UNIVERSITI PUTRA MALAYSIA

EVALUATION OF POTENTIAL PROBIONTS FROM MARINE SHELLFISH FOR BLACK TIGER SHRIMP Penaeus monodon (FABRICIUS,1978) LARVICULTURE AGAINST VIBRIOSIS

NUR JASMIN MOHD YAMINUDIN

FP 2017 61

EVALUATION OF POTENTIAL PROBIONTS FROM MARINE SHELLFISH FOR BLACK TIGER SHRIMP *Penaeus monodon* (FABRICIUS, 1978) LARVICULTURE AGAINST VIBRIOSIS

By

NUR JASMIN MOHD YAMINUDIN

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

March 2017

DEDICATION

To my beloved father and mother

En. Mohd Yaminudin bin Yahaya and Pn. Rozimah bt Md. Hashim

Family members:

Nurul Syafiqah binti Mohd Yaminudin

Safwan Hadi bin Mohd Yaminudin

Nur Fatin Ameera binti Mohd Yaminudin

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

EVALUATION OF POTENTIAL PROBIONTS FROM MARINE SHELLFISH FOR BLACK TIGER SHRIMP *Penaeus monodon* (FABRICIUS, 1978) LARVICULTURE AGAINST VIBRIOSIS

By

NUR JASMIN MOHD YAMINUDIN

March 2017

Chair: Murni Marlina Abd Karim, PhD
Faculty: Agriculture

Penaeid shrimp farming is one of the major contributors in annual world shrimp production. However, vibriosis has been one of the main diseases problems which often cause high mortality and decrease of production. Probiotics are now a leading new alternative in preventing vibriosis in *Penaeus monodon* culture. This study was carried out to discover new potential probiotics strain isolated from shellfish (shrimp and oyster) for tiger shrimp, *Penaeus monodon* larviculture culture. Total of 144 isolates of bacteria were successfully isolated from the intestine, hind gut and stomach of *P. monodon* and 136 isolates from shell, gills and digestive gland of oyster *Crassostrea iredalei.* In *in vitro* screening tests using series of plate assays, co-culture assay and pathogenicity test on TCBS agar, one isolate from shrimp and oysters each, showed potential as probiotics.

Two potential probionts were identified as *Bacillus megaterium* (I24, from shrimp) and *Exiguobacterium acetylicum* (S66, from oyster) using 16S rRNA. *Bacillus* I24 able to excrete lipases, proteases and amylase meanwhile *Exiguobacterium* S66 was positive in excreting amylase and lipases only. Both potential probionts were able to produce biofilm, slightly reduced haemolysis and did not showed anti-quorum sensing properties.

A preliminary *in vivo* assay for the two potential probionts were carried out on *Artemia salina* challenged with *Vibrio harveyi* and *Vibrio alginolyticus.* Results demonstrated a significant survival of *Artemia* after treated with probiont *Bacillus* I24 at 10^8 CFU mL^{-1} and challenged with *V. harveyi* (73.33±0.88%) and *V. alginolyticus* (98.33±0.33%) compared to the control *Artemia* with pathogen only (*V. harveyi* 23.33±0.23%, *V. alginolyticus* 36.67±0.33%). Moreover, *Artemia* treated with *Exiguobacterium* S66, at concentration 10^8 CFU mL^{-1} also showed high

survival (95±0.58%) after challenged with *V. harveyi* (*V. harveyi* only 23.33±0.33%). There was a significant reduction of vibrios in *Artemia* treated with *Bacillus* I24 and *Exiguobacterium* S66 at 10^8 CFU mL^{-1} compared to the control group.

Similar results were observed in *in vivo* test by using *P. monodon* postlarvae (PL) as a host. Postlarvae treated with *Bacillus* I24 at 10^6 CFU mL^{-1} had significant high survival after challenged with the pathogens *V. harveyi* (88.33±1.45%) and *V. alginolyticus* (78.33±1.33%) at 10^6 CFU mL^{-1}. Meanwhile, PL treated with *Exiguobacterium* S66 at concentration 10^6 CFU mL^{-1} showed significant high survivals (93.33±0.88%) after challenged with *V. harveyi.* Vibrios counts were significantly lower in PL and culture water treated with *Bacillus* I24 and *Exiguobacterium* S66 at the end of challenged assay. Specific growth rate in terms of weight and length were shown to be higher in larvae treated with both potential probionts compared to control groups with vibrios only. Results indicate PL treated with potential probionts had faster growth rate.

This research suggests that *Bacillus* I24 and *Exiguobacterium* S66 have potential to be commercialized as probiotics for aquaculture industry specifically for shrimp culture.

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

PENILAIAN CALON PROBIOTIK YANG DIPENCIL DARIPADA KERANG KERANGAN UNTUK TERNAKAN UDANG HARIMAU *Penaeus monodon* (FABRICIUS, 1978) TERHADAP VIBRIOSIS

Oleh

NUR JASMIN MOHD YAMINUDIN

Mac 2017

Pengerusi: Murni Marlina Abd Karim, PhD
Fakulti: Pertanian

Ternakan udang Penaeid adalah salah satu penyumbang utama dalam pengeluaran tahunan udang dunia. Walau bagaimanapun, vibriosis telah menjadi salah satu masalah penyakit utama yang sering menyebabkan kematian yang tinggi dan mengurangkan hasil pengeluaran. Probiotik kini merupakan alternatif baru dalam mencegah vibriosis di dalam kultur *Penaeus monodon.* Kajian ini telah dijalankan untuk mencari potensi probiotik baru yang dipencil daripada kerangan (udang dan tiram) untuk kultur larva udang harimau. Sebanyak 144 bakteria telah berjaya dipencil daripada usus, usus belakang dan perut *P. monodon* dan 136 pencilan dari permukaan kerang, insang dan saluran pencernaan tiram. Di dalam beberapa ujian saringan *in vitro* serta ujian kepatogenan menggunakan agar TCBS, satu bakteria daripada udang dan satu daripada tiram menunjukkan potensi sebagai probiotik.

Kedua bakteria tersebut telah dikenal pasti menggunakan 16S rRNA sebagai *Bacillus megaterium* (I24, dari udang) dan *Exiguobacterium acetylicum* (S66, dari tiram). *Bacillus* I24 mampu menghasilkan enzim lipases, protease dan amilase Sementara *Exiguobacterium* S66 positif dalam menghasilkan amilase dan lipase sahaja. Kedua bakteria probiotik ini dapat menghasilkan biofilm, dapat mengurangkan sedikit hemolisis tetapi tidak mempunyai ciri-ciri *anti-quorum sensing*

Ujian *in vivo* oleh kedua bakteria probiotik telah dijalankan ke atas *Artemia salina* dan mencabar dengan *Vibrio harveyi* dan *Vibrio alginolyticus*. Keputusan menunjukkan kemandirian *Artemia* yang tinggi selepas dirawat dengan probiotik *Bacillus* I24 10^8 CFU mL^{-1} dan dicabar dengan *V. harveyi* (73.33 ± 0.88%) dan *V. alginolyticus* (98.33 ± 0.33%) berbanding dengan *Artemia* dengan pathogen sahaja (*V. harveyi* 23.33±0.33%, *V. alginolyticus* 36.67±0.33%). Sementara itu *Artemia*

yang dirawat dengan *Exiguobacterium* S66, pada kepekatan 10^8 CFU mL^{-1} juga menunjukkan tahap kemandirian yang tinggi (95 ± 0.58%) selepas dicabar dengan *V. harveyi* (23.33±0.33). Terdapat juga pengurangan ketara bilangan bakteria vibrios dalam *Artemia* yang dirawat dengan *Bacillus* I24 dan *Exiguobacterium* S66 10^8 CFU mL^{-1} berbanding dengan kumpulan kawalan.

Keputusan yang sama diperhatikan di dalam ujian *in vivo* dengan menggunakan larva *P. monodon* sebagai perumah. Larva yang dirawat dengan *Bacillus* I24 10^6 CFU mL^{-1} menunjukkan kadar hidup yang paling tinggi selepas dicabar dengan patogen *V. harveyi* (88.33 ± 1.45%) dan *V. alginolyticus* (78.33 ± 1.33%) pada kepekatan 10^6 CFU mL^{-1}. Larva yang dirawat dengan *Exiguobacterium* S66 pada kepekatan 10^6 CFU mL^{-1} menunjukkan tahap kemandirian yang paling tinggi (93.33 ± 0.88%) apabila dicabar dengan *V. harveyi*. Bilangan vibrios adalah lebih rendah dalam larva dan air yang dirawat dengan *Bacillus* I24 dan *Exiguobacterium* S66. Kadar pertumbuhan yang khusus dari segi berat badan dan panjang menjadi lebih tinggi dalam larva yang dirawat dengan *Bacillus* I24 dan *Exiguobacterium* S66.

Keputusan menunjukkan *Bacillus* I24 dan *Exiguobacterium* S66 berpotensi untuk menjadi probiotik di dalam industri akuakultur dan dikomersialkan khusus untuk kultur udang.

ACKNOWLEDGEMENT

First of all, I would like to express my gratitude to Allah SWT for giving me the opportunities, grace and health to further and accomplish my Master studies

I would like to express my deepest gratitude to my dearest supervisor, Dr. Murni Marlina Abd Karim for her continuous support, guidance, advices and help, throughout the experiment period. I also want to thank her, for spending her precious time to read, refine my writing and review each of my method used in this experiment. This research would not be complete by Allah will and her kind cooperation.

I also want to thank my co – supervisors; Dr. Ina Salwany Md Yasin and Assoc. Prof. Dr. Hassan Mohd Daud for their continuing advices and helps. Special thanks to staff and Fish Health labmates, Puan Shafika, Cik Fiza, Fathiah, Zeti, Azrin, Ifa, Tasya and others for their patience, valuable time and information which helped me in completing my master. Not to forget my close friends, Sofea, Zalikha and Puvaneswari who keep supporting me throughout the hard time.

Lastly, thanks to my family for always standing behind me and keep on encouraging me through my ups and downs to finish this thesis successfully. This is for them.

I certify that a Thesis Examination Committee has met on 29 March 2017 to conduct the final examination of Nur Jasmin binti Mohd Yaminudin on her thesis entitled "Evaluation of Potential Probionts from Marine Shellfish for Black Tiger Shrimp *Penaeus monodon* (Fabricius, 1978) Larviculture Against Vibriosis" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Annie Christianus, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Natrah Fatin binti Mohd Ikhsan, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Lee Seong Wei, PhD
Associate Professor
Universiti Malaysia Kelantan
Malaysia
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 6 July 2017

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

Murni Marlina Abd Karim, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Ina Salwany Md Yasin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Hassan Hj. Mohd. Daud, PhD
Associate Professor
Faculty of Veterinary medicine
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Nur Jasmin Mohd Yaminudin/ GS38647

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Member of Supervisory Committee: Murni Marlina Abd Karim

Signature: _____
Name of Member of Supervisory Committee: Ina Salwany Md Yasin

Signature: _____
Name of Member of Supervisory Committee: Hassan Hj Mohd Daud

TABLE OF CONTENTS

		Page
ABSRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENT		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xiii
LIST OF FIGURES		xvii
LIST OF ABBREVIATION/SYMBOLS		xxii
CHAPTER		
1	**INTRODUCTION**	
1.1	Background studies	1
1.2	Problem statement	2
1.3	Significant of study	3
1.4	Objectives of study	3
1.5	Hypothesis of study	4
2	**LITERATURE REVIEW**	5
2.1	*Penaeus monodon*	5
2.1.1	Taxonomy	5
2.1.2	Development and distribution	6
2.2	Status of *Penaeus monodon* culture	7
2.3	Problems associated with *Penaeus monodon* culture	9
2.4	Vibriosis in aquaculture	10
2.5	Management of diseases problems	11
2.5.1	Water quality management	11
2.5.2	Feed management	12
2.5.3	Treatment using chemicals and antibiotics	13
2.6	Environmentally method: Probiotics in aquaculture	14
2.6.1	Definition of probiotics	14
2.6.2	Modes of action of probiotics	15
2.6.2.1	Competitive exclusion	15
2.6.2.2	Source of nutrients and enzymatic contribution to digestion	16
2.6.2.3	Improvement of water quality	16
2.6.2.4	Enhance immune response	17
2.7	Properties of probiotics	18
2.7.1	Extracellular enzyme production	18
2.7.2	Ability to attach and form biofilm	18

