UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF MICROBIAL-FORTIFIED RICE STRAW COMPOST FOR AEROBIC RICE CULTIVATION

NG LEE CHUEN

ITA 2012 3
DEVELOPMENT OF MICROBIAL-FORTIFIED RICE STRAW COMPOST FOR AEROBIC RICE CULTIVATION

By

Ng Lee Chuen

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

August 2012
DEDICATION

To my beloved parents, husband, daughter, sister and brothers for their inspiration, love and support
Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

DEVELOPMENT OF MICROBIAL-FORTIFIED RICE STRAW COMPOST FOR AEROBIC RICE CULTIVATION

By

NG LEE CHUEN

August, 2012

Chairperson: Professor Sariah Meon, PhD

Institute: Institute of Tropical Agriculture

Rice production consumes lots of water. Due to increasing water scarcity, research on aerobic rice cultivation has been intensified. Poor root anchorage, unavailable phosphorus (P), low soil organic matter and blast disease caused by Pyricularia oryzae are the major constraints of aerobic rice cultivation systems.

A study was carried out with the aim to inoculate commercially prepared rice straw compost (RSC) with a consortium of microbes in order to produce a microbial-fortified product, and evaluate its bio-efficacy on rice blast severity, growth, yield and soil health of rice variety M4 using an aerobic cultivation system. Four bacterial isolates [Pseudomonas aeruginosa (P1), Corynebacterium agropyri (P7), Enterobacter gergoviae (P9) and Bacillus amyloliquefaciens (S3)] and two Trichoderma spp. [(Trichoderma harzianum (T1) and Trichoderma virens (T2)] isolated from rice rhizospheres have been isolated and selected based on in vitro tests for the production of indole-acetic acid (IAA), siderophore, chitinase, volatile compounds, abilities to solubilize
phosphate and suppress mycelia growth of *P. oryzae*. These isolates were proven to be compatible as a mixed culture. *Enterobacter gergoviae*, *B. amyloliquefaciens*, *T. harzianum* and *T. virens* had significantly increased seed germination and seedling establishment, due to their ability to produce IAA and solubilize phosphate. Seedlings pre-inoculated with *P. aeruginosa*, *C. agropyri* and *T. harzianum* have higher production of peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase corresponding to lower incidence of blast disease under greenhouse conditions. Total microbial activity based on fluorescein diacetate (FDA) hydrolysis measured in rhizosphere soil was positively correlated ($r = 0.76$, $P = 0.04$) to the seedling vigor index. These isolates were further inoculated into commercial rice straw compost (RSC) as carriers. The stability and the survival of the introduced microbes were evaluated based on total culturable plate count and FDA hydrolysis in storage up to 38 weeks. The viability of all the introduced bacteria in the microbial-fortified RSC in both sterilized and non-sterilized conditions decreased with time of storage. At week 38th, in sterilized RSC, all introduced bacteria was detected at $1.00 \log_{10} \text{cfu/g}$ and none of the introduced bacteria was detected in unsterilized RSC. The viability of *T. harzianum* and *T. virens* remained constant ($5.48–7.78 \log_{10} \text{cfu/g}$) throughout the storage period and was associated with the better colonization and proliferation due to no competition from the indigenous *Trichodema* spp. A greenhouse experiment indicated that soil amended with the microbial-fortified RSC significantly improved yield (1768.42, 1052.00 and 2233.33%) and decreased rice blast severity based on AUDPC (area under disease progress curve) (74.52, 86.31 and 86.70%) compared to the control (mineral soil alone),
when *P. oryzae* inoculated at 14, 56 and 80 DAS (days after sowing), respectively. In soil amended with microbial-fortified RSC also improved soil total microbial activity (4.49 µg/g/0.5h in control; 7.32 µg/g/0.5h in soil amended with microbial-fortified RSC) at harvest, as well as physico-chemical properties included soil EC, bulk density and moisture content. Microbial-fortified RSC introduced during soil preparation was effective in reducing rice blast severity, promoting plant growth, yield and improving soil health for rice variety M4 under aerobic cultivation system.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PEMBANGUNAN KOMPOS JERAMI PADI YANG DIPERKAYAKAN DENGAN MIKROB UNTUK PENANAMAN PADI AEROBIK

