ISOLATION OF PHOTOSYNTHETIC BACTERIA AND POTENTIALITY AS AN AQUACULTURE FEED

SANKARI D/O RANJAPAN

THIS DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGRRE OF BACHELOR OF SCIENCE WITH HONOURS

AQUACULTURE PROGRAM
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SABAH

MAY, 2008

PUMS99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: ISOLATION OF PHOTOSYNTHETIC BACTERIA AND POTENTIALITY AS AN AQUACULTURE ~~FEED~~ FEED

IJAZAH: DEGREE OF BACHELOR OF SCIENCE WITH HONOURS (AQUACULTURE)

SAYA SANKARI A/P RANJAPAN (HURUF BESAR) SESI PENGAJIAN: 2005/2006

mengaku membenarkan tesis (LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institutsi pengajian tinggi.
4. Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☑	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☐	TIDAK TERHAD	

Disahkan Oleh

NURULAIN BINTI IS...
LIBRARIAN
UNIVERSITI MALAYSIA SABAH

(TANDATANGAN PENULIS)

Alamat Tetap: 3234 TAMAN INDAH, 73000 DAMPIN, NEGERI SEMBILAN

Tarikh: 13/5/08

(TANDATANGAN PUSTAKAWAN)

DR. SUJJAT AL-AZAD
Nama Penyelia

Tarikh: 13/5/08

CATATAN:- *Potong yang tidak berkenaan.
**Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa /organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
@Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

RECOGNITION

I agree and recognise that this thesis is fully my work except for any article or tables that have been identified its sources and expressed clearly.

Signature

12 MAY 2008 .....

SANKARI D/O RANJAPAN

HS2005-1891

AUTHENTICATION

Signature

1. **SUPERVISOR**

   (DR. Sujjat Al-Azad)

2. **EXAMINER 1**

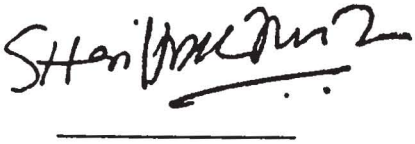
   (Mr. Kennedy Aaron Aguol)

3. **EXAMINER 2**

   (Dr. Normawaty Mohammad Noor)

4. **DEAN**

   (SUPT./KS Profesor Madya Dr. Shariff AK Omang)



ACKNOWLEDGEMENT

I take pleasure in thanking the many people who have offered help and suggestions during my study. First of all, I would like to express my special gratitude to my supervisor; Dr. Sujjat Al-Azad, who provided encouragement and guidance through out my research and contributes to my research design. All advises, help and guidance provided by him during my research are heartedly appreciated.

I am very grateful to the Director of Marine Borneo Research Institute, Prof.Saleem Mustafa, Prof.Shegeharu Senoo, to all staffs and master students for their friendly cooperation, guidance and giving me necessary help. My next appreciation goes to the research assistance for their willingness to participate the research and giving extra feedbacks regarding the research.

All help from my friends during my research are truly appreciated especially Thinakharasi, Priatharisini, Jeevitha and Lechman. I also would like thank my beloved parent who gives me unlimited support and courage through out my studies. Here I would like to thank everyone who had involved directly or indirectly in helping me completing this research.