2.7.3 Anti-quorum sensing 20
2.7.4 Inhibition of hemolytic activity of *Vibrio* spp. 21
2.8 Previous research on probiotics 21

3 ISOLATION, IDENTIFICATION AND SCREENING OF POTENTIAL PROBIONTS ISOLATED FROM SHELLFISH 25

3.1 Introduction 25
3.2 Materials and methods 27
3.2.1 Isolation of potential probionts from shellfish; tiger shrimp (*Penaeus monodon*) and slipper cupped oyster (*Crassostrea iredalei*) 27
3.2.1.1 Samples collection 27
3.2.1.2 Preparation of media 27
3.2.1.3 Glycerol stock preparation 28
3.2.1.4 Isolation of potential probionts 28
3.2.1.5 Subculture of bacterial colonies 28
3.2.2 *In vitro* screening of potential probionts 29
3.2.2.1 Bacterial isolates and pathogens 29
3.2.2.2 Broth culture 29
3.2.2.3 Spot assay 29
3.2.2.4 Colony on top assay 29
3.2.2.5 Elimination of pathogenic probionts 29
3.2.2.6 Minimal inhibition of pathogens by potential probionts 30
3.2.2.7 Co-culture assay 30
3.2.3 Identification of potential probionts 30
3.2.3.1 Morphology observation 30
3.2.3.2 Gram staining technique 31
3.2.3.3 Polymerase Chain Reaction (PCR) 31
3.2.4 Statistic analysis 33
3.3 Results 34
3.4 Discussion 50
3.5 Conclusion 52

4 INVESTIGATION ON *Bacillus megaterium* I24 AND *Exiguobacterium acetylicum* S66 PROPERTIES AS PROBIONTS 53

4.1 Introduction 53
4.2 Materials and methods 55
4.2.1 Extracellular enzymes production 55
4.2.1.1. Probiont cultures 55
4.2.1.2 Amylase test 55
4.2.1.3 Gelatinase test 55
4.2.1.4 Lipase test 55
4.2.1.5 Proteases test 55
4.2.2 Biofilm production 56

4.2.3 Inhibition of hemolytic activity of *Vibrio* species 56
4.2.4 Anti quorum sensing (QS) procedure 56
4.2.5 Statistic analysis 57
4.3 Results 57
4.4 Discussion 67
4.5 Conclusion 69

5 PROTECTION EFFECT OF POTENTIAL PROBIONTS ON *Artemia salina* NAUPLII AND BLACK TIGER SHRIMP (*Penaeus monodon*) POSTLARVAE AGAINST VIBRIOSIS IN *IN VIVO* CHALLENGE ASSAY 70

5.1 Introduction 69
5.2 Materials and Methods 72
5.2.1 Probionts cultures 72
5.2.2 Preliminary *in vivo* assay using *Artemia* 72
5.2.2.1 *Artemia* culture 72
5.2.2.2 Experimental design 72
5.2.2.3 Survival and vibrios count 73
5.2.3 Safety test of potential probionts on *Penaeus monodon* postlarvae 73
5.2.3.1 Experimental design 73
5.2.4 *Penaeus monodon* postlarvae challenge 74
5.2.4.1 Experimental design 74
5.2.4.2 Survival and vibrios count 75
5.2.5 Growth Rate 75
5.2.6 Statistical analysis 75
5.3 Results 76
5.4 Discussion 94
5.5 Conclusion 97

6 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH 98

6.1 General Discussion 98
6.2 Conclusion 104
6.3 Recommendation for future research 104

REFERENCES 106
APPENDICES 126
BIODATA OF STUDENT 129
LIST OF PUBLICATIONS 130

LIST OF TABLES

Table		Page
2.1	Production of *Penaeus monodon* in Malaysia from Year 2012 to 2015 (Source: DOF Malaysia, 2016)	9
2.2	Summary of previous research on probiotics in aquaculture	23
3.1	Primers sequences used for PCR amplification	32
3.2	Volume in microliters (µl) of master mix to perform PCR.	33
3.3	Total isolates obtained from 20 tiger shrimp *Penaeus monodon*	34
3.4	Total isolates obtained from 20 slipper cupped oyste*r Crassostrea iredalei*	34
3.5	Diameter of inhibition zone ± colony size by potential probionts from *Penaeus monodon* against *Vibrio harveyi* and *Vibrio alginolyticus* in spot assay	35
3.6	Diameter of inhibition zone ± colony size by potential probionts from *Crassostrea iredalei* against *Vibrio harveyi* and *Vibrio alginolyticus* in spot assay	37
3.7	Diameter of inhibition zone ± colony size by potential probionts from *Penaeus monodon* against *Vibrio harveyi* and *Vibrio alginolyticus* in colony on top assay	38
3.8	Diameter of inhibition zone ± colony size by potential probionts from *Crassostrea iredalei* against *Vibrio harveyi* and *Vibrio alginolyticus* in colony on top assay	39
3.9	The growth of potential probionts isolated from *Penaeus monodon* on Thiosulphate Citrate-Bile Salt (TCBS).	40
3.10	The growth of potential probionts isolated from *Crassostrea iredalei* on Thiosulphate Citrate-Bile Salt (TCBS)	41
3.11	Morphology characteristics of potential probionts	47
3.12	Identification of potential probionts strain I24 and S66 with region gene sequences accession no. based on NCBI gene bank	49
4.1	Absorbance (OD) of biofilm production from 0 to 70 hour	61
4.2	Hemolysis zones by pathogens and potential probionts diameter	64

(mm)

4.3 Summary on the properties of potential probionts *Bacillus megaterium* I24 and *Exiguobacterium acetylicum* S66. (+) indicate positive and (-) results 66

5.1 Treatment in *Artemia* challenge test 72

5.2 Treatment used in safety test assay 74

5.3 Treatment of *Penaeus monodon* PL15 challenge test 74

5.4 Survival of *Artemia* after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Different alphabet indicates significant differences among treatments ($p<0.05$) 77

5.5 Survival of *Artemia* after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio alginolyticus* (VAL) after 7 days of observation. Different alphabet indicates significant differences among treatments ($p<0.05$) 78

5.6 *Vibrio* counts in *Artemia* after pre-treated at different concentrations of *Bacillus* I24 (10^4, 10^6, 10^8 CFU mL^{-1}) and challenged with 10^5 CFU mL^{-1} of *Vibrio harveyi.* Different alphabet indicates significant differences among treatments ($p<0.05$) 78

5.7 *Vibrio* counts in *Artemia* after pre-treated at different concentrations of *Bacillus* I24 (10^4, 10^6, 10^8 CFU mL^{-1}) and challenged with 10^5 CFU mL^{-1} of *Vibrio alginolyticus*. Different alphabet indicates significant differences among treatments ($p<0.05$) 79

5.8 Survival of *Artemia* after pre-treated with probiont *Exiguobacterium* S66 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Different alphabet indicates significant differences among treatments ($p<0.05$) 80

5.9 *Vibrio* counts in *Artemia* after pre-treated at different concentrations of *Exiguobacterium* S66 (10^4, 10^6, 10^8 CFU mL^{-1}) and challenged with 10^5 CFU mL^{-1} of *Vibrio harveyi.* Different alphabet indicates significant differences among treatments ($p<0.05$) 81

5.10 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$) 84

5.11 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio alginolyticus* (VAL) after 7 days of observation. Different alphabet indicates significant differences among treatments ($p<0.05$) 85

5.12 *Vibrio* counts in culture water after pre-treated at different concentrations of *Bacillus* I24 (10^4, 10^6 CFU mL^{-1}) and challenged with 10^6 CFU mL^{-1} of *Vibrio harveyi.* Different alphabet indicates significant differences among treatments ($p<0.05$) 86

5.13 *Vibrio* counts in *Penaeus monodon* PL15 after pre-treated at different concentrations of *Bacillus* I24 (10^4, 10^6 CFU mL^{-1}) and challenged with 10^6 CFU mL^{-1} of *Vibrio harveyi.* Different alphabet indicates significant differences among treatments ($p<0.05$) 86

5.14 *Vibrio* counts in culture water after pre-treated at different concentrations of *Bacillus* I24 (10^4, 10^6 CFU mL^{-1}) and challenged with 10^6 CFU mL^{-1} of *Vibrio alginolyticus.* Different alphabet indicates significant differences among treatments ($p<0.05$) 87

5.15 *Vibrio* counts in *Penaeus monodon* PL15 after pre-treated at different concentrations of *Bacillus* I24 (10^4, 10^6 CFU mL^{-1}) and challenged with 10^6 CFU mL^{-1} of *Vibrio alginolyticus.* Different alphabet indicates significant differences among treatments ($p<0.05$) 87

5.16 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Exiguobacterium* S66 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Different alphabet indicates significant differences among treatments ($p<0.05$) 88

5.17 *Vibrio* counts in culture water after pre-treated at different concentrations of *Exiguobacterium* S66 (10^4, 10^6, 10^8 CFU mL^{-1}) and challenged with 10^6 CFU mL^{-1} of *Vibrio harveyi.* Different alphabet indicates significant differences among treatments ($p<0.05$) 89

5.18 *Vibrio* counts in *Penaeus monodon* PL15 after pre-treated at different concentrations of *Exiguobacterium* S66 (10^4, 10^6, 10^8 CFU mL^{-1}) and challenged with 10^6 CFU mL^{-1} of *Vibrio harveyi.* Different alphabet indicates significant differences among treatments ($p<0.05$) 89

LIST OF FIGURES

Figure		Page
2.1	Black tiger shrimp *Penaeus monodon* (Source: Food and Agriculture Organization FAO, 2015)	5
2.2	Taxonomy of black tiger shrimp *Penaeus monodon*	6
2.3	Shrimp aquaculture world production from 2007- 2016 and expected production in 2017 (Source of data: Global Aquaculture Advocate, 2016)	8
3.1	Maps of Malaysia showing the location of Pulau Sayak, Kedah and Kuala Muda, Selangor	27
3.2	Clear zones of potential probionts isolated from intestine of *Penaeus monodon* against (A) *Vibrio harveyi* and (B) *Vibrio alginolyticus* in *in vitro* spot assay	36
3.3	Clear zone of potential probionts isolated from the outer shell of *Crassostrea iredalei* against (A) *Vibrio harveyi* and (B) *Vibrio alginolyticus* in *in vitro* spot assay	36
3.4	Clear zones of potential probionts isolated from intestine of *Penaeus monodon* against (A) *Vibrio harveyi* and (B) *Vibrio alginolyticus* and in *in vitro* colony on top assay	38
3.5	Clear zone of potential probionts isolated from the outer shell of *Crassostrea iredalei* against (A) *Vibrio harveyi* and (B) *Vibrio alginolyticus* in *in vitro* colony on top assay	39
3.6	Inhibition of *Vibrio alginolyticus* by potential probiont I24 at different concentrations after 24 hr of pre incubation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T2 (I24 10^8 CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T3 (I24 10^7 CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T4 (I24 10^6 CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1}),T5 (I24 10^5CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T6 (I24 10^4 CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T7 (I24 10^3 CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T8 (I24 10^8 CFU mL^{-1}+*Vibrio alginolyticus* 10^2 CFU mL^{-1}), T9 (I24 10^1 CFU mL^{-1}+*Vibrio alginolyticus* 10^5 CFU mL^{-1})	42
3.7	Inhibition of *Vibrio harveyi* by potential probiont I24 at different concentrations after 24 hr of pre incubation. Error	43

bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (*Vibrio harveyi* 10^5 CFU mL^{-1}), T2 (I24 10^8 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T3 (I24 10^7 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T4 (I24 10^6 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T5 (I24 10^5CFU mL^{-1}+*Vibrio harveyi s* 10^5 CFU mL^{-1}), T6 (I24 10^4 CFU mL^{-1}+*Vibrio harveyi s* 10^5 CFU mL^{-1}), T7 (I24 10^3 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T8 (I24 10^8 CFU mL^{-1}+*Vibrio harveyi* 10^2 CFU mL^{-1}), T9 (I24 10^1 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1})