Oleh

NG LEE CHUEN

Ogos, 2012

Pengerusi: Profesor Sariah Meon, PhD
Institut : Institut Pertanian Tropika

Tanaman padi adalah tanaman yang memerlukan air yang banyak. Seiring dengan peningkatan kebimbangan terhadap kekurangan air, penyelidikan ke atas penanaman padi aerobik telah pun dipergiatkan. Kelemahan cengkaman akar pokok, ketersediaan fosforus (P) dan bahan organik yang rendah dalam tanah bersamaan dengan masalah penyakit karah yang disebabkan oleh Pyricularia oryzae menjadi halangan utama dalam tanaman padi aerobik. Kajian ini bertujuan menghasilkan kompos jerami padi yang diperkayakan dengan mikrob melalui inokulasi konsortium mikrob ke dalam kompos komersial, dan menilai keberkesanan biologinya terhadap kawalan penyakit karah, pertumbuhan, hasil and kesihatan tanah pada padi varieti M4 yang ditanam dengan sistem aerobik. Empat bakteria [Pseudomonas aeruginosa (P1), Corynebacterium agropyri (P7), Enterobacter gergoviae (P9) dan Bacillus amyloliquefaciens (S3)] dan dua Trichoderma spp. [(T. harzianum (T1) dan T. virens (T2)] telah dipencilkan dan dipilih daripada rizosfera padi berdasarkan
ujian *in vitro* terhadap penghasilan indole-asetik asid (IAA), siderofor, kitinase

dan sebatian mudah merupa, keupayaan untuk melarutkan fosfat dan menyekat

dan pertumbuhan miselia *P. oryzae*. Pencilan telah dibuktikan serasi untuk dijadikan

terekam. *Enterobacter gergoviae, B. amyloliquefaciens, T. harzianum*


dan *T. virens* telah menunjukkan peningkatkan secara signifikan dalam

ischebahan biji benih dan pertumbuhan awal anak benih berdasarkan

keupayaan menghasilkan IAA dan melarutkan fosfat. Anak benih pra-inokulasi

dengan *P. aeruginosa, C. agropyri* dan *T. harzianum* pula menunjukkan

pengeluaran peroksidase, polifenol oksidase dan fenilalanine ammonia-liase yang

lebih tinggi sepadan dengan kejadian penyakit karah yang lebih rendah

dalam keadaan rumah hijau. Jumlah aktiviti mikrob berasaskan hidrolisis
diasetat pendarfluor (FDA) pada rizosfera berkorelasi secara positif (*r* = 0.76, *P*

= 0.04) dengan indek kecergasan anak benih. Pencilan selanjutnya

ditambahkan ke dalam kompos jerami padi (KJP) komersial yang berfungsi

sebagai pembawa. Kestabilan KJP yang diperkayakan dengan mikrob dan

diterkaitkan dengan kemandirian mikrob yang diperkenalkan telah dinilai berdasarkan jumlah kiraan

koloni yang tumbuh dan hidrolisis FDA setelah menyimpan selama 38 minggu.

Pada minggu ke-38, pada KJP yang telah disteril, kesemua bakteria yang

diperkenalkan telah dikesan dengan 1.00 log$_{10}$ cfu/g dan tiada bakteria yang

diperkenalkan dikesan dalam KJP tanpa disteril. Kebernasan *T. harzianum* dan

*T. virens* didapati berkekalan (5.48–7.78 log$_{10}$ cfu/g) sepanjang tempoh

simpanan adalah berkaitan dengan kolonisasi and proliferasi yang lebih baik

akibat tanpa persaingan dengan spesies *Trichoderma* asli. Kajian di rumah hijau

menunjukkan tanah yang ditambahkan dengan KJP yang diperkayakan dengan
mikrob menunjukkan peningkatan hasil (1768.42, 1052.00 dan 2233.33%) dan pengurangan penyakit berdasarkan KBLPP (kawasan di bawah lengkung perkembangan penyakit) (74.52, 86.31 dan 86.70%) secara signifikan berbanding dengan kawalan (tanah mineral sahaja) apabila inokulatan dengan *P. oryzae* dilakukan pada 14, 56 dan 80 HLT (hari lepas tabur). Penambahan KJP yang diperkayakan dengan mikrob juga meningkatkan jumlah aktiviti mikrob (4.49 µg/g/0.5h pada kawalan; 7.32 µg/g/0.5h pada tanah yang ditambah dengan KJP yang diperkayakan dengan mikrob) dan menambahbaik sifat-sifat fizik-kimia tanah termasuk kekonduksian elektrik, ketumpatan pukal dan kandungan air. Penyediaan tanah bersama dengan KJP yang diperkayakan dengan mikrob berkesan untuk mengurangkan penyakit karah, menggalakkan pertumbuhan, hasil (t/ha) dan kesihatan tanah pada tanaman padi varieti M4 yang ditanam dengan sistem aerobik.
ACKNOWLEDGEMENTS

