UNIVERSITI PUTRA MALAYSIA

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

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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

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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 andS48) 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 mgl^{-1} and tanks treated with *Penicillium* sp. S6 (final

concentration 0.829 mgl^{-1}) also showed significant reduction of TAN compared to control tanks (final concentration 2.153 mgl^{-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

PENGGUNAAN *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

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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 berpontensi sebagai bioremediasi yang ekonomi, mesra alam sekitar dan membersihkan air kolam udang, mengekalkan kualiti air yang baik, biokawalan pada patogenik vibrios dan meningkatan 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 menunjukan 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 $0.721 mgl^{-1}$ dan tangki-tangki dikultur dengan *Penicillium* sp. S6 kepekatan akhir (0.829 mgl-1) juga menunjukkan penurunan TAN yang signifikan dibandingkan dengan tangki kawalan (akhir 2.153 mgl 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.

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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:

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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:

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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.

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MURNI MARLINA BT ABD KARIM

Date: 24 September 2008

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

$(NH_4)_2SO_4$	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
NH_3-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	*Vibrio alginolyticus* Malaysia 11
VhI	*Vibrio harveyi* Indonesia
VpM1	*Vibrio parahaemolyticus* Malaysia 1