3.8 Inhibition of *Vibrio harveyi* by potential probiont S66 at different concentrations after 24 hr of pre incubation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$) T1 (*Vibrio harveyi* 10^5 CFU mL^{-1}), T2 (S66 10^8 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T3 (S66 10^7 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T4 (S66 10^6 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T5 (S66 10^5CFU mL^{-1}+*Vibrio harveyi s* 10^5 CFU mL^{-1}), T6 (S66 10^4 CFU mL^{-1}+*Vibrio harveyi s* 10^5 CFU mL^{-1}), T7 (S66 10^3 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}), T8 (S66 10^8 CFU mL^{-1}+*Vibrio harveyi* 10^2 CFU mL^{-1}), T9 (S66 10^1 CFU mL^{-1}+*Vibrio harveyi* 10^5 CFU mL^{-1}) 44

3.9 Growth pattern of *Vibrio alginolyticus* incubated with different concentrations of potential probiont I24 (10^4, 10^6, 10^8 CFU mL^{-1}) against time. T1 (*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T2 (I24 10^4 CFU mL^{-1}+ *Vibrio alginolyticus* 10^5 CFU mL^{-1}), T3 (I24 10^6 CFU mL^{-1}+ *Vibrio alginolyticus* 10^5 CFU mL^{-1}), T4 (I24 10^8 CFU mL^{-1}+ *Vibrio alginolyticus* 10^5 CFU mL^{-1}). Different alphabet indicates significant differences among treatments ($p<0.05$) 45

3.10 Growth pattern of *Vibrio harveyi* incubated with different concentrations of potential probiont I24 (10^4, 10^6, 10^8 CFU mL^{-1}) against time. T1 (*Vibrio harveyi* 10^5 CFU mL^{-1}), T2 (I24 10^4 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}), T3 (I24 10^6 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}), T4 (I24 10^8 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}). Different alphabet indicates significant differences among treatments ($p<0.05$) 46

3.11 Growth pattern of *Vibrio harveyi* incubated with different concentrations of potential probiont S66 (10^4, 10^6, 10^8 CFU mL^{-1}) against time. T1 (*Vibrio harveyi* 10^5 CFU mL^{-1}), T2 (S66 10^4 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}), T3 (S66 10^6 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}), T4 (S66 10^8 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}). Different alphabet indicates significant differences among treatments ($p<0.05$) 47

3.12	Potential probiont I24 under light microscope (40x)	48
3.13	Potential probiont S66 under light microscope (40x)	48
3.14	Agarose gel electrophoresis analysis of PCR amplification of the 16S rRNA gene of isolate I24 and S66 using 16S rRNA gene primer combination	49
4.1	Clear zone around bacteria indicates presence of amylase enzyme (A) *Exiguobacterium acetylicum* S66 (B) *Bacillus megaterium* I24	57
4.2	Semi solid gelatinase agar indicates the absence of gelatinase enzymes to hydrolyze gelatin (A) *Bacillus megaterium* I24 and (B) *Exiguobacterium acetylicum* S66.	58
4.3	Clear zone around bacteria indicates presence of lipases enzyme (A) *Exiguobacterium acetylicum* S66 (B) *Bacillus megaterium* I24	59
4.4	Clear zone around bacteria indicates presence of proteases enzyme (A) *Bacillus megaterium* I24 (B) *Exiguobacterium acetylicum* S66	59
4.5	Comparison of biofilm production for *Bacillus* I24, *Exiguobacterium* S66 with pathogen *Vibrio alginolyticus* and *Vibrio harveyi*. Biofilm formation was measured every 10 hours interval. Different alphabet indicates significant differences among treatments ($p<0.05$)	62
4.6	Crystal violet stain indicate presence of biofilm	63
4.7	Hemolysis on 24 Hour. (A) *Vibrio alginolyticus* 10^5 CFU mL^{-1} (B) *Vibrio harveyi* 10^5 CFU mL^{-1}	63
4.8	Absent of inhibition zone around purple area indicate the potential probionts *Bacillus* I24 and *Exiguobacterium* S66 did not display quorum sensing property.	65
5.1	Survival of *Artemia* after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Vibrio harveyi* 10^5 CFU mL^{-1}), T3 (*Bacillus* I24 10^4 CFU mL^{-1}), T4 (*Bacillus* I24 10^6 CFU mL^{-1}), T5 (*Bacillus* I24 10^8 CFU mL^{-1}), T6 (*Bacillus* I24 10^4 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}), T7 (*Bacillus* I24 10^6 CFU mL^{-1} + *Vibrio harveyi* 10^5 CFU mL^{-1}) and T8 (*Bacillus* I24 10^8 CFU mL^{-1} + *Vibrio harveyi* 10^5 CFU mL^{-1})	76

5.2 Survival of *Artemia* after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio alginolyticus* (VAL) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Vibrio alginolyticus* 10^5 CFU mL^{-1}), T3 (*Bacillus* I24 10^4 CFU mL^{-1}), T4 (*Bacillus* I24 10^6 CFU mL^{-1}), T5 (*Bacillus* I24 10^8 CFU mL^{-1}), T6 (*Bacillus* I24 10^4 CFU mL^{-1}+ *Vibrio alginolyticus* 10^5 CFU mL^{-1}), T7 (*Bacillus* I24 10^6 CFU mL^{-1} + *Vibrio alginolyticus* 10^5 CFU mL^{-1}) and T8 (*Bacillus* I24 10^8 CFU mL^{-1} + *Vibrio alginolyticus* 10^5 CFU mL^{-1}) 77

5.3 Survival of *Artemia* after pre-treated with probiont *Exiguobacterium* S66 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Vibrio harveyi* 10^5 CFU mL^{-1}), T3 (*Exiguobacterium* S66 10^4 CFU mL^{-1}), T4 (*Exiguobacterium* S66 10^6 CFU mL^{-1}), T5 (*Exiguobacterium* S66 10^8 CFU mL^{-1}), T6 (*Exiguobacterium* S66 10^4 CFU mL^{-1}+ *Vibrio harveyi* 10^5 CFU mL^{-1}), T7 (*Exiguobacterium* S66 10^6 CFU mL^{-1} + *Vibrio harveyi* 10^5 CFU mL^{-1}) and T8 (*Exiguobacterium* S66 10^8 CFU mL^{-1} + *Vibrio harveyi* 10^5 CFU mL^{-1}) 80

5.4 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Bacillus* I24 after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Bacillus* I24 10^4 CFU mL^{-1}), T3 (*Bacillus* I24 10^6 CFU mL^{-1}), T4 (*Bacillus* I24 10^8 CFU mL^{-1}) 82

5.5 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Exiguobacterium* S66 after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Exiguobacterium* S66 10^4 CFU mL^{-1}), T3 (*Exiguobacterium* S66 10^6 CFU mL^{-1}), T4 (*Exiguobacterium* S66 10^8 CFU mL^{-1}) 83

5.6 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Bacillus* I24 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control),T5 (*Vibrio harveyi* 10^6 CFU mL-1), T6 (*Bacillus* I24 10^4 CFU mL^{-1}+ *Vibrio harveyi* 10^6 CFU mL^{-1}) and T7 (*Bacillus* I24 10^6 CFU mL^{-1} + *Vibrio harveyi* 10^6 CFU mL^{-1}) 84

5.7 Survival of *Penaeus monodon* PL15 after pre-treated with 85

probiont *Bacillus* I24 and challenged with *Vibrio alginolyticus* (VAL) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T5 (*Vibrio alginolyticus* 10^6 CFU mL^{-1}), T6 (*Bacillus* I24 10^4 CFU mL^{-1}+ *Vibrio alginolyticus* 10^6 CFU mL^{-1}) and T7 (*Bacillus* I24 10^6 CFU mL^{-1} + *Vibrio alginolyticus* 10^6 CFU mL^{-1})

5.8 Survival of *Penaeus monodon* PL15 after pre-treated with probiont *Exiguobacterium* S66 and challenged with *Vibrio harveyi* (VH) after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T5 (*Vibrio harveyi* 10^6 CFU mL^{-1}), T6 (*Exiguobacterium* S66 10^4 CFU mL^{-1}+ *Vibrio harveyi* 10^6 CFU mL^{-1}), T7 (*Exiguobacterium* S66 10^6 CFU mL^{-1} + *Vibrio harveyi* 10^6 CFU mL^{-1}) and T8 (*Exiguobacterium* S66 10^8 CFU mL^{-1} + *Vibrio harveyi* 10^6 CFU mL^{-1}). 88

5.9 Specific Growth Rate of *Penaeus monodon* PL15 after pre-treated with probiont *Bacillus* I24 after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Bacillus* I24 10^4 CFU mL^{-1}), T3 (*Bacillus* I24 10^6 CFU mL^{-1}) 90

5.10 Specific Growth Rate (SGR) of *Penaeus monodon* PL15 after pre-treated with probiont *Exiguobacterium* S66 after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Exiguobacterium* S66 10^4 CFU mL^{-1}), T3 (*Exiguobacterium* S66 10^6 CFU mL^{-1}), T4 (*Exiguobacterium* S66 10^8 CFU mL^{-1}) 91

5.11 Mean length increment of *Penaeus monodon* PL15 after pre-treated with probiont *Bacillus* I24 after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Bacillus* I24 10^4 CFU mL^{-1}), T3 (*Bacillus* I24 10^8 CFU mL^{-1}) 92

5.12 Mean length increment of *Penaeus monodon* PL15 after pre-treated with probiont *Exiguobacterium* S66 after 7 days of observation. Error bars indicate standard error (SE). Different alphabet indicates significant differences among treatments ($p<0.05$). T1 (Control), T2 (*Exiguobacterium* S66 10^4 CFU mL^{-1}), T3 (*Exiguobacterium* S66 10^6 CFU mL^{-1}), T4 (*Exiguobacterium* S66 10^8 CFU mL^{-1}) 93

6.1 Summary on the selection process of probionts *Bacillus* I24 and *Exiguobacterium* S66 103

LIST OF ABBREVIATIONS

VH	*Vibrio harveyi*
VAL	*Vibrio alginolyticus*
PL	Postlarvae
NaCl	Sodium Chloride
TCBS	Thiosulfate citrate bile salt sucrose
TSB	Tryptic soy broth
TSA	Tryptic soy agar
SSW	Sterile seawater
CFU	Colony forming unit
CFU mL^{-1}	Colony forming unit per mililiter
rpm	Revolution per minute
µl	microliter
hr	hour
Kb	Kilobase
bp	basepair
16S rRNA	16 subunit ribosomal ribonucleic acid
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
sp. or spp.	Species (for singular or plural term)
SGR	Specific growth rate
H_2O	Dihydrogen monoxide
QS	Quorum sensing
AHL	N-Acylhomoserine lactone
FCR	Feed Conversion Ratio
TAN	Total Ammonia Nitrate
NH_3	Ammonia
NH_4	Ammonium

CHAPTER 1

INTRODUCTION

1.1 Background of study

World global population is expected to reach 9.6 billion by 2050 (FAO 2012). Aquaculture is one of the fastest growing food producing sectors that contributes to the majority of human food. According to FAO (2015), global aquaculture production in year 2014 was recorded at 73.8 million tonnes where the majority of the productions came from Asian region which contributes 65 million tonnes from the total production.

