

IDENTIFICATION OF BACTERIA ISOLATED ON TCBS  
AGAR FROM WHITE-LEG SHRIMP *Litopenaeus vannamei*  
CULTURED IN THE WEST COAST OF PENINSULAR MALAYSIA

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Identification of bacteria isolated on TCBS agar from the white leg shrimp *Litopenaeus vannamei* cultured in the west coast of peninsular Malaysia / by Shahzia Shah Rasool.

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**IDENTIFICATION OF BACTERIA ISOLATED ON TCBS AGAR FROM  
THE WHITE LEG SHRIMP *Litopenaeus vannamei* CULTURED IN THE  
WEST COAST OF PENINSULAR MALAYSIA**

**By**

**Shahzia Binti Shah Rasool**

**Research Report submitted in partial fulfillment of  
the requirements for the degree of  
Bachelor of Science (Marine Biology)**

**School of Marine Science and Environment  
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**2014**



SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
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**DECLARATION AND VERIFICATION REPORT**  
**FINAL YEAR RESEARCH PROJECT**

It is hereby declared and verified that this research report entitled **Identification of Bacteria Isolated on TCBS from White-Leg Shrimp *Litopenaeus vannamei* Cultured in the West Coast of Peninsular Malaysia** by **Shahzia binti Shah Rasool**, Matric No. **UK26633** 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

mg	-	miligram
ml	-	mililiter
M	-	molarity
rpm	-	revolutions per minute
mM	-	milimolar
$\mu$ L	-	microliter
TCBS	-	Thiosulphate –Citrate Bile Sucrose
EDTA	-	ethylenediaminetetraacetic acid
DMSO	-	dimethyl sulfoxide
TAE	-	tris-acetate EDTA

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Appendix 1 : Sequences of isolates from 5'-3' and the accession number from BLAST GenBank

## ABSTRACT

In this present study, eight isolates of bacteria associated with marine shrimp aquaculture were collected from the species *Litopenaeus vannamei*. Mysis and water samples were collected at Gertak Sanggul Aquaculture farm and inoculated on Thiosulphate-Citrate-Bile Salts agar (TCBS) for 72 hours at 37 °C . All of the strains were identified by biochemical test which is Gram staining, followed by 16S ribosomal RNA gene sequence analysis. All the isolated were cultured on Thiosulphate-Citrate Bile Sucrose agar which have been accepted as the selective medium for isolation of *Vibrio* species. . The identification of bacteria amplified with BaF, (5'-AGAGTTTGATCCTGGCTCAG-3') and BaR, (5'-GTTTACCTTGTTACGACTT-3') were conducted using BLAST and EZTaxon. The results revealed the absence of *Vibrio* sp in all the water samples and shrimp tissue examined . Instead, five species of *Bacillus* namely *B. amyloliquefaciens*, *B. megatrium*, *B.methylotrophicus*, and *B. vietnamensis* were found, with *B. amyloliquefaciens* being the major species, representing 62.5% of the total bacteria isolated. These *Bacillus* spp. are probably the probiotic bacteria used in *L. vannamei* culture. *Bacillus*, gives a beneficial effect in controlling microbial infections through competition with harmful microorganisms, produce inhibitory compounds or by stimulation of the immune response of the host. It is noted that Gram –positive bacteria such as *Bacillus* sp are able to grow well on TCBS agar and this possibly indicates that TCBS agar is not a reliable selective media for *Vibrio* isolation.

# IDENTIFIKASI BAKTERIA YANG DIKULTUR KE AGAR TCBS DARIPADA *Litopenaeus vannamei* YANG DITERNAK DI BARAT SEMENANJUNG MALAYSIA

## ABSTRAK

Di dalam kajian ini , lapan sampel bakteria yang berkaitan dengan akuakultur udang laut telah dikumpulkan daripada spesis *Litopenaeus Vannamei* . Mysis dan air sampel telah dikumpulkan di ladang Gertak Sanggul Akuakultur dan dikultur pada Tiosulfat - Citrate - Hempedu Garam agar ( TCBS ) selama 72 jam pada 37 ° C . Semua strain telah dikenal pasti oleh ujian biokimia iaitu Gram staining , diikuti oleh 16S ribosom RNA gen analisis urutan. Semua terencil telah dikultur pada Tiosulfat - Citrate hempedu Sukrosa agar yang telah diterima sebagai medium terpilih untuk pengasingan spesies *Vibrio* . Pengenalpastian bakteria dikuatkan dengan BAF , ( 5'-AGAGTTTGATCCTGGCTCAG -3 ') dan BaR , ( 5'- GTTTACCTTGTTACGACTT -3 ') telah dijalankan menggunakan BLAST dan EZTaxon . Keputusan menunjukkan ketiadaan *Vibrio* sp di semua dalam sampel air dan tisu udang yang diperiksa. Sebaliknya, lima spesies *Bacillus* iaitu *B. amyloliquefaciens* , *B. megatrium* , *B.methylotrophicus* , dan *B. vietnamensis* ditemui, dengan *B. amyloliquefaciens* menjadi spesis utama , mewakili 62.5 % daripada jumlah bakteria dikaji. Spesies *Bacillus* ini mungkin adalah daripada bakteria probiotik digunakan dalam akuakultur *L. Vannamei* . *Bacillus* memberikan kesan yang baik dalam mengawal jangkitan mikrob melalui persaingan dengan mikroorganisma yang berbahaya , menghasilkan sebatian yg melarang atau rangsangan gerak balas imun tuan rumah. Didapati juga bahawa bakteria Gram- positif seperti *Bacillus* sp dapat tumbuh dengan baik di TCBS agar dan ini mungkin menunjukkan bahawa TCBS agar bukan media terpilih boleh dipercayai untuk pengasingan spesies *Vibrio*.