



UNIVERSITI PUTRA MALAYSIA

**UTILISATION OF LOCAL *PENICILLIUM* SPP. IN CONSORTIUM WITH
BACILLUS SPP. AS BIOREMEDIATORS FOR SHRIMP CULTURE**

MURNI MARLINA BT ABD KARIM

IB 2008 4

**UTILISATION OF LOCAL *PENICILLIUM* SPP. IN CONSORTIUM WITH *BACILLUS*
SPP. AS BIOREMEDIATORS FOR SHRIMP CULTURE**

MURNI MARLINA BT ABD KARIM

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

MAY 2008



**UTILISATION OF LOCAL *PENICILLIUM* SPP. IN CONSORTIUM WITH *BACILLUS*
SPP. AS BIOREMEDIATORS FOR SHRIMP CULTURE**

MURNI MARLINA BT ABD KARIM

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfillment of the Requirement for the Degree of Master of Science**

August 2008



Specially dedicated to my parents for their unconditional love and support



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

UTILISATION OF LOCAL *PENICILLIUM* SPP. IN CONSORTIUM WITH *BACILLUS* SPP. AS BIOREMEDIATORS FOR SHRIMP CULTURE

By

Murni Marlina Bt Abd Karim

May 2008

Chairman: Professor Mohamed Shariff Mohamed Din, PhD

Faculty : Institute of Bioscience

Shrimp aquaculture industry is suffering from severe disease outbreaks, environmental degradation and poor management practices. This project was undertaken to investigate the use of *Penicillium* isolates as bioremediation consortium with potential *Bacillus* spp. for economical and environmental-friendly clean-up of shrimp culture tank water, maintenance of good water quality, biocontrol against pathogenic vibrios and enhancement of shrimp production in shrimp hatchery with zero water exchange.

Two potential *Penicillium* spp. S6 and S48 were originally isolated from sediment samples. S6 was collected from Sungai Dina while S48 was collected from Teluk Adang, Johor. The *Penicillium* isolates were identified up to genus level based on colony morphology and were coded as *Penicillium* sp. S6 and



Penicillium sp. S48. The *Penicillium* species S6 and S48 showed no inhibitory effect towards *B. pumilus*, *B. subtilis* and *B. licheniformis* and no mycotoxins were detected when the isolates were run on thin-layer chromatography against vomitoxin, aflatoxin B1, B2, G1 and G2 standard. The S6 colony produced amylase enzymes while S48 produced four types of major extracellular enzymes viz., amylase, protease, lipase and gelatinase.

In a preliminary biocontrol experiment using disc diffusion methods, S6 showed a significant inhibitory effect on the growth of the pathogenic vibrios tested. Both potential isolates passed the non-pathogenicity test against shrimp postlarvae (PL15). Preliminary ammonia reduction experiment showed that S6 in its mycelial form and S48 in the spore forms reduced the total ammonia nitrogen (TAN) concentration better in the flasks. A cocktail of microorganisms containing S6 and S48 could reduce ammonia significantly than other cocktails when combination of *Penicillium* spp. (S6 and S48) and *Bacillus* spp. was tested. Results revealed that a microorganism cocktail containing S6 reduced ammonia significantly higher ($p < 0.05$) than other combination of isolates.

Hatchery tanks containing PL 15 to 36 grown for 3 weeks and treated with combination of *Penicillium* spp. (S6 and S48) showed the highest survival rate (41.17%) compared to other treatments. The TAN concentration of the hatchery tank treated with a combination of *Penicillium* spp. (S6 and S48) with final concentration of 0.721 mg l^{-1} and tanks treated with *Penicillium* sp. S6 (final

concentration 0.829 mg l^{-1}) also showed significant reduction of TAN compared to control tanks (final concentration 2.153 mg l^{-1}), at 21 days of growth.