I wish to express my deepest and sincere gratitude to Prof. Dr. Sariah Meon, the chairman of my supervisory committee for her dedicated effort, support, intellectual guidance throughout the entire period of research and preparation of this dissertation.

I would also thank my committee members Assoc. Prof. Dr. Zainal Abidin Mior Ahmad, Assoc. Prof. Dr. Radziah Othman and Dr. Sariam Othman for the constructive comments, advices and helps at various stages of my research. My sincere appreciation to the Universiti Malaysia Terengganu and the Ministry of Higher Education Malaysia through a doctoral fellowship, the Ministry of Science, Technology and Innovation (MOSTI) for the research grant administered through the science fund of MOA and also to the Institute of Tropical Agriculture and the Faculty of Agriculture, Universiti Putra Malaysia for providing the research facilities.

My grateful thanks to numerous colleagues, friends and laboratory officers of the Plant Pathology, Plant Physiology and Soil Science departments for their excellent assistance and co-operation.

Finally, my heartfelt thanks go to my beloved parents, husband and daughter for their love, understanding, patience and spiritual support during this research.
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follow:

**Sariah Meon, PhD**  
Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**Radziah Othman, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Zainal Abidin Mior Ahmad, PhD**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**Sariam Othman, PhD**  
Malaysian Agricultural Research and Development Institute  
(External member)

__________________________________________

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td>ACKNOWLEDGEMENTS</td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td>APPROVAL</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>DECLARATION</td>
<td>xii</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xix</td>
</tr>
<tr>
<td></td>
<td>LIST OF PLATES</td>
<td>xxiii</td>
</tr>
<tr>
<td></td>
<td>LIST OF ABBREVIATIONS</td>
<td>xxiv</td>
</tr>
<tr>
<td></td>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>8</td>
</tr>
<tr>
<td>2.1</td>
<td>Rice</td>
<td></td>
</tr>
<tr>
<td>2.1.1</td>
<td>Economic Importance</td>
<td>8</td>
</tr>
<tr>
<td>2.1.2</td>
<td>Challenges in Rice Production</td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Aerobic Rice Cultivation</td>
<td>13</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Potential of Aerobic Rice</td>
<td>13</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Risk and Limitations of Aerobic Rice</td>
<td>14</td>
</tr>
<tr>
<td>2.3</td>
<td>Rice Blast Disease</td>
<td>15</td>
</tr>
<tr>
<td>2.3.1</td>
<td><em>Pyricularia oryzae</em></td>
<td>15</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Disease Symptoms</td>
<td>17</td>
</tr>
<tr>
<td>2.3.3</td>
<td>Favorable Environmental Conditions</td>
<td>18</td>
</tr>
<tr>
<td>2.3.4</td>
<td>Infection Mechanisms</td>
<td>19</td>
</tr>
<tr>
<td>2.3.5</td>
<td>Distribution and Economic Impact</td>
<td>20</td>
</tr>
<tr>
<td>2.3.6</td>
<td>Control Measures</td>
<td>21</td>
</tr>
<tr>
<td>2.4</td>
<td>Agricultural Wastes Management</td>
<td>27</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Rice Wastes Management</td>
<td>27</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Advantages of Bio-compost</td>
<td>32</td>
</tr>
<tr>
<td>2.5</td>
<td>Plant Growth Promoting Microorganisms and Mechanisms</td>
<td>34</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Indole-acetic Acid Production</td>
<td>35</td>
</tr>
<tr>
<td>2.5.2</td>
<td>Phosphate Solubilization</td>
<td>37</td>
</tr>
<tr>
<td>2.5.3</td>
<td>Siderophore Production</td>
<td>39</td>
</tr>
<tr>
<td>2.5.4</td>
<td>Chitinase Production</td>
<td>41</td>
</tr>
<tr>
<td>2.5.5</td>
<td>Antibiotics Production</td>
<td>42</td>
</tr>
</tbody>
</table>

xiii
3 ISOLATION AND SCREENING OF RHIZOSPHERE AND PHYLLOSPHERE MICROORGANISMS FROM RICE FOR PLANT GROWTH PROMOTION AND PYRICULARIA ORYZAE CONTROL