Shrimp aquaculture is among the major contributor in international markets. Annual world shrimp production is approximately 6 million metric tonnes. The production is expected to expand due to the decreases in natural resources and high demand from the consumers. In 2014, black tiger shrimp (*Penaeus monodon)* is among the major species that received high demand from the consumers (FAO, 2015).

Due to the increases in demands, farmers are obligated to have high production of shrimps which results in high densities culture. However, this practice often leads to disease outbreaks (Rahman *et al.,* 2009). Marine infectious diseases have caused major losses in aquaculture (Lafferty *et al.,* 2015). *Vibrio* spp. is one of the significant bacterial pathogen that affects marine species cultures (Flegel, 2012). This species is a gram negative bacterium, ubiquitous in marine and estuarine ecosystem as well as aquaculture farm and cause vibriosis. Among significant pathogenic *Vibrio* spp. are *Vibrio harveyi, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus* and *Vibrio anguillarum.*

Infectious bacterial diseases in aquaculture are of major concern to the industry and are typically controlled by killing of the pathogen either by doing treatment with antibiotics or chemotherapeutics; or vaccination. Although the prevention of disease by vaccination is increasing, disease control using antibiotics remains the method of choice in many areas. Antimicrobial drugs have been one of the solutions chosen for treatment. Despite its obvious benefit in treating animals infected by bacterial diseases, antimicrobial drugs have been serve as prophylactic (preventive) or for growth enhancement (Van den Bogaard and Stobberingh, 2000). However, the uses of these drugs have risen up the emergence of antibiotic resistance bacteria (Schwarz *et al.*, 2001) which make these bacteria immune to the antibiotic given. There are certain antibiotic resistant bacteria that could pass on the resistance genes to human pathogens which could be a health risking problem to human (Van den Bogaard and Stobberingh, 2000).

Environmentally friendly methods for controlling microbial pathogenesis in aquaculture with the use of probiotic bacteria have gained considerable research interest and becoming increasingly preferred as viable, alternative management practices for disease prevention. In 1965, Lilly and Stillwell stated the original probiotic concept which is protozoans producing substances that stimulated other protozoans. This concept is then improvised by Fuller (1989) when he stated probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance.

The probiotic organisms must not be pathogenic to the host and, furthermore, it is considered advantageous if the probiont is indigenous to the environment in which it will be used. It should also be accepted by the host through ingestion and potential colonization and replication within the host digestive system. The probiont must also work *in vitro* as well as *in vivo* and lastly, it must be free from virulence resistance genes or antibiotic resistance genes (Verschuere *et al.*, 2000).

Bacteria that have been successfully proven to be used as probiotic such as *Bacillus* spp. (Moriarty, 1998; Rengpipat *et al.*, 1998) and *Thalassobacter utilis* (Maeda and Liao, 1992). These bacteria strain were isolated from shrimp culture water (Tanasomwang *et al*., 1998) and from the intestine of different penaeid species (Rengpipat *et al.*, 2000). Gomez-Gil *et al*. (1998) stated that there is wide diversity of bacteria species in the hepatopancreas of healthy *Penaeus vannamei.* Thus, it is interesting to isolate probiotics from the wide diversity bacteria in either culture water or from the host and examine its possibility as potential probiotics for commercial use.

1.2 Problem statement

Black tiger shrimp *Penaeus monodon* was one of the leading species for the previous five years in Malaysia which value USD 160,186. However, productions have been lowered since due to diseases (FAO, 2015). Vibriosis has caused high mortalities and major economic losses to some countries (Estes *et al.*, 2004). This is proven when one of *Vibrio* spp; *V. harveyi* had caused luminescent vibriosis, which contributed to mass mortality in shrimp culture especially *Penaeus monodon* (Lavilla - Pitogo *et al.*, 1990). Wang *et al.* (2015) reported on the luminescent disease outbreaks in *Litopenaeus vannamei* farm in Zhangpu County, Southern China.

In Taiwan, *V. alginolyticus* has been the main causative agent for mass mortality of *L. vannnamei* (Liu *et al.,* 2004). Whereas in Malaysia, Ransangan (2012) reported an outbreaks of vibriosis in seabass *Lates calcarifer* which occur in year 2008 in open cage in Sabah.

Vibriosis is highly infectious to the early stages of shrimp culture and the administration of probiotics appears to be a very promising research area for

nutrition, biocontrol and disease prevention in aquaculture (Balcazar *et al.,* 2006). The role of probiotics bacteria in farming of shrimp was attempted but such studies in hatchery are not much reported especially in black tiger shrimp*, P. monodon* (Soundarapandian and Rabu, 2010). Hence, it is interesting to develop potential probiotic bacteria isolated from the shellfish species (black tiger shrimp, *P. monodon* and slipper cup oyster, *Crassostrea iredalei*) for improvement of shrimp health.

1.3 Significant of the study

Today, probiotics have been widely applied in aquatic farms as a method to control diseases. This environment-friendly approaches is known to be able to produce enzymes which aids in digestion process, provides nutrients, improve water quality and immune system (Qi *et al.,* 2009). Probiotics can be introduced to the host and culture water by various ways and one of them is through feed. This can help to establish balance communities full of indigenous microbes in gastrointestinal tract (Rengpipat, 2005).

Probiotics are considered as the new therapeutic agents and several farmers have already started to use probiotics in their farm to replace chemicals and antibiotics (Tuan *et al.,* 2013). Probiotics have variety of mechanisms to protect host from diseases. Hence, it is important to determine the characteristics of the probiont which will lead to the knowledge on its mechanisms. Furthermore, new local probiotic is needed for black tiger shrimp larviculture to combat vibriosis as foreign probiotics strain have difficulty in adjusting to local environment and usually ended up being pathogenic (Wang *et al.,* 2000).

1.4 Objectives of study

It is essential to find local strain of probiotics that are able to be used in *P. monodon* culture in order to yield high production of healthy shrimp. Hence, the objectives of this research were:

1. To isolate, screen and identify beneficial bacteria as biocontrol agent (probiotics) from adult *Penaeus monodon* and *Crassostrea iredalei* against pathogenic *Vibrio* spp. (*Vibrio harveyi* and *Vibrio alginolyticus*).

2. To determine the characteristics of selected potential probionts in producing biofilms, expression of extracellular enzymes, reducing haemolysis and anti-quorum sensing properties.

3. To evaluate the protection effects of selected potential probionts on *Artemia salina* and *Penaeus monodon* postlarvae culture in *in vivo* challenge assay.

1.5. Hypothesis of study

The hypothesis of the study:

Null hypothesis: Isolated bacteria unable to inhibit the growth of pathogen in *in vitro* assay, did not portray any probiotics characteristics and incapable in protecting *Artemia salina* nauplii and *P. monodon* PL from *V. harveyi* and *V. alginolyticus* infections.

Alternative hypothesis: Isolated bacteria able to inhibit the growth of *V. harveyi* and *V. alginolyticus* in *in vitro* assay, showed positive characteristics as probiotics and able to confer protection to *Artemia salina* nauplii and *P. monodon* PL against *V. harveyi* and *V. alginolyticus* infection.

REFERENCES

Abdallah, F. B., Chaieb, K., Zmantar, T., Kallel, H., and Bakhrouf, A. (2009). Adherence assays and slime production of *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. *Brazilian Journal of Microbiology*, 40(2): 394-398.

Abdelhamid AM, Mehrim AI, El-Barbary MI, Ibrahim SM and Abd El-Wahab AI (2009). Evaluation of a New Egyptian probiotic by African catfish fingerlings. *Environ Science and Technology,* 2:133–145.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of antimicrobial Chemotherapy*, 48:5-16.

Annual Fisheries Statistics (2015). Department of Fisheries: Malaysia.

Ashokkumar, S., Mayavu, P. (2013). Screening, identification and antagonistic activity of halo stable *Bacillus* sp. Mk22 used as probiotic in *Penaeus monodon Fabricius*, 1798. *African Journal and Food Science*, 8: 48-53.

Askarian, F., Zhou, Z., Olsen, R. E., Sperstad, S., and Ringø, E. (2012). Culturable autochthonous gut bacteria in Atlantic salmon (*Salmo salar L.*) fed diets with or without chitin. Characterization by 16S rRNA gene sequencing, ability to produce enzymes and *in vitro* growth inhibition of four fish pathogens. *Aquaculture*, 326: 1-8.

Austin B, Pride AC, Rhodie GA (2003). Association of a bacteriophage with virulence in *Vibrio harveyi*. *Journal of Fish Diseases,* 26: 55-58.

Austin B, Austin D, Southerland R, Thompson F, Swings J (2005). Pathogenicity of vibrios to rainbow traut (*Oncothynchus mykiss, Walbaum*) and *Artemia* nauplii. *Environmental Microbiology,* 7: 1488-1495.

Bacon, C. W., and Hinton, D. M. (2002). Endophytic and biological control potential of *Bacillus mojavensis* and related species. *Biological Control*, 23(3): 274-284.

Bagheri, T., Hedayati, S. A., Yavari, V., Alizade, M., & Farzanfar, A. (2008). Growth, survival and gut microbial load of rainbow trout (Onchorhynchus mykiss) fry given diet supplemented with probiotic during the two months of first feeding. *Turkish Journal of Fisheries and Aquatic Sciences*, 8(1), 43-48.

Balca' zar, E., (2003). Anti-infectious immune effectors in marine invertebrates: potential tools for disease control in larviculture. *Aquaculture,* 227: 427-438.

Balcázar, J. L., De Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., and Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary microbiology*, 114(3):173-186.

Balcázar, J. L., and Rojas-Luna, T. (2007). Inhibitory activity of probiotic *Bacillus subtilis* UTM 126 against *Vibrio* species confers protection against vibriosis

in juvenile shrimp (*Litopenaeus vannamei*). *Current microbiology*, 55(5): 409-412.

Barbosa, T.M., Serra, C.R., La Ragione, R.M., Woodward, M.J. and Henriques, A.O. (2005). Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Applied Environmental Microbiology*, 71: 968– 978.

Barman, P., Banerjee, A., Bandyopadhyay, P., Mondal, K. C., and Das Mohapatra, P. K. (2011). Isolation, identification and molecular characterization of potential probiotic bacterium, *Bacillus subtilis* PPP 13 from *Penaeus monodon*. *Biotechnology of Bioinformatics and Bioengineering*, 1(4): 473-482.

Biofilm Bacteria, The Marshall Protocol Knowledge Base. Retrieved 31 May 2016 from *https://mpkb.org/home/pathogenesis/microbiota/biofilm*.

Bjarnsholt, T., Jensen, P. Ø., Burmølle, M., Hentzer, M., Haagensen, J. A., Hougen, H. P., and Høiby, N. (2005). *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. *Microbiology*, 151(2): 373-383.

Bogino, P. C., Oliva, M. D. L. M., Sorroche, F. G., and Giordano, W. (2013). The role of bacterial biofilms and surface components in plant-bacterial associations. *International journal of molecular sciences*, 14(8):15838-15859.

Boonthai, T., Vuthiphandchai and V., Nimrat, S. (2011). Probiotic bacteria effects on growth and bacterial composition of black tiger shrimp (*Penaeus monodon*). *Aquaculture Nutrition*, 17: 634-644.

Bruhn, J. B., Gram, L., and Belas, R. (2007). Production of antibacterial compounds and biofilm formation by *Roseobacter* species are influenced by culture conditions. *Applied and environmental microbiology*, 73(2): 442-450.

Burbank, D. R., Shah, D. H., LaPatra, S. E., Fornshell, G., and Cain, K. D. (2011). Enhanced resistance to coldwater disease following feeding of probiotic bacterial strains to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 321(3): 185-190.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology*, 8(7):1137-1144.

Campaña-Torres, A., Martínez-Córdova, L. R., Villarreal-Colmenares, H., & Cortés-Jacinto, E. (2010). Evaluation of different concentrations of adult live *Artemia* (*Artemia franciscana*, Kellogs 1906) as natural exogenous feed on the water quality and production parameters of *Litopenaeus vannamei* (Boone 1931) pre-grown intensively. *Aquaculture Research*, 42(1):40-46.

