DETECTION OF AQUATIC POLLUTION USING LUMINESCENT BACTERIA

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SCHOOL OF MARINE SCIENCE AND ENVIRONMENT
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DETECTION OF AQUATIC POLLUTANTS USING LUMINESCENT BACTERIA

By

Aty Atiqah Muhassan

Research Report submitted in partial fulfillment of

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DECLARATION AND VERIFICATION REPORT

FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled Detection of Aquatic Pollutants Using Luminescent Bacteria by Aty Atiqah Binti Muhassan. Matric No. UK26386 have been examined and all errors identified have been corrected. This report is submitted to the School of Marine Science and Environment as partial fulfillment towards obtaining the Degree of Marine Biology School of Marine Science and Environment, Universiti Malaysia Terengganu.

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LIST OF ABBREVIATIONS

5KG	-	5-KETO-D-GLUCONATE
Abs	-	Absorbance
AchE	-	Acetylcholinesterase
ADO	-	ADONITOL
AGAL	-	ALPHA-GALACTOSIDASE
AGLTp	-	Glutamyl Arylamidase Pna
AGLU	-	ALPHA-GLUCOSIDASE
APPA	-	Ala-Phe-Pro-ARYLAMIDASE
As	-	Arsenic
Balap	-	BETA-Alanine arylamidase pNA
BGAL	-	BETA-GALACTOSIDASE
BGLU	-	BETA-GLUCOSIDASE
BGUR	-	BETA-GLUCURONIDASE
BNAG	-	BETA-N-ACETYL-GLUCOSAMINIDASE
BOD	-	Biochemical Oxygen Demand
BXYL	-	BETA-XYLOSIDASE
Cd	-	Cadmium
CIT	-	CITRATE (SODIUM)
CMT	-	COURMARATE

Co	-	Cobalt
COD	-	Chemical Oxygen Demand
Cr	-	Chromium
Cu	-	copper
dCEL	-	D-CELLOBIOSE
DDT	-	Dichlorodiphenyltrichloroethane
dGLU	-	D-GLUCOSE
dMAL	-	D-MALTOSE
dMAN	-	D-MANNITOL
dMNE	-	D-MANNOSE
dSOR	-	D-SORBITOL
dTAG	-	D-TAGATOSE
dTRE	-	D-TREHALOSE
ELLM	-	ELLMAN
g	-	gram
GGAA	-	Glu-Gly-Arg-ARYLAMIDASE
GGT	-	GAMMA-GLUTAMYL-TRANSFERASE
GlyA	-	Glycine ARYLAMIDASE
H_2O	-	Water
H2S	-	H2S PRODUCTION
HCH	-	Hexachlorocyclohexane
IARL	-	L-ARABITOL

IHISa	-	I-HISTIDINE assimilation
ILATa	-	L-LACTATE assimilation
ILATk	-	I-LACTATE alkalinization
IMLTa	-	L-MALATE assimilation
kg	-	kilogram
LDC	-	LYSINE DECARBOXYLASE
LIP	-	LIPASE
mg	-	milligram
ml	-	millilitre
MNT	-	MALONATE
NaCl	-	sodium chloride
NAGA	-	Beta-N-ACETYL-GALACTOSAMINIDASE
nm	-	nanometre
O129R	-	O/129 RESISTANCE (comp. Vibrio)
O_2	-	Oxygen
°C	-	degree Celsius
ODC	-	ORNITHINE DECARBOXYLASE
ODEC	-	DECARBOXYLASE BASE
OFF	-	FERMENTATION/GLUCOSE
Pb	-	lead
PHOS	-	PHOSPHATE
PLE	-	PALATINOSE
ppm	-	Part per thousands

ProA	-	L-Proline ARYLAMIDASE
PyrA	-	L-Pyrrolydonyl-ARYLAMIDASE
rpm	-	Revolutions per minute
SAC	-	SACCHAROSE/SUCROSE
ß	-	beta
SUCT	-	SUCCINATE alkalinization
TBT	-	tributyltin
TSS	-	Total Suspended Solid
TyrA	-	Tyrosine ARYLAMIDASE
URE	-	UREASE
v	-	volume
w	-	weight
α	-	alpha