The PL grown in *Penicillium* sp. S6 tanks and microorganism cocktail tanks (*Penicillium* spp. and *Bacillus* spp.) showed better stress tolerance (90%) compared to other treatments and control tanks (67%). Vibrio counts were significantly lower in tanks treated with *Bacillus* spp. ($p < 0.05$) compared to other treatments. In addition, the vibrio counts for tanks treated with *Penicillium* sp. S6 also shown significant reduction ($p < 0.05$) and good specific growth rate (15.32%) compared to the control (11.41%). Results showed that selected *Penicillium* spp. satisfied the criteria to qualify as bioremediation agent in marine shrimp culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGUNAAN *PENICILLIUM* SPP. TEMPATAN KONSORTIUM DENGAN
BACILLUS SPP. SEBAGAI BIOREMEDIATOR UNTUK TERNAKAN UDANG**

Oleh

Murni Marlina Bt Abd Karim

Mei 2008

Pengerusi: Professor Mohamed Shariff Mohamed Din, PhD

Fakulti: Institute of Bioscience

Industri akuakultur udang sedang menderita teruk disebabkan wabak penyakit, penurunan alam sekitar dan amalan pengurusan yang tidak baik. Projek ini telah dijalankan untuk mengkaji penggunaan isolat *Penicillium* konsortium dengan *Bacillus* spp. yang berpotensi sebagai bioremediasi yang ekonomi, mesra alam sekitar dan membersihkan air kolam udang, mengekalkan kualiti air yang baik, biokawalan pada patogenik vibrios dan meningkatkan pengeluaran udang tanpa penukaran air.

Dua potensi *Penicillium* spp. S6 dan S48 telah di pencilkan daripada sedimen. S6 dipencilkan dari Sungai Dina manakala S48 dari Teluk Adang, Johor. Isolat telah dikenalpasti sehingga paras genus berdasarkan morfologi dan dikodkan sebagai *Penicillium* sp. S6 dan *Penicillium* sp. S48. *Penicillium* S6 dan S48 menunjukkan tiada kesan perencatan pada bakteria gabungan iaitu *B. pumilus*, *B. subtilis* dan *B. licheniformis*. dan tiada pengeluaran mycotoxins dikesan pada



isolat *Penicillium* apabila di uji dengan kromatografi lapisan nipis menggunakan vomitoxin, aflatoksin B1, B2, G1 sebagai piawaian. Koloni S6 merembeskan enzim amilase manakala S48 menrembeskan empat jenis enzim luar sel iaitu amilase, protease, lipase dan gelatinase.

Ujikaji biokawalan di makmal menggunakan kaedah cakera resapan, S6 menunjukkan rencatan ke atas pertumbuhan patogenik vibrios. Kedua-dua isolat diuji tidak patogenik kepada udang (PL15). Ujikaji makmal bagi penurunan ammonia menunjukkan S6 dalam bentuk mycelial dan S48 bentuk spora adalah lebih baik dalam menurunkan TAN. Koktel mikroorganisma yang mengandungi S6 dan S48 menurunkan ammonia lebih signifikan daripada koktel *Penicillium* sp. (S6 and S48) dan *Bacillus* spp. Keputusan menunjukkan koktel mikroorganisma yang mengandungi S6 dapat menurunkan kepekatan ammonia dengan signifikan ($p < 0.05$) berbanding gabungan isolat lain.

Tangki-tangki yang mengandungi PL 15 hingga PL36 di besarkan selama 3 minggu dan dikultur dengan gabungan *Penicillium* spp. (S6 dan S48) membuktikan kadar kemandirian tertinggi (41.17%) berbanding dengan rawatan lain. Kepekatan TAN dalam tangki yang dikultur dengan kombinasi *Penicillium* spp. (S6 dan S48) mempunyai kepekatan akhir 0.721 mg l^{-1} dan tangki-tangki dikultur dengan *Penicillium* sp. S6 kepekatan akhir (0.829 mg l^{-1}) juga menunjukkan penurunan TAN yang signifikan dibandingkan dengan tangki kawalan (akhir 2.153 mg l^{-1}), selepas 21 hari pengkulturan.

Postlarva dikulturkan dalam tangki dengan *Penicillium* sp. S6 dan tangki koktel (*Penicillium* spp. dan *Bacillus* spp.) menunjukkan tekanan toleransi lebih baik (90%) berbanding dengan rawatan lain dan tangki kawalan (67%). Bilangan vibrio direncat dengan signifikan dalam tangki yang dikultur dengan *Bacillus* spp. ($p < 0.05$) berbanding tangki rawatan yang lain. Selain itu, bilangan vibrio untuk tangki *Penicillium* sp. S6 turut menunjukkan perencatan yang signifikan ($p < 0.05$) dan kadar pertumbuhan tentu baik (15.32%) yang signifikan berbanding dengan kawalan (11.41%). Keputusan menunjukkan *Penicillium* spp. memenuhi kriteria bagi melayakkan mereka sebagai ejen bioremediasi dalam pembiakan udang laut.