3.1 Introduction 46
3.2 Materials and Methods 48
  3.2.1 Isolation and Pathogenicity Testing of *Pyricularia oryzae* 48
  3.2.2 Isolation of Rhizosphere Soil, Root and Phyllosphere Microorganisms 50
  3.2.3 *In vitro* Screening of Plant Growth-promoting and Antagonistic Activities 53
  3.2.4 Compatibility Testing 59
  3.2.5 Identification and Selection of Potential Microorganisms 60

3.3 Results 64
  3.3.1 Isolation and Pathogenicity Testing of *Pyricularia oryzae* 64
  3.3.2 Isolation of Rhizosphere Soil, Root and Phyllosphere Microorganisms 67
  3.3.3 *In vitro* Screening for Plant Growth-promoting and Antagonistic Activities 70
  3.3.4 Compatibility Testing 75
  3.3.5 Identification and Selection of Potential Isolates 76

3.4 Discussion 81
3.5 Conclusion 88

4 EFFICACY OF PGPM ON EARLY RICE SEEDLING ESTABLISHMENT AND ANTAGONISTIC ACTIVITY AGAINST PYRICULARIA ORYZAE

4.1 Introduction 90
4.2 Materials and Methods 92
  4.2.1 Effect of PGPM on Early Establishment of Rice Seedlings 92
  4.2.2 Evaluation of Rice Blast Disease Development 97
  4.2.3 Evaluation of Defense Related Enzymatic Activities on *Pyricularia oryzae* Challenged Rice Seedlings 98
  4.2.4 Determination of Total Microbial Activity in Rhizosphere Soil 100
  4.2.5 Experimental Design and Data Analysis 102

4.3 Results 103
  4.3.1 Evaluation of PGPM on Early Establishment of Rice Seedlings 103
  4.3.2 Evaluation of Rice Blast Disease Development 111
  4.3.3 Evaluation of Defense Related Enzymatic Activities on *Pyricularia oryzae* Challenged Rice Seedlings 114
  4.3.4 Total Microbial Activity in Rhizosphere Soil 117

4.4 Discussion 118
4.5 Conclusion 123
DEVELOPMENT AND STABILITY OF THE MICROBIAL-FORTIFIED RICE STRAW COMPOST

5.1 Introduction 125
5.2 Materials and Methods 127
  5.2.1 Determination of Compost Maturity 127
  5.2.2 Determination of Physico-chemical and Microbiological Properties of Rice Straw Compost 131
  5.2.3 Preparation of Microbial-fortified Rice Straw Compost 135
  5.2.4 Evaluation the Stability of Microbial-fortified Rice Straw Compost 137
  5.2.5 Experimental Design and Data Analysis 139
5.3 Results 140
  5.3.1 Compost Maturity 140
  5.3.2 Physico-chemical and Microbiological Properties of Rice Straw Compost 141
  5.3.3 Stability of Microbial-fortified Rice Straw Compost 144
5.4 Discussion 154
5.5 Conclusion 163

6 BIO-EFFICACY OF MICROBIAL-FORTIFIED RICE STRAW COMPOST ON RICE BLAST DISEASE SEVERITY, GROWTH, YIELD AND SOIL HEALTH OF AEROBIC RICE

6.1 Introduction 164
6.2 Materials and Methods 166
  6.2.1 Plant Materials and Growth Conditions 166
  6.2.2 Inoculum Preparation and Disease Assessment 167
  6.2.3 Evaluation of Plant Growth-promoting Traits 169
  6.2.4 Determination of Physico-chemical and Microbiological Properties of Soil Treatments 170
  6.2.5 Experimental Design and Data Analysis 172
6.3 Results 173
  6.3.1 Correlation of Rice Blast Severity, Growth and Yield 173
  6.3.2 Estimation of Growth Performance, Yield Loss and Disease Development 184
  6.3.3 Evaluation of Physico-chemical and Microbiological Properties of Soil Treatments 191
6.4 Discussion 196
6.5 Conclusion 202

7 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

REFERENCES R.1
APPENDICES A.1
BIODATA OF STUDENT B.1
LIST OF PUBLICATIONS L.1