed palm kernel olein, which is a lauric acid oil, poorly. Results obtained indicated that these organisms probably elaborated extracellular lipases that possessed some degree of fatty acid specificity.

The cultural conditions for the maximal hydrolysis of palm olein and for the production of extracellular lipases, both soluble and cell-bound, in submerged culture were determined for one of the *G. candidum* strains. The optimal pH for lipolysis and maximal production of lipases occurred at pH 7.0 - 7.2. It was discovered that the soluble lipase of this organism was produced constitutively. The cell-bound lipase, however, was found to be an inducible enzyme where production took place only when an oil was added to the culture medium. The type of oil used did not affect production significantly but the presence of sugars and glycerol decreased lipase productivity markedly. High levels of glycerol suppressed growth of the organism.

The cell-bound lipase was characterised and was shown to be most active at 43°C and preferred p-nitrophenylcaprylate as the substrate. The kinetics of the hydrolysis of various fatty acid esters of p-nitrophenol were studied and the Km and Vmax values are presented. When the enzyme was stored at 4°C, a second temperature optimum developed at 30°C. Continued storage resulted in an increase in the activity at 30°C with a concomittant decrease in activity at 43°C. After 6 days, the temperature optimum at 43°C was completely lost. The shift in temperature optimum from 43°C to 30°C could be quickly achieved by heating the cell-bound lipase at 40°C for 2 h.

The extraction of the cell-bound lipase of *G. candidum* was simply and easily achieved by shaking induced cells in a buffer solution. Complete extraction could be accomplished in 4-5 h and the total enzyme activity recovered was 4.6-fold greater than what was initially measured and found to be bound to the cells. Magnesium ions when added to the extraction buffer caused a delay in the release of the enzyme from the

cells. The most efficient pH for extraction was pH 8.4. The extracted lipase was most active at pH 7.8. This enzyme had two temperature optima : 33°C and 40°C. The temperature optimum at 33°C was observed only upon storage of the enzyme extract at 4°C. When in the soluble form, the cell-bound lipase preferred p-nitrophenylpalmitate as the substrate, instead of p-nitrophenylcaprylate. The Km and Vmax values of the enzyme for this ester was 6.7 mM and 6.3 x $10^3$ umol/min, respectively. The rate of hydrolysis of olive oil exceeded the rate of hydrolysis of tributyrin by 4 times. The profiles of the hydrolysis of a number of fatty acid esters of p-nitrophenol, olive oil and tributyrin of the extracted lipase and those of several commercial lipases were obtained and compared.

Purification of both the extracted lipase and soluble lipase was performed and the results obtained are presented. Gel filtration of cell-bound extract and soluble lipase extract on Sephadex G-150 revealed that the *G. candidum* produced at least two cell-bound lipase and two soluble lipase isozymes. The molecular weights of the bound lipases were estimated to be 75,000 and 58,000 - 61,000, respectively.

Abstrak yang dikemukakan Kepada Senat
Universiti Pertanian Malaysia Sebagai
Memenuhi Syarat Keperluan Untuk Ijazah
Doktor Falsafah

**PENGHASILAN, PENGEKSTRAKAN, PENULINAN DAN PENCIRIAN LIPASE-LIPASE TERIKAT SEL YANG DIREMBES OLEH STRAIN *Geotrichum candidum***

Oleh

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Mikroorganisma lipolitik tempatan telah berjaya diasingkan dari sampel-sampel tanah yang diambil dari sebuah ladang kelapa sawit dan dikenalpasti schingga kc paras genera. Lebih dari tujuh ratus koloni mikroorganisma telah disaring dan lima belas didapati positif terhadap penghidrolisisan triolein. Tiga daripadanya adalah spesi-spesi yis, tiga lagi adalah strain-strain *Geotrichum candidum* dan yang lain adalah bakteria. Kajian-kajian mengenai lipolisis beberapa minyak asli diatas media pepejal and didalam media kultur cecair oleh yis-yis dan strain-strain *G. candidum* tersebut menunjukkan bahawa strain-strain *G. candidum* merupakan organisma lipolitik yang terhandal. Salah satu dari yis-yis didapati organisma lipolitik yang lemah Organisma-organisma ini berkongsi dua sifat umum. Mereka tidak berupaya menghidrolisis tributirin dan kurang

baik dalam menghidrolisis minyak olein isirong kelapa sawit, sejenis minyak asid laurik. Keputusan yang diperolehi menunjukkan bahawa organisma-organisma tersebut berkemungkinan merembes lipase-lipase ekstrasel yang mempunyai beberapa darjah kekhususan terhadap asid lemak.

Keadaan pengkulturan untuk hidrolisis minyak olein kelapa sawit dan penghasilan lipase-lipase ekstrasel iaitu kedua-dua lipase terlarut dan terikat-sel dalam kultur terendam, yang maksimum ditentukan untuk salah satu dari strain-strain *G. candidum.* pH optimum untuk lipolisis dan penghasilan maksimum lipase berlaku pada pH 7.0 -7.2. Lipase terlarut untuk organisma ini adalah dihasilkan secara juzukan (konstitutif). Akan tetapi, lipase terikat-sel merupakan sejenis enzim teraruh. Penghasilan berlaku hanya apabila minyak ditambah kepada media kultur. Jenis minyak yang ditambah tidak memberi kesan yang bererti kepada penghasilan tetapi kehadiran gula dan gliserol mengurangkan penghasilan lipase dengan nyata. Paras gliserol yang tinggi menyekat tumbesaran mikroorganisma tersebut.

Lipase terikat-sel dicirikan dan ditunjukkan paling aktif pada 43°C dan menyukai p-nitrofenilkaprilat sebagai substrat. Kinetik penghidrolisan beberapa ester asid lemak kepada p-nitrofenol dikaji dan nilai-nilai Km dan Vmax dibentangkan. Apabila enzima tersebut distor pada 4°C, suhu optimum yang kedua membangun pada 30°C. Penstoran seterusnya mengakibatkan aktiviti pada 30°C meningkat dan pada masa yang sama, aktiviti pada 43°C menurun. Selepas 6 hari, suhu optimum pada 43°C terhapus sama sekali. Perubahan suhu optimum dari 43°C kepada 30°C boleh diperolehi dengan cepat dengan memanaskan lipase terikat-sel pada 40°C selama 2 jam.