ACKNOWLEDGEMENTS

All the praise and admiration for Allah, the Almighty, Beneficial and the most Merciful, who has enabled me to submit this thesis.

I would like to express sincere gratitude to everybody who helped me to get through this three and halves years. This feeling of grateful and blessed goes to my supervisor, Professor Dato' Dr. Mohamed Shariff Mohamed Din for his enthusiasm towards my work, for giving fruitful suggestions and ideas, untiring support both morally and financially, motivation, helpful comments, suggestions and encouragement.

Many thanks go to my co-supervisors, Dr. Seri Intan Mokhtar for the optimistic views on my work when things get tougher, her counseling and constant encouragement has helped me a lot during my study. Not to forget Professor Dr. Fatimah Md. Yusoff and Professor Dr. Faridah Abdullah for their invaluable advice on my research and for providing all the expertise and critical suggestions that I required.

Special thanks go to the staffs and students of Aquatic Animal Health Unit, Noraini, Azrin, Dr. Lee, Azmi, Zainal, Kak Azah, Dr. Sanjoy, Rashidah, Syahril, Safura, Wan and Dora for their technical assistance, support and friendship. My heartiest appreciation also goes to all my dearest friends, Suriana, Dzarifah,



Zarirah, Ina Salwany, Izza, Adilah, and Hanim for their unconditional friendship, love and prayers.

My sincere respect to my family (Papa, Mak, Kak Long, Kakak, Ayu) who I owed the most for their patience, understanding and support throughout the long and winding road of my master study. I am totally indebted to Zulakmal Marzuki for standing with me through good times and bad times.

This work was sponsored by MOSTI, whom I like to express my gratitude for the financial support.



I certify that an Examination committee met on 16 October 2008 to conduct the final examination of Murni Marlina binti Abd Karim on her Master of Science thesis entitled “Utilisation Of Local *Penicillium* spp. in Consortium With *Bacillus* spp. as Bioremediators for Shrimp Culture” 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 Examination Committee were as follows:

Chairman

Abdul Rani Bahaman, PhD

Professor

Faculty of Veterinary Science,
Universiti Putra Malaysia

Suriani Abd Aziz, PhD

Assoc. Professor

Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mariana, PhD

Professor

Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Claude E. Boyd, PhD

Professor

College of Agriculture
Auburn University, USA
(External Examiner)

HASANAH MOHD GHAZALI, Ph.D.

Professor/Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date :



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Dato' Mohamed Shariff Mohamed Din, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Fatimah Md. Yusoff, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Faridah Abdullah, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Seri Intan Mokhtar, PhD

Researcher
SIRIM Berhad
(Member)

AINI IDERIS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :



DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MURNI MARLINA BT ABD KARIM

Date: 24 September 2008



TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	lx
APPROVAL	
DECLARATION	xi
LIST OF TABLES	xii
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
CHAPTERS	
1 GENERAL INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 <i>Penaeus monodon</i>	8
2.2 The economic importance of shrimp industry	10
2.3 Sensitivity of <i>Penaeus monodon</i> culture to water quality	12
2.4 Problems associated with <i>Penaeus monodon</i>	
2.4.1 Water and sediment quality in <i>Penaeus monodon</i> culture	14
2.4.2 Toxicity of ammonia and nitrite to shrimp	17
2.4.3 Vibriosis in shrimp larvae culture	18
2.5 Bioremediation as a viable alternative technology for pollution treatment	22
2.5.1 Bioremediation	23
2.5.2 Fundamental approaches in bioremediation	25
2.5.3 Bioremediation in aquaculture	27
2.6 Fungi	29
2.6.1 Bioremediation and extracellular enzymes	32
2.6.2 Mycotoxins	35
2.6.3 Fungi as potential bioremediation agent	36
2.6.4 The Significance of the Use of Microbial Products in Aquaculture	38
3 SCREENING, ISOLATION AND IDENTIFICATION OF SELECTED <i>PENICILLIUM</i> ISOLATES AS BIOREMEDIATION AGENTS	
3.1 Introduction	42
3.2 Materials and methods	



