

SPELMAK FOLKLÆRTE BÅND OG SØS
BACTERIA ISOLATED FROM CORAL ANGUS

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SCREENING FOR LIPASE ACTIVITY FROM MD022 BACTERIA ISOLATED
FROM CORAL MUCUS

By

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the requirements for the degree of
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LIST OF ABBREVIATIONS

A	-	absorbance
BSA	-	bovine serum albumin
CaCl ₂	-	calcium chloride
g	-	gram
km ²	-	kilometer square
M	-	molar
mL	-	milliliter
µg	-	microgram
µm	-	micrometer
NaOH	-	sodium hydroxide
nm	-	nanometer
OD	-	optical density
rpm	-	rotation per minute
v/v	-	volume per volume
°C	-	degree celcius
%	-	percentage

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ABSTRACT

At the moment, the information on lipase-producing marine bacteria is quite limited. In this study, the ability to produce this enzyme by marine bacteria MD022, isolated from coral mucus at Pulau Bidong, Terengganu was investigated. In order to confirm the identification of the bacteria, biochemical tests based on their morphological and phenotypic characteristics have been carried out. The results suggested that MD022 bacterium is *Klebsiella pneumoniae*. Prior to the induction of lipase by the bacteria, three different media have been tested to determine the best culture medium: Nutrient Broth added with distilled water, Nutrient Broth added with sea water and ZoBell's modified broth. ZoBell's medium was found to contain the highest bacterial growth and needed four hours and 15 minutes to reach the log phase of bacterial growth. In order to induce the lipase production, an investigation on the optimum culture conditions was held: incubation time, temperature and amount of substrate. For the effect of temperature, there was no significant difference ($P>0.05$) observed between 15 and 27°C , but there was significant difference ($P<0.05$) observed between 27 and 37°C . No significance differences were observed for different incubation time tested and also for different amount of substrates. Therefore, the optimum conditions chosen for hydrolysis of olive oil were in the range of 15 to 27°C , using 35 $\mu\text{g}/\text{ml}$ of enzyme for lipase assay, while optimum incubation time and amount of substrate could not yet be determined. The results indicated that *Klebsiella pneumoniae* have produced lipase which was able to catalyze the hydrolysis of olive oil. It is hoped that in the future, marine bacterial lipase can be an alternative to chemical catalysts to meet the demand of industries.

**PENYARINGAN AKTIVITI LIPASE DARI BAKTERIA MARIN MD022
YANG DIPENCILKAN DARI LENDIR BATU KARANG**

ABSTRAK

Sehingga kini, maklumat mengenai bakteria marin yang menghasilkan enzim lipase adalah sangat terhad. Dalam kajian ini, keupayaan bakteria marin MD022 yang dipencilkan dari lendir batu karang di Pulau Bidong, Terengganu untuk menghasilkan enzim lipase telah dikaji. Bagi memastikan identiti bakteria ini, ujian-ujian biokimia berdasarkan ciri-ciri bentuk dan fisiologi telah pun dilakukan. Keputusan yang telah dicadangkan kepada bakteria MD022 adalah *Klebsiella pneumoniae*. Sebelum rangsangan pengeluaran enzim lipase oleh bakteria ini, tiga jenis media telah diuji bagi menentukan media kultur yang terbaik: broth nutrien dalam air suling, broth nutrien dalam air laut dan media ZoBell diubahsuai. Media ZoBell diubahsuai didapati mengandungi pertumbuhan bakteria yang paling tinggi dan memerlukan selama empat jam 15 minit untuk mencapai pertumbuhan bakteria optimum pada fasa log. Bagi merangsang pengeluaran enzim lipase, satu kajian ke atas keadaan kultur yang optimum telah dilakukan: masa pengerman, suhu dan amaun substrate. Untuk kesan ke atas suhu, tiada perbezaan yang bererti ($P>0.05$) diperhatikan antara 15 dan 27°C , tetapi terdapat perbezaan yang bererti ($P<0.05$) diperhatikan antara 27 dan 37°C . Tiada perbezaan yang bererti yang diperhatikan untuk perbezaan masa pengerman dan juga amaun substrat. Oleh itu, keadaan yang optimum yang dipilih untuk menghidrolisiskan minyak zaitun adalah di dalam julat 15 ke 27°C , dengan menggunakan $35\mu\text{g}/\text{ml}$ enzim untuk asai lipase, manakala optimum masa pengerman dan amaun substrat tidak boleh ditentukan lagi. Keputusan menunjukkan *Klebsiella pneumoniae* telah menghasilkan lipase di mana boleh memangkinkan pemecahan minyak zaitun. Adalah diharapkan pada masa hadapan, bakteria lipase dari marin boleh dijadikan salah satu alternatif kepada pemangkin kimia untuk memenuhi permintaan industri.