Castex, M., Chim, L., Pham, D., Lemaire, P., Wabete, N., Nicolas, J.L., Schmidely, P., Mariojouls, C., (2008). Probiotic *P. acidilactici* application in shrimp

Litopenaeus stylirostris culture subject to vibriosis in New Caledonia. *Aquaculture,* 275: 182-193.

C.E. Boyd, C.S. Tucker (1998). Pond *Aquaculture Water Quality Management*, Kluwer Academic Publishers, USA.

Chang, C. I., Liu, W. Y., and Shyu, C. Z. (2000). Use of prawn blood agar hemolysis to screen for bacteria pathogenic to cultured tiger prawns *Penaeus monodon*. *Diseases of aquatic organisms*, 43(2):153-157.

Chatterjee, S., and Haldar, S. (2012). *Vibrio* related diseases in aquaculture and development of rapid and accurate identification methods. *Journal of Marine Science Research and Development,* S1:002

Chu, W.-H. (2007). Optimization of extracellular alkaline protease production from *Bacillus*. *Journal of Industrial. Microbiology and Biotechnology*, 34:241–245.

Chu W, Lu F, Zhu W, Kang C (2011). Isolation and characterization of new potential probiotic bacteria based on quorum- sensing system. *Journal of Applied Microbiology*, 110:202–208.

Clements, K.D. (1997). Fermentation and gastrointestinal microorganisms in fishes. Gastrointestinal Ecosystems and Fermentations (Mackie, R.I. & White, B.A. eds). *Gastrointestinal Microbiology*, 1:156–198.

Cole, B. A., and Boyd, C. E. (1986). Feeding rate, water quality, and channel catfish production in ponds. *The Progressive Fish-Culturist*, 48(1):25-29.

Collado MC, Grzeskowiak L, Salminen S. (2007). Probiotic strains and their combination inhibit *in vitro* adhesion of pathogens to pig intestinal mucosa. *Current Microbiology*, 55:260e5.

Costerton, J. W., Stewart, P. S., and Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, 284(5418):1318-1322.

Cruz PM, Ana LI, Oscar AM, Hugo CR (2012). Use of probiotics in aquaculture. International Scholarly Research Network, ISRN. *Microbiology,* 916845:1–14.

Cutting, S. M. (2011). *Bacillus* probiotics. *Food microbiology*, 28(2), 214-220.

Das, P., Mandal, S. C., Bhagabati, S. K., Akhtar, M. S., and Singh, S. K. (2012). Important live food organisms and their role in aquaculture. *Frontiers in Aquaculture*, 5:69-86.

Davey, M. E., and O'toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiology and molecular biology reviews*, 64(4):847-867.

Davis, D. A., Amaya, E., Venero, J., Zelaya, O., & Rouse, D. B. (2006). A case study on feed management to improving production and economic returns for the semi-intensive pond production of *Litopenaeus vannamei*. In *Avances en Nutrición Acuícola VIII. Memorias del Octavo Simposio Internacional de Nutrición Acuícola* (Vol. 15).

de Azevedo, R. V., and Braga, L. G. T. (2012). Use of probiotics in aquaculture. INTECH Open Access Publisher.

Deeseenthum, S., Leelavatcharamas, V., and Brookes, J. D. (2007). Effect of feeding *Bacillus* sp. as probiotic bacteria on growth of giant freshwater prawn (*Macrobrachium rosenbergii* de Man). *Pakistan journal of biological sciences,* 10(9):1481-1485.

Donio, M. B. S., Velmurugan, S., Raman, K., Babu, M. M., and Citarasu, T. (2014). Antagonistic *Bacillus cereus* TC-1 isolated from solar salt work in southern India. *Journal of Microbiology, Biochemical and Technology*, 6:242-246.

Duc, L. H., Hong, H. A., Barbosa, T. M., Henriques, A. O., and Cutting, S. M. (2004). Characterization of *Bacillus* probiotics available for human use. *Applied and environmental microbiology*, 70(4):2161-2171.

El-Haroun, E. R., Goda, A. S., and Chowdhury, K. (2006). Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). *Aquaculture Research*, 37(14): 1473-1480.

Estes, R. M., Friedman, C. S., Elston, R. A., and Herwig, R. P. (2004). Pathogenicity testing of shellfish hatchery bacterial isolates on Pacific oyster *Crassostrea gigas* larvae. *Diseases of aquatic organisms*, 58(2-3): 223-230.

Fabricius, J. C. (1798). *Joh. Christ. Fabricicus. Supplementum entomologiae systematicae*. apud Proft et Storch.

FAO (2012). *Food and Agricultural Organization.* The State of World Fisheries and Aquaculture.

FAO (2014). *Food and Agricultural Organization*. The State of World Fisheries and Aquaculture.

FAO (2015). *Food and Agricultural Organization*. The State of Food and Agriculture.

Flegel, T. W. (2012). Historic emergence, impact and current status of shrimp pathogens in Asia. *Journal of invertebrate pathology*, 110(2): 166-173.

Fuller, R. (1989). A review: probiotics in man and animals. *Journal of Applied Bacteriology,* 66:365–378.

Fuller, R. (1992). Problems and prospects in probiotics. *Springer Netherlands*, 377-386.

Garriques, D., and Arevalo, G. (1995). An evaluation of the production and use off five bacterial isolates to manipulate production of *P. vannamei* post larvae in Eucador. *World Aquaculture meeting*, 1-14.

Gatesoupe, F.J.(1999). The use of probiotics in aquaculture. *Aquaculture,* 180:147–165.

Ghosh, K., Sen, S. K., and Ray, A. K. (2002). Characterization of *Bacilli* isolated from the gut of rohu, *Labeo rohita*, fingerlings and its significance in digestion. *Journal of Applied Aquaculture*, 12(3):33-42.

Global shrimp survey: GOAL (2016). Global Aquaculture Advocate GAA. Portsmouth:USA

Gismondo, M. R., Drago, L., and Lombardi, A. (1999). Review of probiotics available to modify gastrointestinal flora. *International journal of antimicrobial agents*, 12(4):287-292.

Gomez-Gil, B., Tron-Mayen, L., Roque, A., Turnbull, J. F., Inglis, V., and Guerra-Flores, A. L. (1998). Species of *Vibrio* isolated from hepatopancreas, haemolymph and digestive tract of a population of healthy juvenile *Penaeus vannamei. Aquaculture*, 163(1):1-9.

Gómez-León, J., Villamil, L., Lemos, M. L., Novoa, B., and Figueras, A. (2005). Isolation of *Vibrio alginolyticus* and *Vibrio splendidus* from aquaculture carpet shell clam (*Ruditapes decussatus*) larvae associated with mass mortalities. *Applied and environmental microbiology*, 71(1): 98-104.

Gram, L., Melchiorsen, J., Spanggaard, B., Huber, I., and Nielsen, T. F. (1999). Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a Possible Probiotic Treatment of Fish. *Applied and Environmental Microbiology*, 65(3):969-973.

Grossart, H. P., Schlingloff, A., Bernhard, M., Simon, M., and Brinkhoff, T. (2004). Antagonistic activity of bacteria isolated from organic aggregates of the German Wadden Sea. *FEMS microbiology ecology*, 47(3):387-396

Gulig, P. A., Bourdage, K. L., and Starks, A. M. (2005). Molecular pathogenesis of *Vibrio vulnificus*. *Journal of Microbiology*, 43(1):118-131.

Gullian, M., Thompson, F., and Rodriguez, J. (2004). Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. *Aquaculture*, 233(1):1-14.

Guo, Z., Wang, J., Yan, L., Chen, W., Liu, X. M., and Zhang, H. P. (2009). *In vitro* comparison of probiotic properties of *Lactobacillus casei* Zhang, a potential

new probiotic, with selected probiotic strains. *LWT-Food Science and Technology*, 42(10):1640-1646.

Hagi, T., Tanaka, D., Iwamura, Y., and Hoshino, T. (2004). Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. *Aquaculture*, 234(1):335-346.

Hameed, A.S.S., 1993. A study of the aerobic heterotrophic bacterial flora of hatchery-reared eggs, larvae and post larvae of *Penaeus indicus*. *Aquaculture,* 117: 195-204.

Haq, M. B., Nazar, A. R., Ali, B. A., Somnath, C., and Shalini, R. (2011). Assessment of *Artemia franciscana* as a probable vector for WSSV transmission to *Macrobrachium idella idella* (Hilgendorf 1898). *International Journal of Chemical Analysis Science*, 2(9):1159-1170.

. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria (2001). *Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria*. FAO / WHO.

Heidelberg, J. F., Heidelberg, K. B., and Colwell, R. R. (2002). Seasonality of Chesapeake Bay bacterioplankton species. *Applied and Environmental Microbiology*, 68(11):5488-5497.

Hemaiswarya, S., Raja, R., Kumar, R. R., Ganesan, V., and Anbazhagan, C. (2011). Microalgae: a sustainable feed source for aquaculture. *World Journal of Microbiology and Biotechnology*, 27(8):1737-1746.

Hill, J.E., Baiano, J.C.F., Barnes, A.C., 2009. Isolation of a novel strain of *Bacillus pumilus* from penaeid shrimp that is inhibitory against marine pathogens. *Journal of Fish Diseases* 32: 1007-1016.

Høj, L., Bourne, D. G., & Hall, M. R. (2009). Localization, abundance and community structure of bacteria associated with *Artemia:* Effects of nauplii enrichment and antimicrobial treatment. *Aquaculture*, *293*(3), 278-285.

Holthuis, L. B. (1949). The caridean crustacea of the Canary Islands. *Zoologische Mededelingen, Leiden*, 30(15):227-255.

Hossain, M. I., Kamal, M. M., Mannan, M. A., and Bhuyain, M. A. B. (2013). Effects of Probiotics on Growth and Survival of Shrimp (*Penaeus monodon*) in Coastal Pond at Khulna, Bangladesh. *Journal of Scientific Research*, 5(2): 363-370.

Hsueh, Y. H., Somers, E. B., Lereclus, D., and Wong, A. C. L. (2006). Biofilm formation by *Bacillus cereus* is influenced by PlcR, a pleiotropic regulator. *Applied and environmental microbiology*, 72(7):5089-5092.

Irianto, A., and Austin, B. (2002). Probiotics in aquaculture. *Journal of fish diseases*, 25(11):633-642.

Irie, T., Watarai, S., Iwasaki, T., and Kodama, H. (2005). Protection against experimental *Aeromonas salmonicida* infection in carp by oral immunisation with bacterial antigen entrapped liposomes. *Fish and shellfish immunology*, 18(3):235-242.

Itami, T., Asano, M., Tokushige, K., Kubono, K., Nakagawa, A., Takeno, N., and Takahashi, Y. (1998). Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. *Aquaculture,* 164(1):277-288.

Janeo, R.L., Corre Jr, V.L., (2011). Bacterial change in the gut and hepatopancreas of tiger shrimp reared in bioaugmented system. *Journal of World Aquaculture Society,* 42: 863-872.

Jiqiu L, Beiping T, Kangsen M (2006). Comparative study between probiotic bacterium *Arthrobacter* XE-7 and chloramphenicol on protection of *Penaeus chinensis* postlarvae from pathogenic Vibrios. *Aquaculture,* 253:140–147.

Jöborn, A., Olsson, J. C., Westerdahl, A., Conway, P. L., and Kjelleberg, S. (1997). Colonization in the fish intestinal tract and production of inhibitory substances in intestinal mucus and faecal extracts by *Carnobacterium* sp. strain K1. *Journal of Fish Diseases*, 20(5):383-392.

Joint, F. A. O. (2001). WHO Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Córdoba, Argentina. October*, 1-4.