SANKARI D/O RANJAPAN

HS2005-1891

12 MAY 2008

ABSTRACT

An indigenous strain of the phototrophic bacteria, isolated from the Kingfisher mangrove area (UMSPSB1) and seabass tank in UMS Hatchery (UMSPSB2) was mass cultured under anaerobic light conditions. The bacterial cell mass was analyzed for carotenoids (mgg^{-1} dry cell), bacteriochlorophyll and dry cell weight (mgl^{-1}). UMSPSB1 strain shows higher dry cell weight of 24.3 mgl^{-1} and carotenoid of 22.73 mgg^{-1} dry cell contents compare to the bacterial strain UMSPSB2, 3.7 mgl^{-1} dry cell weight and carotenoid 16.36 mgg^{-1} dry cell weight. The strain UMSPSB1 was tested for toxicity and acceptability as an aquaculture feed additives. Feeding trial on brine shrimp (*Artemia*) larvae showed that the mix feed (UMSPSB1+ *Spirulina*) gives the highest growth in length 3500µm of the *Artemia* compare to *Spirulina* solely 1200µm in length and UMSPSB1 solely fed 876µm in *Artemia*. Other than this, brine shrimp fed by UMSPSB1+*Spirulina* gives the highest survival rate of 88% which is comparable with bacteria free mixed diet and *Spirulina*. The UMSPSB1 strain was also fed to the Asian sea bass (*Lates calcarifer*) larvae to observe the growth and survival in the sea bass larvae. Larvae of Asian seabass fed by bacteria as a solely diet was died on the second day of the experiment and the larvae reared solely by green water (*Nanno chloropsis*) were died on the third day of the experiment. The larvae fed by UMSPSB1 enriched *Artemia* showed the highest growth of 8.9mm and survival rate of 75% rate after 10 days of experiment.

ABSTRAK

Satu strain bakteria fototropik diekstrak dari kawasan paya bakau Kingfisher dan dari tanki ikan Siakap di Hatceri UMS. Strain bakteria tersebut dimaskulturkan dibawah cahaya dalam keadaan anaerobic. Sel-sel bakteria tersebut dianalisa untuk kehadiran karotenoid (mgg^{-1} sel kering), bakteriokIorofil dan berat sel kering mgl^{-1}. Strain UMSPSB1 menunjukkan berat sel yang tinggi iaitu 24.3 mgl^{-1} dan karotenoid iaitu 22.73 mgg^{-1} sel kering berbanding strain bakteria yang satu lagi, UMSPSB2, iaitu 3.7 mgl^{-1} berat sel kering dan karotenoid 16.36 mgg^{-1} berat sel kering yang diekstrak dari tanki ikan siakap di Hatceri UMS. Strain UMSPSB1 diuji untuk kehadiran toksik dan penerimaannya sebagai aditif makanan akuakultur. Ujian pemakanan keatas larva Artemia menunjukkan bahawa makanan campuran iaitu UMSPSB1 dan Spirulina yang memberikan pertumbuhan tinggi dalam panjang larva Artemia tersebut iaitu 3500µm berbanding dengan Spirulina sahaja iaitu 1200µm panjangnya serta UMSPSB1 sahaja dengan panjang Artemia 876µm. Selain daripada itu, Artemia yang diberi makanan UMSPSB1 dan Spirulina memberi kadar jangka hayat yang lebih tinggi iaitu 88% berbanding dengan diet yang bebas dari bakteria dan Spirulina. Strain UMSPSB1 juga diberi kepada larva ikan siakap Asia untuk membuat pemerhatian keatas pertumbuhan dan kadar jangka hayat larva ikan siakap.Larva ikan siakap Asia yang diberi makan dengan bakteria mati pada hari kedua eksperimen manakala larva yang dibela dalam air hijau sahaja mati pada hari ketiga eksperimen. Larva UMSPSB1 diberi makan dengan Artemia yang diperkaya menunjukkan pertumbuhan yang tinggi iaitu 8.9mm dan kadar jangka hayat 75% selepas 10 hari eksperimen dijalankan.

CONTENTS

CHAPTER 3 METHODOLOGY

CHAPTER 4 RESULT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

μm	micrometer
%	percentage
^0C	degree Celsius
g	gram
mL	milliliter
rpm	revolution per minute
TSA	Tryticase Soy Agar
UMSPSB1	University Malaysia Sabah Phototrophic Bacteria Strain1
UMSPSB2	University Malaysia Sabah Phototrophic Bacteria Strain2
PUFA	polyunsaturated fatty acids
HUFA	high unsaturated fatty acids
Mgg^{-1}	Milligram per gram
Mgl^{-1}	Milligram per litre