3.2.1	Isolation and identification of potential <i>Penicillium</i> isolates	44
3.2.2	Interaction effect of <i>Bacillus</i> and <i>Penicillium</i> isolates	49
3.2.3	Screening of mycotoxin gene using thin layer chromatography (TLC)	52
3.2.4	Secretion of extracellular enzymes	55
3.3	Results	
3.3.1	Sampling, isolation and identification	58
3.3.2	Growth inhibition of <i>Bacillus</i> by <i>Penicillium</i> isolates	59
3.3.3	Screening of mycotoxin by thin layer chromatography	65
3.3.4	Screening of extracellular enzymes production from fungal isolates	67
3.4	Discussion	73
4	BIOCONTROL OF PATHOGENIC <i>VIBRIO</i> SPP. BY <i>PENICILLIUM</i> SPP.	
4.1	Introduction	80
4.2	Materials and methods	
4.2.1	Fungal strains and culture conditions	82
4.2.2	Source of pathogenic vibrios	82
4.2.3	Media preparation	83
4.2.4	Diffusion disc method	83
4.3	Results	
4.3.1	Inhibition by diffusion disc method	84
4.4	Discussion	86
5	USE OF INDIVIDUAL AND CONSORTIUM OF <i>PENICILLIUM</i> AND <i>BACILLUS</i> SPECIES FOR IMPROVING WATER QUALITY AND SURVIVAL OF SHRIMP LARVAE	
5.1	Introduction	89
5.2	Materials and methods	
5.2.1	Microorganisms	91
5.2.2	Experimental animals	92
5.2.3	Measurement of total ammonia-nitrogen (TAN) concentrations	92
5.2.4	Degradation of TAN by fungal mycelia and spores during preliminary studies	94
5.2.5	Degradation of TAN consortium of <i>Penicillium</i> spp. and <i>Bacillus</i> spp. during preliminary studies	96
5.2.6	Pathogenicity of fungal isolates against <i>P. monodon</i> postlarvae during preliminary studies in flask	98



5.2.7	Evaluation on the potential use of <i>Penicillium</i> and <i>Bacillus</i> isolates as a bioremediator for <i>P.monodon</i> cultured in hatchery trial	100
5.3	Results	
5.3.1	Degradation of TAN in flask treated with individual bioremediator such as <i>Penicillium</i> S6, S48 and <i>Bacillus</i> spp. in preliminary studies	109
5.3.2	Degradation of TAN in flask treated with combination of potential bioremediator <i>Penicillium</i> spp. S6, S48 and <i>Bacillus</i> spp. in preliminary studies	111
5.3.3	Preliminary studies on percentage survival of <i>P. monodon</i> PLs treated with potential bioremediator <i>Penicillium</i> spp. S6 and S48 in conical flasks	113
5.3.4	Physical properties, total ammonia nitrogen, total plate count and survival percentage of <i>P.monodon</i> PL treated with <i>Penicillium</i> S6, S48 and <i>Bacillus</i> spp. as bioremediators in hatchery tanks	114
5.4	Discussion	124
6	GENERAL DISCUSSION AND CONCLUSION	131
	REFERENCES	138
	APPENDICES	160
	BIODATA OF THE STUDENT	164
	PUBLICATIONS AND AWARDS	165



LIST OF TABLES

Table		Page
1	Water quality parameters for penaeid shrimp grow-out ponds.	16
2	Summary of recent findings on biodegradation of a variety of pollutants by fungi. The efficiency of pollutant biodegradation depended on both the type of pollutant and the fungus involved in the process.	34
3	Number of selected fungal isolates for interaction study between <i>Bacillus</i> and <i>Penicillium</i> isolates	50
4	Number of non-inhibitory fungal isolates for screening of mycotoxin.	53
5	Number of fungal colonies isolated from water and sediment samples in various sampling location in Malaysia.	60
6	Clear zone or 'halo' (mm) formed due to protein degradation by extracellular enzymes produced by <i>Penicillium</i> sp. S6 and <i>Penicillium</i> sp. S48 culture grown for 5 days at 25 °C.	67
7	Pathogenic vibrios used in biocontrol experiments.	82
8	Inhibition zones diameter (mm) formed by <i>Penicillium</i> S6 against mat culture of vibrios grown on NA plates incubated at 30 °C for 5 days.	84
9	Summary of the experimental design for preliminary study on degradation of TAN by individual <i>Penicillium</i> isolates in conical flasks.	95
10	Summary of the experimental design for preliminary study on degradation of TAN by consortium of <i>Penicillium</i> and <i>Bacillus</i> isolates in conical flasks.	97
11	Summary of the experimental design for preliminary study of pathogenicity of <i>Penicillium</i> spp. S6 and S48 against <i>P. monodon</i> (PL16).	99
12	Experimental design to evaluate the pathogenicity of <i>Penicillium</i> spp. (S6 & S48) and <i>Bacillus</i> spp. (<i>B. subtilis</i> , <i>B. pumilus</i> and <i>B. licheniformis</i>) on <i>P. monodon</i> postlarvae.	101