Jory, D.E., Cabrera, T.R., Dugger, D.M., Fegan, D., Lee, P.G., Lawrence, A.L., Jackson, C.J., McIntosh, R.P. and Castaneda, J. (2001). A global review of shrimp feed management: status and perspectives. *In* C.L. Browdy & D.E. Jory, eds. *The New Wave. Proceedings of the Special Session on Sustainable Shrimp Culture*, 104–152.

Karim, M (2012). *Mechanism of Protection of Probiotics against Bacterial Pathogens in Oyster Aquaculture*, PhD Thesis, University of Rhode Island.

Karunasagar, I., and Ababouch, L. (2012). Shrimp viral diseases, import risk assessment and international trade. *Indian Journal of Virology*, 23(2):141-148.

Kasana, R. C., and Yadav, S. K. (2007). Isolation of a psychrotrophic *Exiguobacterium* sp. SKPB5 (MTCC 7803) and characterization of its alkaline protease. *Current microbiology*, 54(3):224-229.

Keysami, M. A., Mohammadpour, M., and Saad, C. R. (2012). Probiotic activity of *Bacillus subtilis* in juvenile freshwater prawn, *Macrobrachium rosenbergii*

(de Man) at different methods of administration to the feed. *Aquaculture International*, 20(3), 499-511.

Kolndadacha, O.D., I.A. Adikwu, A.N. Okaeme, R.Y. Atiribom, A. Mohammed and Y.M. Musa, (2011). The role of probiotics in aquaculture in Nigeria- a review. *Continental Journal of Fisheries and Aquatic Science*, 5(1):8- 15.

Kozasa, M. (1986). Toyocerin (*Bacillus toyoi*) as growth promotor for animal feeding. *Microbiology. Alimenst. Nutrition*, 4(1):121-135.

Kumar, V., Roy, S., Meena, D. K., and Sarkar, U. K. (2016). Application of probiotics in shrimp aquaculture: importance, mechanisms of action, and methods of administration. *Reviews in Fisheries Science & Aquaculture*, 24(4):342-368.

Kureshy, N., & Davis, D. A. (2002). Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei. Aquaculture*, 204(1):125-143.

Lafferty, K. D., Harvell, C. D., Conrad, J. M., Friedman, C. S., Kent, M. L., Kuris, A. M., and Saksida, S. M. (2015). Infectious diseases affect marine fisheries and aquaculture economics. *Annual review of marine science*, 7:471-496.

Lakshmi B, Viswanath B, SaiGopal DVR (2013). Probiotics as antiviral agents in shrimp aquaculture. *Journal of Pathogens,* Article ID:424123.

Lamari, F., Sadok, K., Bakhrouf, A., and Gatesoupe, F. J. (2014). Selection of lactic acid bacteria as candidate probiotics and *in vivo* test on *Artemia* nauplii. *Aquaculture international*, 22(2):699-709.

Lara-Flores, M., Olvera-Novoa, M. A., Guzmán-Méndez, B. E., and López-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 216(1):193-201.

Lavilla-Pitogo CR, Baticados MCL, Cruz-Lacierda ES, de la Pena LD (1990). Occurrence of luminous bacterial disease of *Penaeus monodon* nauplii in Philippines.

Lazado, C. C., Caipang, C. M. A., and Kiron, V. (2012). Enzymes from the gut bacteria of Atlantic cod, *Gadus morhua* and their influence on intestinal enzyme activity. *Aquaculture Nutrition*, 18(4):423-431.

Lazado, C. C., Lacsamana, J. I., and Caipang, C. M. (2015). Mechanisms of probiotic actions in shrimp: Implications to tropical aquaculture. In *Biotechnological Advances in Shrimp Health Management in the Philippines*. Research Signpost.

Lekshmy, S., Nansimole, A., Mini, M., Athira, N., and Radhakrishnan, T. (2014). Occurrence of *Vibrio cholerae* in shrimp culture environments of Kerala, India. *Indian Journal of Scientific Research*, 5(2):151.

Licciano, M., Terlizzi, A., Giangrande, A., Cavallo, R. A., and Stabili, L. (2007). Filter-feeder macroinvertebrates as key players in culturable bacteria biodiversity control: a case of study with *Sabella spallanzanii* (Polychaeta: Sabellidae). *Marine environmental research*, 64(4):504-513.

Lilly, D. M., and Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science*, 147(3659):747-748.

Lin, H. Z., Guo, Z., Yang, Y., Zheng, W., and Li, Z. J. (2004). Effect of dietary probiotics on apparent digestibility coefficients of nutrients of white shrimp *Litopenaeus vannamei* Boone. *Aquaculture research*, 35(15):1441-1447.

Liu, C. H., Cheng, W., Hsu, J. P., and Chen, J. C. (2004). *Vibrio alginolyticus* infection in the white shrimp *Litopenaeus vannamei* confirmed by polymerase chain reaction and 16S rDNA sequencing. *Diseases of aquatic organisms*, 61(1-2):169-174.

Liu, C. H., Chiu, C. S., Ho, P. L., and Wang, S. W. (2009). Improvement in the growth performance of white shrimp, *Litopenaeus vannamei,* by a protease-producing probiotic, *Bacillus subtilis* E20, from natto. *Journal of applied microbiology*, 107(3):1031-1041.

Liu, K. F., Chiu, C. H., Shiu, Y. L., Cheng, W., and Liu, C. H. (2010). Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. *Fish and shellfish immunology*, 28(5): 837-844.

Liu, H., Li, Z., Tan, B., Lao, Y., Duan, Z., Sun, W., and Dong, X. (2014). Isolation of a putative probiotic strain S12 and its effect on growth performance, non-specific immunity and disease-resistance of white shrimp, *Litopenaeus vannamei*. *Fish and shellfish immunology*, 41(2):300-307.

Luis-Villaseñor, I. E., Macías-Rodríguez, M. E., Gómez-Gil, B., Ascencio-Valle, F., and Campa-Córdova, Á. I. (2011). Beneficial effects of four *Bacillus* strains on the larval cultivation of *Litopenaeus vannamei. Aquaculture*, 321(1):136-144.

Lumare, F., Di Muro, P., Tenderini, L., and Zupo, V. (1993). Experimental intensive culture of *Penaeus monodon* in the cold-temperate climate of the north-east coast of Italy (a fishery 'valle'of the River Po Delta). *Aquaculture*, 113(3): 231-241.

Maeda, M. and Liao, I.C. (1992). Effect of bacterial population on the growth of a prawn larva, *Penaeus monodon*. *Bulletin of National Research Institute of Aquaculture,* 21:25–29.

Mahdhi, A., F. Amoun, and A. Bakhrouf (2011). Inhibitory activity and adhesive ability of potential probiotics *Bacillus* sp. to confer protection for *Artemia* gnotobiotic culture against pathogenic *Vibrio* sp. *Turkey Journal of Veterinary Animals and Science,* 35(4): 227– 233.

Maldonado-Montiel T.D.N. and Rodriguez-Cache. L.G. (2005). Biomass production and nutritional value of *Artemia* sp. (*Anostraca:Artemiidae*) in Campeche, Mexico. *Revista de Biologia Tropical* 53:447-454.

Martin-Laurent, F., Philippot, L., Hallet, S., Chaussod, R., Germon, J. C., Soulas, G., and Catroux, G. (2001). DNA extraction from soils: old bias for new microbial diversity analysis methods. *Applied and environmental microbiology*, 67(5):2354-2359.

Marques, A., François, J. M., Dhont, J., Bossier, P., and Sorgeloos, P. (2004). Influence of yeast quality on performance of gnotobiotically grown *Artemia*. *Journal of Experimental Marine Biology and Ecology*, 310(2):247-264.

Marques, A., Thanh, T. H., Sorgeloos, P., & Bossier, P. (2006). Use of microalgae and bacteria to enhance protection of gnotobiotic *Artemia* against different pathogens. *Aquaculture*, 258(1):116-126.

Martinez Cruz, P., Ibañez, A.L., Monroy Hermosillo, O.A., Ramirez Saad, H.C., (2012). Use of probiotics in aquaculture. *ISRN Microbiology,* 2012:13.

McClean, K. H., Winson, M. K., Fish, L., Taylor, A., Chhabra, S. R., Camara, M., ... and Stewart, G. S. (1997). Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology*, 143(12):3703-3711.

McLean, R. J., Pierson, L. S., & Fuqua, C. (2004). A simple screening protocol for the identification of quorum signal antagonists. *Journal of Microbiological Methods*, 58(3):351-360.

Metchnikoff, E. (1905). *Immunity in infective diseases*. University Press.

Microbiology Australia (2003). Official Journal of the Australian Society of Microbiology, Australia.

Mohapatra S, Chakraborty T, Kumar V, DeBoeck G, Mohanta KN (2013). Aquaculture and stress management: a review of probiotics intervention. *Journal of Animal Physiology Animals and Nutrition,* 97(3):405–430.

Moriarty, D. J. W. (1996). Probiotics and bioremediation in aquaculture. *Asian shrimp news*, 26(3).

Moriarty, D. J. W. (1998). Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture*, 164(1):351-358.

Moriarty, D. J. (2003). Probiotics in aquaculture. *Microbiology Australia*, 24(1):15.

Moriarty, P. B., and Lovell, C. J. (1998). Groundwater resource development in the context of farming systems intensification and changing rainfall regimes: A case study from south east Zimbabwe. *Network Paper-Agricultural Administration, Research and Extension Network (United Kingdom)*.

Morikawa, M. (2006). Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. *Journal of bioscience and bioengineering*, 101(1): 1-8.

Motoh, H. (1981). Studies on the fisheries biology of the giant tiger prawn, *Penaeus monodon* in the Philippines. Aquaculture Department, Southeast Asian Fisheries Development Center.

Munoz-Atienza, E., Araújo, C., Lluch, N., Hernández, P. E., Herranz, C., Cintas, L. M., and Magadán, S. (2015). Different impact of heat-inactivated and viable lactic acid bacteria of aquatic origin on turbot (*Scophthalmus maximus L.*) head-kidney leucocytes. *Fish and shellfish immunology*, 44(1):214-223.

Myers, D, (2007). "Probiotics", *Journal of Exotic PetMedicine*,, 16(3):195–197.

Nadell, C. D., Xavier, J. B., and Foster, K. R. (2009). The sociobiology of biofilms. *FEMS microbiology reviews*, 33(1):206-224.

Nakayama, T., Lu, H., and Nomura, N. (2009). Inhibitory effects of *Bacillus* probionts on growth and toxin production of *Vibrio harveyi* pathogens of shrimp. *Letters in applied microbiology*, 49(6):679-684.

Nealson, K. H., Platt, T., and Hastings, J. W. (1970). Cellular control of the synthesis and activity of the bacterial luminescent system. *Journal of bacteriology*, 104(1):313-322.

Nealson, K. H., and Hastings, J. W. (1979). Bacterial bioluminescence: its control and ecological significance. *Microbiological reviews*, 43(4):496.

Nejad, P., Ramstedt, M., and Granhall, U. (2004). Pathogenic ice-nucleation active bacteria in willows for short rotation forestry. *Forest pathology*, 34(6):369-381.

Nimrat, S., Boonthai, T., and Vuthiphandchai, V. (2011). Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (*Litopenaeus vannamei*) larvae and postlarvae. *Animal feed science and technology*, 169(3):244-258.

Ninawe A.S. and Selvin J. (2009). Probiotics in shrimp aquaculture: avenues and challenges. *Critical Reviews in Microbiology,* 35:43–66.

Nithya, Chari, Mansur Farzana Begum, and Shunmugiah Karutha Pandian (2010). Marine bacterial isolates inhibit biofilm formation and disrupt mature

biofilms of *Pseudomonas aeruginosa* PAO1. *Applied Microbiology and Biotechnology,* 88(1): 341-358.

Nithya, C., and Pandian, S. K. (2010). *Bacillus pumilus* of Palk Bay origin inhibits quorum-sensing-mediated virulence factors in Gram-negative bacteria. *Research in Microbiology*, 161(4):293-304.