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ABSTRACT

Nowadays, luminescent bacteria as a sensing organism to detect the aquatic pollutants have been widely used. In this study, luminescent bacteria were identified and the effect of different concentrations of pollutants towards the density of luminescent bacteria was carried out. The luminescent bacteria were isolated from the ink of the squids. Then, the Gram staining was done to observe the shape of the luminescent bacteria. The bacteria were identified using VITEK. Besides that, the bacteria were tested with different NaCl concentration (0, 1, 2, 3, 4 and 5 % (w/v)) and at temperature (4, 26, 28, 37 and 40°C). Then, toxicity test was carried out with 1.5 ml of toxicants Cd, $ZnCl_2$, $HgSO_4$, $Pb(NO_3)_2$, diazinon and malathion (2, 4, 6, 8 and 10 ppm) were added to 1.5 ml of bacterial suspension in a cuvette. As for control, 1.5 ml of saline solution was added to 1.5 ml of bacterial suspensions. The Optical density (OD) was measured using spectrophotometer at 600 nm. Based on the result, the Gram staining showed it was Gram-negative bacteria with rod shaped or coccobacillus. The VITEK showed that the bacterium was 89% probability for *Pseudomonas fluorescens*. At different NaCl concentration showed that the bacteria were able to grow at all concentration and the bioluminescence emitted was brightly as the concentration increased and at different temperature, there was no growth and bioluminescence emitted at temperature 4 and 40°C. The bioluminescence emitted by bacteria was most brightly at 26°C. Meanwhile, for the toxicity test as the toxicant concentration increased, the bacterial cells density decreased. The overall study showed that most probability the bacteria were under the genus *Photobacterium* and can be used to detect aquatic pollutants.

PENGESANAN BAHAN PENCEMARAN AKUATIK MENGGUNAKAN BAKTERIA LUMINESEN

ABSTRAK

Pada masa kini, bakteria luminesen telah digunakan secara meluas sebagai organisma penderiaan untuk mengesan pencemaran akuatik. Dalam kajian ini , bakteria luminesen dikenal pasti dan kesan kepekatan bahan toksik pada ketumpatan bakteria luminesen telah dijalankan. Bakteria luminesen telah diambil dari dakwat sotong . Kemudian , pewarnaan Gram dilakukan dan bakteria telah dikenal pasti menggunakan sistem VITEK. Bakteria telah diuji dengan kepekatan NaCl yang berbeza (0, 1, 2, 3, 4 dan 5% (w / v)) dan pada suhu (4, 26, 28, 37 dan 40 ° C). Kemudian, ujian ketoksikan dijalankan dengan 1.5 ml bahan toksik Cd, $ZnCl_2$, $HgSO_4$, Pb $(NO_3)_2$, diazinon dan malathion (2, 4, 6, 8 dan 10 ppm) telah ditambah kepada 1.5 ml larutan bakteria dalam kuvet. Bagi kawalan, 1.5 ml larutan garam ditambah kepada 1.5 ml larutan bakteria. Ketumpatan Optik (OD) telah diukur dengan menggunakan spektrofotometer pada 600 nm. Berdasarkan keputusan pewarnaan Gram ia adalah bakteria Gram- negatif dengan berbentuk rod atau coccobacillus . Keputusan daripada VITEK menunjukkan bakteria adalah 89% *Pseudomonas fluorescens*. Daripada ujian kemasinan menunjukkan bahawa pertumbuhan bakteria pada semua kepekatan dan bioluminesen yang dipancarkan adalah terang apabila kepekatan meningkat. Pada suhu yang berbeza, tidak ada pertumbuhan dan bioluminesen dipancarkan pada suhu 4 dan 40 ° C. Bioluminesen paling terang pada 26 °C. Sementara itu, bagi ujian ketoksikan semakin meningkat kepekatan bahan toksik, ketumpatan sel-sel bakteria berkurangan. Kajian secara keseluruhannya, menunjukkan

bahawa bakteria diidentifikasikan dalam genus *Photobacterium* dan bakteria ini boleh digunakan untuk mengesan bahan pencemar akuatik.