13	Standardized feeding for 1 million <i>P. monodon</i> larvae reared in the hatchery tank for 21 days.	104
14	Physical water quality parameters taken in all the tanks throughout the experiment.	114
15	Total plate count, TPC (cfu/ml) of seawater samples in all tanks during the experiment.	119
16	<i>Vibrio</i> spp. count (cfu/ml) of seawater samples in all tanks during the experiment.	120



LIST OF FIGURES

Figure		Page
1	Map of Malaysia showing the sampling sites for water and sediment collection for isolation of marine indigenous fungi.	45
2	(a) Mature surface of fungus isolate S6 from Sg. Dina sediment on PDA and (b) typical conidial head and mycelium (10x magnification). Colony is yellowish on PDA.	61
3	(a) Mature surface of isolate fungus (S48) from Teluk Adang sediment on PDA and (b) typical conidial head and mycelium (10x magnification). Colony is greenish on PDA.	62
4	No inhibition of <i>Bacillus</i> spp. by <i>Penicillium</i> S6 on NA plates with lawn of a) <i>Bacillus subtilis</i> ; b) <i>Bacillus pumilus</i> ; and c) <i>Bacillus licheniformis</i> . The cultures were grown at 30 °C and observed for 5 days.	63
5	No inhibition of <i>Bacillus</i> spp. by <i>Penicillium</i> S48 on NA plates with lawn of a) <i>Bacillus subtilis</i> ; b) <i>Bacillus pumilus</i> ; and c) <i>Bacillus licheniformis</i> . The cultures were grown at 30 °C and observed for 5 days.	64
6	Schematic presentation for determination of TLC silica plate showing the retention factor (R _f) value of fungal broth isolates.	66
7	Clear zone due to production of extracellular proteases which degrade protein was only visible on skim milk agar after 5 days incubation at 25 °C with a) <i>Penicillium</i> sp. S48 and not b) <i>Penicillium</i> sp. S6.	69
8	Clear zone due to production of extracellular lipases which hydrolyse fat in the trybutyrin was only visible on trybutyrin agar after 5 days incubation at 25 °C with a) <i>Penicillium</i> sp. S48 and not b) <i>Penicillium</i> sp. S6.	70
9	Clear zone due to production of extracellular amylases which degrade starch was only visible on starch agar after 5 days incubation at 25 °C with a) <i>Penicillium</i> sp. S48 and not b) <i>Penicillium</i> sp. S6.	71

10	Clear zone due to production of extracellular gelatinase which degrade gelatin was only visible on gelatin agar after 5 days incubation at 25 °C with a) <i>Penicillium</i> sp. S48 and not b) <i>Penicillium</i> sp. S6.	72
11	Growth inhibition of <i>Vibrio</i> spp. by <i>Penicillium</i> S6 on NA plates with a) <i>Vibrio alginolyticus</i> (Vam 11); b) <i>Vibrio parahaemolyticus</i> (VpM1); and c) <i>Vibrio harveyi</i> (Vhl). The cultures were grown at 30 °C for 5 days.	85
12	Summary of methodology from the sampling of isolates and going through various stages of screening before the isolates were tested in hatchery trial.	108
13	Total ammonia-nitrogen (mg l ⁻¹) concentration in flasks treated with individual isolates during preliminary studies. The readings were obtained from C=control, S6 (m)= <i>Penicillium</i> sp. S6 (mycelia), S6 (s)= <i>Penicillium</i> sp. S6 (spore), S48(s)= <i>Penicillium</i> sp. S48 (spore) and S48(m)= <i>Penicillium</i> sp. S48 (mycelia) grown in seawater at room temperature (25-30 °C), at salinity 30 ppt. shaken at 100 rpm.	110
14	Total ammonia-nitrogen (mg l ⁻¹) concentration in flasks treated with combination isolates during preliminary studies. The readings were obtained from C=control, S6+B= <i>Penicillium</i> sp. S6+ <i>Bacillus</i> spp., S48+B= <i>Penicillium</i> sp. S48+ <i>Bacillus</i> spp., S6+S48= <i>Penicillium</i> sp. S6+ <i>Penicillium</i> sp. S48, S6+S48+B= <i>Penicillium</i> sp. S6+ <i>Penicillium</i> sp. S48+ <i>Bacillus</i> spp. grown in seawater at room temperature (25-30 °C), at salinity 30 ppt. shaken at 100 rpm.	112
15	Survival percentage of <i>P. monodon</i> in the flask containing sterile seawater with salinity 30 ppt and continuous aeration for 5 days treated with <i>Penicillium</i> spp. S6 and S48.	113
16	TAN concentration (mg l ⁻¹) measured from culture filtrate treated with C=control, B= <i>Bacillus</i> spp., F= <i>Penicillium</i> S6 (mycelia), FF= <i>Penicillium</i> sp. S6 (mycelia)+ <i>Penicillium</i> sp. S48 (spore) and FB= <i>Penicillium</i> sp. S6 (mycelia)+ <i>Penicillium</i> sp. S48 (spore)+ <i>Bacillus</i> spp. in hatchery tanks with <i>P.monodon</i> grown at 27-28 °C, salinity 31-32 ppt, dissolve O ₂ 6.0-6.5 mg L ⁻¹ and pH 7.0-8.0.	117