Niu, Y., Defoirdt, T., Baruah, K., Van de Wiele, T., Dong, S., and & Bossier, P. (2014). *Bacillus* sp. LT3 improves the survival of gnotobiotic brine shrimp (*Artemia franciscana*) larvae challenged with *Vibrio campbellii* by enhancing the innate immune response and by decreasing the activity of shrimp-associated vibrios. *Veterinary microbiology*, 173(3):279-288.

Nogami, K., and Maeda, M. (1992). Bacteria as biocontrol agents for rearing larvae of the crab *Portunus trituberculatus*. *Canadian Journal of fisheries and aquatic sciences*, 49(11):2373-2376.

Nurhidayu, A. (2012). *Selection of Potential Bacterial Probiotics for Tiger Grouper (Epinephelus fuscoguttatus Forsskal) Larviculture.* Master Thesis, Universiti Putra Malaysia.

Ochoa-Solano, J. L., and Olmos-Soto, J. (2006). The functional property of *Bacillus* for shrimp feeds. *Food microbiology*, 23(6):519-525.

Oggioni, M. R., Ciabattini, A., Cuppone, A. M., and & Pozzi, G. (2003). *Bacillus* spores for vaccine delivery. *Vaccine*, 21:S96-S101.

Pai, S. S. (2006*). Biocontrol of Vibrio harveyi in Penaeus monodon larval rearing systems employing probiotics and vibriophages*, PhD Thesis, Cochin University of Science and Technology.

Pai, S. S., Anas, A., Jayaprakash, N. S., Priyaja, P., Sreelakshmi, B., Preetha, R., and Singh, I. S. B. (2010). *Penaeus monodon* larvae can be protected from *Vibrio harveyi* infection by pre-emptive treatment of a rearing system with antagonistic or non-antagonistic bacterial probiotics. *Aquaculture Research*, 41(6):847-860.

Pandiyan, P., Balaraman, D., Thirunavukkarasu, R., George, E. G. J., Subaramaniyan, K., Manikkam, S., and Sadayappan, B. (2013). Probiotics in aquaculture. *Drug Invention Today*, 5(1):55-59.

P.A.W. Robertson, C. O'Dowd, C. Burrells, P. Williams, B. Austin (2000). Use of *Carnobacterium* sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) *Aquaculture,* 185:235–243.

Pearson, J. P., Passador, L., Iglewski, B. H., and Greenberg, E. P. (1995). A second N-acylhomoserine lactone signal produced by Pseudomonas maeruginosa. *Proceedings of the National Academy of Sciences*, 92(5):1490-1494.

Pirarat, N., Pinpimai, K., Endo, M., Katagiri, T., Ponpornpisit, A., Chansue, N., and Maita, M. (2011). Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus GG*. *Research in veterinary science*, 91(3):92-97.

Portley, N (2016) *Asian Farmed Shrimp Trade and Sustainability*. SFP Report on the Shrimp Sector.

Porubcan, R. S. (1991). *Reduction in chemical oxygen demand and improvement in Penaeus monodon yield in ponds inoculated with aerobic Bacillus bacteria*. Proceedings of the Program and Abastracts of the 22nd Annual Conference and Exposition. World Aquaculture Society.

Prieur, D., Mevel, G., Nicolas, J. L., Plusquellec, A., and Vigneulle, M. (1990). Interactions between bivalve molluscs and bacteria in the marine environment. *Oceanography Marine Biology Annual Review*, 28:277-352.

Priyadarsani, L., and Abraham, T. J. (2016). Water and sediment quality characteristics of medium saline traditional shrimp culture system (bheri). *Journal of Fisheries*, 4(1):309-318.

Pushparajan, N., and Soundarapandian, P. (2010). Recent farming of marine black tiger shrimp, *Penaeus monodon* (Fabricius) in South India. *African Journal of Basic and Applied Sciences*, 2(1-2):33-36.

Qi, Z.Z., X.H. Zhang, N. Boon and P. Bossier, (2009). Probiotics in aquaculture of China- Current state, problems and prospect. *Aquaculture*, 290:15-21.

Qiu, J., Wang, D., Xiang, H., Feng, H., Jiang, Y., Xia, L. and Deng, X. (2010). Subinhibitory concentrations of thymol reduce enterotoxins A and B and α-hemolysin production in *Staphylococcus aureus* isolates. *PLoS One*, 5(3): e9736.

Rahman, S., Khan, S. N., Naser, M. N., and Karim, M. M. (2009). Application of probiotic bacteria: a novel approach towards ensuring food safety in shrimp aquaculture. *Journal of Bangladesh Academy of Sciences*, 33(1):139-144.

Ramaiah, N. (2006). A review on fungal diseases of algae, marine fishes, shrimps and corals. *Indian Journal of Marine Sciences,* 35(4):380-387.

Ramanathan, N., Padmavathy, P., Francis, T., Athithian, S., and Selvaranjithan, N. (2005). Manual on polyculture of tiger shrimp and in freshwater. Tamil Nadu

Ran C, Carrias A, Williams MA, Capps N, Dan BCT (2012). Identification of *Bacillus* strains for biological control of catfish pathogens. *Plos one,* 7(9):e45793.

Ransangan, J., Lal, T. M., and& Al-Harbi, A. H. (2012). Characterization and experimental infection of *Vibrio harveyi* isolated from diseased Asian seabass (*Lates calcarifer*). *Malaysian Journal of Microbiology*, 8(2):104-115.

Raul, D., Biswas, T., Mukhopadhyay, S., Kumar Das, S., and Gupta, S. (2014). Production and partial purification of alpha amylase from *Bacillus subtilis* (MTCC 121) using solid state fermentation. *Biochemistry research international*, 12(2014):568141.

Ray, A. K., Ghosh, K., and Ringø, E. (2012). Enzyme-producing bacteria isolated from fish gut: a review. *Aquaculture Nutrition*, 18(5):465-492.

Reddy, J. (2014). Influence of vitamin c on growth indices, survival rate and moulting of the juveniles of *Macrobrachium rosenbergii*. *International Journal of Bioassays*, 3(07):3111-3118.

Reed, P. A., and Francis-Floyd, R. (1996). *Vibrio infections of fish*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

Rengpipat, S., Phianphak, W., Piyatiratitivorakul, S. and Menasveta, P. (1998). Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. *Aquaculture,* 167:301–313.

Rengpipat, S., Rukpratanporn, S., Piyatiratitivorakul, S. and Menasaveta, P. (2000). Immunity enhancement in black tiger shrimp (*Penaeus monodon)* by a probiont bacterium (*Bacillus* S11). *Aquaculture,* 191:271–288.

Rengpipat, S., (2005). Biocontrol of bacteria pathogens in aquaculture with emphasis on chage therapy. *In:* P. Walker, R. Lester and M.G. Bondad-Reantaso (Eds.). *Diseases in Asian Aquaculture* V, pp. 543-552. Fish Health Section, Asian Fisheries Society, Manila.

Rinaudi, L. V., and Giordano, W. (2010). An integrated view of biofilm formation in rhizobia. *FEMS Microbiology letters*, 304(1):1-11.

Ringø, E., Olsen, R. E., Gifstad, T. Ø., Dalmo, R. A., Amlund, H., HEMRE, G. I., and Bakke, A. M. (2010). Prebiotics in aquaculture: a review. *Aquaculture Nutrition*, 16(2):117-136.

Romero, J., Feijoó, C. G., and Navarrete, P. (2012). *Antibiotics in aquaculture-use, abuse and alternatives*. INTECH Open Access Publisher.

Rungrassamee, W., Klanchui, A., Chaiyapechara, S., Maibunkaew, S., Tangphatsornruang, S., Jiravanichpaisal, P., and Karoonuthaisiri, N. (2013). Bacterial population in intestines of the black tiger shrimp (*Penaeus monodon*) under different growth stages. *PloS one, 8*(4):e60802.

Rygus, T., and Hillen, W. (1991). Inducible high-level expression of heterologous genes in Bacillus megaterium using the regulatory elements of the xylose-utilization operon. *Applied microbiology and biotechnology*, 35(5):594-599.

Sahoo, P.K. and S.C. Mukherjee, (1999). Influence of the immunostimulant, chitosan on immune responses of healthy and cortisol treated rohu (Labeo rohita). *Journal of Aquaculture in Tropic*, 14:209-215.

Sakai, M., T. Yoshida, S. Astuta, and M. Kobayashi, (1995). Enhancement of resistence to vibriosis in rainbow trout, *Oncorrhynchus mykiss* (Walbaum) by oral administration of *Clostridium butyricum* bacteria. *Journal of Fish Diseases.*, 18: 187–190.

Sakata, T. (1990). Microflora in the digestive tract of fish and shellfish. In: Microbiology in Poecilotherms (Lesel, R. ed.), pp. 171– 176. *Elsevier*, Amsterdam.

Salah Mesalhy A, Yousef A, Ahlam A, Moahmed Fathi M (2008). Studies on *B. subtilis* and, *Lactobacillus acidophilus*, as potential probiotics, on the immune response and resistance of Tilapia nilotica (*Oreochromis niloticus*) to challenge infections. *Fish Shellfish Immunology,* 25:128–136.

S. Schwarz, C. Kehrenberg, T.R. Walsh (2001).Use of antimicrobial agents in veterinary medicine and food animal production. *International Journal of Antimicrobial Agents,* 17:431–437.

Schembri, M. A., and Klemm, P. (2001). Biofilm formation in a hydrodynamic environment by novel FimH variants and ramifications for virulence. *Infection and immunity*, 69(3):1322-1328.

Seenivasan, C., Bhavan, P. S., Radhakrishnan, S., and Shanthi, R. (2012). Enrichment of *Artemia* nauplii with *Lactobacillus sporogenes* for enhancing the survival, growth and levels of biochemical constituents in the post-larvae of the freshwater prawn *Macrobrachium rosenbergii*. *Turkish Journal of Fisheries and Aquatic Sciences*, 12(1).

Selvakumar, G., Joshi, P., Nazim, S., Mishra, P. K., Kundu, S., and Gupta, H. S. (2009). *Exiguobacterium acetylicum* strain 1P (MTCC 8707) a novel bacterial antagonist from the North Western Indian Himalayas. *World Journal of Microbiology and Biotechnology,* 25(1):131-137.

Silva, A. M., Barbosa, F. H. F., Duarte, R., Vieira, L. Q., Arantes, R. M. E., and Nicoli, J. R. (2004). Effect of *Bifidobacterium longum* ingestion on experimental salmonellosis in mice. *Journal of Applied Microbiology*, 97(1): 29-37.

Silva, E. F., Soares, M. A., Calazans, N. F., Vogeley, J. L., do Valle, B. C., Soares, R., & Peixoto, S. (2012). Effect of probiotic (Bacillus spp.) addition during larvae and postlarvae culture of the white shrimp Litopenaeus vannamei. *Aquaculture Research*, *44*(1), 13-21.

Skjermo, J. and Vadstein, O. (1999). Techniques for microbial control in the intensive rearing of marine larvae. *Aquaculture,* 177:333–343.

Soonthornchai, W., Rungrassamee, W., Karoonuthaisiri, N., Jarayabhand, P., Klinbunga, S., Söderhäll, K.,and Jiravanichpaisal, P. (2010). Expression of immune-related genes in the digestive organ of shrimp, *Penaeus monodon*, after an oral infection by *Vibrio harveyi*. *Developmental & Comparative Immunology*, 34(1):19-28.

Sorgeloos P., Dhert P. and Candreva P. (2001) Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture,* 200:147-159.

Soto-Rodriguez, S. A., Roque, A., Lizarraga-Partida, M. L., Guerra-Flores, A. L., and Gomez-Gil, B. (2003). Virulence of luminous vibrios to *Artemia franciscana* nauplii. *Diseases of aquatic organisms*, 53(3):231-240.

Soundarapandian, P., and Babu, R. (2010). Effect of probiotics on the hatchery seed production of black tiger shrimp, *Penaeus monodon* (Fabricius). *International Journal of Animal and Veterinary Advances*, 2(1):9-15.

Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S., and Kaper, J. B. (1999). Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli. Proceedings of the National Academy of Sciences*, 96(26):15196-15201.

Sperandio, V. (2007). Novel approaches to bacterial infection therapy by interfering with bacteria-to-bacteria signaling. *Expert review of anti-infective therapy*, 5(2):271-276.

Stanley, N. R., and Lazazzera, B. A. (2004). Environmental signals and regulatory pathways that influence biofilm formation. *Molecular microbiology*, 52(4): 917-924.

Stentiford, G. D., Neil, D. M., Peeler, E. J., Shields, J. D., Small, H. J., Flegel, T. W. and Lotz, J. (2012). Disease will limit future food supply from the global crustacean fishery and aquaculture sectors. *Journal of invertebrate pathology*, 110(2):141-157.

Sudhakaran, R., Yoganandhan, K., Ahmed, V. I., & Hameed, A. S. (2006). *Artemia* as a possible vector for *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus transmission (XSV) to *Macrobrachium rosenbergii* post-larvae. *Diseases of aquatic organisms*, 70(1-2):161-166.

Sugita, C., Ogata, K., Shikata, M., Jikuya, H., Takano, J., Furumichi, M., and Sugita, M. (2007). Complete nucleotide sequence of the freshwater unicellular cyanobacterium *Synechococcus elongatus* PCC 6301 chromosome: gene content and organization. *Photosynthesis Research,* 93(1-3):55-67.

Sung, H. H., Li, H. C., Tsai, F. M., Ting, Y. Y., and Chao, W. L. (1999). Changes in the composition of *Vibrio* communities in pond water during tiger shrimp (*Penaeus monodon*) cultivation and in the hepatopancreas of healthy and diseased shrimp. *Journal of Experimental Marine Biology and Ecology*, 236(2):261-271.

Sung, H.H., Hsu, S.F., Chen, C.K., Ting, Y.Y. and Chao, W.L. (2001). Relationship between disease outbreaks in cultured tiger shrimp (*Penaeus monodon*) and the composition of *Vibrio* communities in pond water and shrimp hepatopancreas during cultivation. *Aquaculture,* 192:101–110.

Surette, M. G., and Bassler, B. L. (1998). Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proceedings of the National Academy of Sciences*, 95(12):7046-7050.

Sutherland, I. W. (2001). Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology*, 147(1):3-9.

Suzer, C., Çoban, D., Kamaci, H. O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., and Küçüksari, H. (2008). *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata, L.*) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture*, 280(1):140-145.

Swain, S. M., Singh, C., and Arul, V. (2009). Inhibitory activity of probiotics *Streptococcus phocae* PI80 and *Enterococcus faecium* MC13 against vibriosis in shrimp *Penaeus monodon*. *World Journal of Microbiology and Biotechnology*, 25(4):697-703.

Tanasomwang, V., Nakai, T., Nishimura, Y., and Muroga, K. (1998). Vibrio-inhibiting marine bacteria isolated from black tiger shrimp hatchery. *Fish Pathology*, 33(5):459-466.

Taxonomy of Penaeus monodon (2016). Integrated Taxonomic Information System (ITIS). N.d Retrieved 5 January 2016 from https://www.itis.gov/

Tendencia, E.A., de la Peña, L.D., (2001). Antibiotic resistance of bacteria from shrimp ponds. *Aquaculture,* 195: 193-204.

Tejero-Sariñena, S., Barlow, J., Costabile, A., Gibson, G. R., & Rowland, I. (2012). *In vitro* evaluation of the antimicrobial activity of a range of probiotics against pathogens: evidence for the effects of organic acids. *Anaerobe*, 18(5): 530-538.

Touraki, M., Karamanlidou, G., Karavida, P., and Chrysi, K. (2012). Evaluation of the probiotics *Bacillus subtilis* and *Lactobacillus plantarum* bioencapsulated in *Artemia* nauplii against vibriosis in European sea bass larvae (*Dicentrarchus labrax, L.*). *World Journal of Microbiology and Biotechnology*, 28(6):2425-2433.

Tseng, D. Y., Ho, P. L., Huang, S. Y., Cheng, S. C., Shiu, Y. L., Chiu, C. S., and Liu, C. H. (2009). Enhancement of immunity and disease resistance in the white shrimp, *Litopenaeus vannamei*, by the probiotic, *Bacillus subtilis* E20. *Fish and Shellfish Immunology*, 26(2):339-344.

Tuan, T. N., Duc, P. M., & Hatai, K. (2013). Overview of the use of probiotics in aquaculture. *International Journal of Research in Fisheries and Aquaculture*, *3*(3):89-97.

Tzuc, J. T., Escalante, D. R., Herrera, R. R., Cortés, G. G., & Ortiz, M. L. A. (2014). Microbiota from *Litopenaeus vannamei:* digestive tract microbial community of Pacific white shrimp (*Litopenaeus vannamei*). *SpringerPlus*, 3(1):280.

Van den Bogaard, A. E., and Stobberingh, E. E. (2000). Epidemiology of resistance to antibiotics: links between animals and humans. *International journal of antimicrobial agents*, 14(4):327-335.

Van Hai, N., Fotedar, R., and Buller, N. (2007). Selection of probiotics by various inhibition test methods for use in the culture of western king prawns, *Penaeus latisulcatus* (Kishinouye). *Aquaculture*, 272(1):231-239.

Van Hai, N. (2015). Research findings from the use of probiotics in tilapia aquaculture: a review. *Fish & shellfish immunology*, 45(2):592-597.

Vary, P. S. (1994). Prime time for *Bacillus megaterium*. *Microbiology*, 140(5):1001-1013.

Vary, P. S., Biedendieck, R., Fuerch, T., Meinhardt, F., Rohde, M., Deckwer, W. D., and Jahn, D. (2007). *Bacillus megaterium*—from simple soil bacterium to industrial protein production host. *Applied microbiology and biotechnology*, 76(5): 957-967.

Vaseeharan, B.A.R.P., and Ramasamy, P. (2003).Control of pathogenic *Vibrio* spp by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letters in applied microbiology*, 36(2):83-87.

Venkat, H. K., Sahu, N. P., and Jain, K. K. (2004). Effect of feeding *Lactobacillus*-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research*, 35(5):501-507.

Verschuere, L., Rombaut, G., Sorgeloos, P., and Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and molecular biology reviews,* 64(4):655-671.

Vieira, F. N., Buglione, C. C., Mourino, J. P. L., Jatoba, A., Martins, M. L., Schleder, D. D., and Vinatea, L. A. (2010). Effect of probiotic supplemented diet on marine shrimp survival after challenge with *Vibrio harveyi. Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 62(3):631-638.

Villamil, L., Tafalla, C., Figueras, A., and Novoa, B. (2002). Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). *Clinical and Diagnostic Laboratory Immunology*, 9(6):1318-1323.

Vine, N. G., Leukes, W. D., Kaiser, H., Daya, S., Baxter, J., and Hecht, T. (2004). Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. *Journal of fish diseases*, 27(6): 319-326.

Wang, L., Chen, Y., Huang, H., Huang, Z., Chen, H., and Shao, Z. (2015). Isolation and identification of *Vibrio campbellii* as a bacterial pathogen for luminous vibriosis of *Litopenaeus vannamei*. *Aquaculture Research*, 46(2):395-404.

Wang, Y. B., Li, J. R., and Lin, J. (2008). Probiotics in aquaculture: challenges and outlook. *Aquaculture*, 281(1):1-4.

Wang, Y. G., Lee, K. L., Najiah, M., Shariff, M., and Hassan, M. D. (2000). A new bacterial white spot syndrome (BWSS) in cultured tiger shrimp *Penaeus monodon* and its comparison with white spot syndrome (WSS) caused by virus. *Diseases of aquatic organisms*, 41(1):9-18.

Waters, C. M., and Bassler, B. L. (2005). Quorum sensing: cell-to-cell communication in bacteria. *Annual Review on Cell Development Biology*, 21:319-346.

Watnick, P. I., Fullner, K. J., and Kolter, R. (1999). A role for the mannose-sensitive hemagglutinin in biofilm formation by *Vibrio cholerae* El Tor. *Journal of bacteriology*, 181(11):3606-3609.

Wiegand, I., Hilpert, K., and Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nature protocols*, 3(2):163-175.

Williams, P., Camara, M., Hardman, A., Swift, S., Milton, D., Hope, V. J., and Bycroft, B. W. (2000). Quorum sensing and the population-dependent control of virulence. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 355(1397):667-680.

Winzer, K., and Williams, P. (2001). Quorum sensing and the regulation of virulence gene expression in pathogenic bacteria. *International Journal of Medical Microbiology*, 291(2):131-143.

Wu, H., Song, Z., Givskov, M., Doring, G., Worlitzsch, D., Mathee, K., and Høiby, N. (2001). *Pseudomonas aeruginosa* mutations in lasI and rhlI quorum sensing systems result in milder chronic lung infection. *Microbiology*, 147(5): 1105-1113.

Wyban, J. (2007). Thailand's white shrimp revolution. *Global Aquaculture Advocate*, 10(3):56-58.

Xinhua, Y. (2008). Comparative advantage analysis of shrimp production in Asia. *Aquaculture Asia*, 13(1):33.

Yasin, I. S. M., Razak, N. F., Natrah, F. M. I., and Harmin, S. A. (2016). Selection and evaluation of Malaysian *Bacillus* spp. strains as potential probiotics in cultured tiger grouper (*Epinephelus fuscoguttatus*). *Journal of Environmental Biology*, 37(4):791-800.

Yildiz, F. H., and Visick, K. L. (2009). *Vibrio* biofilms: so much the same yet so different. *Trends in microbiology*, 17(3):109-118.

Yip, E. S., Geszvain, K., DeLoney-Marino, C. R., and Visick, K. L. (2006). The symbiosis regulator RscS controls the syp gene locus, biofilm formation and symbiotic aggregation by *Vibrio fischeri*. *Molecular microbiology*, 62(6): 1586-1600.

Yu, M. C., Li, Z. J., Lin, H. Z., Wen, G. L., and Ma, S. (2008). Effects of dietary *Bacillus* and medicinal herbs on the growth, digestive enzyme activity, and serum biochemical parameters of the shrimp *Litopenaeus vannamei. Aquaculture international*, 16(5):471-480.

Zhang, X. H., and Austin, B. (2005). Haemolysins in *Vibrio* species. *Journal of Applied Microbiology*, 98(5):1011-1019.

Zhang G. and Hong H. (2007) Beneficial effects of *Bacillus licheniformis* on the intestinal microflora and immunity of the white shrimp, *Litopenaeus vannamei*. *Biotechnology Letters,* 29:525–530.

Zhao. W (2015). *Characterization of the Probiotic Mechanism of Phaeobacter gallaeciensis Against Bacterial Pathogens*, PhD, University of Rhode Island.

Zhou, X. X., Wang, Y. B., and Li, W. F. (2009). Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. *Aquaculture*, 287(3):349-353.

.Ziaei-Nejad, S., Rezaei, M. H., Takami, G. A., Lovett, D. L., Mirvaghefi, A. R., and Shakouri, M. (2006). The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus. Aquaculture*, 252(2):516-524.

Zokaeifar, H., Balcázar, J. L., Kamarudin, M. S., Sijam, K., Arshad, A., and Saad, C. R. (2012). Selection and identification of non-pathogenic bacteria isolated from fermented pickles with antagonistic properties against two shrimp pathogens. *The Journal of antibiotics*, 65(6):289-294.

Zokaeifar, H., Babaei, N., Saad, C. R., Kamarudin, M. S., Sijam, K., and Balcazar, J. L. (2014). Administration of *Bacillus subtilis* strains in the rearing water enhances the water quality, growth performance, immune response, and resistance against *Vibrio harveyi* infection in juvenile white shrimp, *Litopenaeus vannamei*. *Fish & shellfish immunology*, 36(1):68-74.