- 17 Survival rate of the *P. monodon* postlarvae after treatment with *Bacillus* spp., S6=*Penicillium* S6 (mycelia), S6+S48=*Penicillium* sp. S6 (mycelia)+*Penicillium* sp. S48 (spore) and B+F=*Penicillium* sp. S6 (mycelia)+*Penicillium* sp. S48 (spore)+*Bacillus* spp. and C=control at 27-28 °C, salinity 31-32 ppt, dissolved O₂ 6.0-6.5 mgL⁻¹ and pH 7.0-8.0 in hatchery tanks with seawater. 117
- 18 Reverse salinity stress test to determine the survival rate of *P. monodon* postlarvae after treatment with B=*Bacillus* spp., S6=*Penicillium* S6 (mycelia), S6+S48=*Penicillium* sp. S6 (mycelia)+*Penicillium* sp. S48 (spore) and B+F= *Penicillium* sp. S6 (mycelia)+*Penicillium* sp. S48 (spore)+*Bacillus* spp. and C=control grown in hatchery tanks with seawater at 27-28 °C, salinity 31-32 ppt, dissolved O₂ 6.0-6.5 mgL⁻¹ and pH 7.0-8.0. 121
- 19 Specific growth rate of the *P. monodon* postlarvae after treatment with B=*Bacillus* spp., S6=*Penicillium* S6 (mycelia), S6+S48=*Penicillium* sp. S6 (mycelia)+*Penicillium* sp. S48 (spore) and B+F= *Penicillium* sp. S6 (mycelia)+*Penicillium* sp. S48 (spore)+*Bacillus* spp. and C=control grown in hatchery tanks at 27-28 °C, salinity 31-32 ppt, dissolved O₂ 6.0-6.5 mgL⁻¹ and pH 7.0-8.0 with seawater. 122



LIST OF ABBREVIATIONS

$(\text{NH}_4)_2\text{SO}_4$	Anhydrous ammonium sulphate
AAHU	Aquatic Animal Health Unit
ANOVA	Analysis of variance
BOD	Biological oxygen demand
Cfu	Colony forming units
DDW	Double distilled water
DO	Dissolved oxygen
DW	Distilled water
hr	Hour
L	Liter
NaNO_2	Anhydrous sodium nitrite
NH_3^+	Ammonia
$\text{NH}_3\text{-N}$	Ammonia-nitrogen
NH_4^+	Ammonium
NO_2^-	Nitrite
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PL	Postlarvae
ppt	Parts per thousand
rpm	Rotation per minute
SAS	Statistical analysis system
SGR	Specific growth rate



TAN	Total ammonia nitrogen
TCBS	Thiosulphate citrate bile salt sucrose
TLC	Thin layer chromatography
TPC	Total plate count
TSA	Trypticase soy agar
TSB	Trypticase soy broth
UPM	Universiti Putra Malaysia
VaM11	<i>Vibrio alginolyticus</i> Malaysia 11
VhI	<i>Vibrio harveyi</i> Indonesia
VpM1	<i>Vibrio parahaemolyticus</i> Malaysia 1