CHAPTER 1

INTRODUCTION

1.1 Aquaculture

The definition of aquaculture is the farming of aquatic organisms, including fish, mollusks, crustaceans and aquatic plants. Farming implies some form of *intervention* in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. Farming also implies individual or corporate ownership of stock being cultivated (John *et al.*, 2003).The aquaculture industry in Malaysia is a one of the important industry which provides seafood. The word 'aquaculture' though used rather widely for over a decade to denote all forms of culture of aquatic animals and plants in fresh, brackish and marine environments , is still used by many in a more restrictive sense. Aquaculture is at an exciting stage of development. World aquaculture production is increasing at a very rapid rate. It is increasing much more rapidly than animal husbandry and capture fisheries and the other two sources of animal protein for the world's population. There is widespread recognition that seafood production from fisheries is at or near its peak, and that aquaculture will become increasingly important as a source of seafood production, and ultimately the main source.

In aquaculture, heavily stocked fish ponds can become hypereutrophic. High and toxic levels of ammonia and nitrates and high levels of organic wastes will upset the balance of the water's chemistry. Unwanted bacteria and other microorganisms may begin to grow. This makes the aquatic occupants vulnerable to many diseases, including *Vibrio harveyi*. Algal blooms begin to dominate the environment. The system can become anoxic. The ponds natural ecosystem cannot be maintained.

By using the probiotic formula, will address ammonia and reduce the organic sludge within the system. The pro-biotics assists in feed conversion and digestion. Excess food, feces build up is reduced. Turbidity is reduced, water become cleaner. This pro-biotic method can be used in fish hatcheries, fish and prawn/shrimp farms, lobsters pens, and aquaria to combat the most common problems associated with high nutrient levels. This will improves the water quality in aquaculture systems, reduce ammonia levels, nitrates, algae and sludge buildup, reduce risk of infection and improves survival rates, reduce stock losses due to toxic ammonia, and reduce or eliminates time required to oxidize the pond bottoms between growing cycles (Rehana, 2003).

1.1.1 Phototrophic bacteria and their distribution

Ecological niches of anoxygenic phototrophic bacteria are those anoxic regions of waters and sediments that receive light of sufficient quantity and quality to allow phototrophic development. Representatives of the purple nonsulfur bacteria are widely distributed in nature and are found in all kinds of stagnant water bodies, in lakes, waste water ponds, coastal lagoons, and other aquatic habitats, but also in sediments, moist soils, and paddy fields. They prefer to live in aquatic habitats (freshwater, marine, and hypersaline environments) with significant amounts of soluble organic matter and low oxygen tension, but they rarely form colored blooms, like those that are characteristically formed by representatives of purple sulfur bacteria (Imhoff *et al.*, 1991).

The greatest variety of species and the largest numbers of cells have been found in mud and water of eutrophic ponds, ditches and lakes. In eutrophic lakes, purple nonsulfur bacteria are more or less restricted to the flat shore area (where 10^3 to more than 10^6 cells/ml) have been found in mud and water samples. In pelagic water, no more than 1-10 cells/ml are usually found (Imhoff *et al.*, 1991).

Some purple nonsulfur bacteria also occur in acidic, boggy waters and soils. Most frequently, *Rhodopseudomonas acidophila*, which grows optimally at pH 5.5, is found in such environments, often accompanied by *Rps.palustris*. *Rps.palustris* is commonly found in wet decaying leaves (Imhoff *et al.*, 1991) and in rotting leaves from zinc plated

roof gutters. It is also the most frequent species encountered in soils, although isolation attempts from soils rarely show satisfactory results.

There are many applications by using the photosynthetic bacteria. Photosynthetic bacteria are useful for the removal of ptomaines contained in foul water such as hydrogen sulphide, putrescine, cadaverine, and other amines; the efficiency of such removal was found to be higher under illuminated conditions than dark conditions (Michiharu *et al.*, 1975). It has become clear that photosynthetic bacteria growing actively in foul water contain anti-virus substances, contributing to the extinction of viruses. Their activity was found to be higher under illuminated conditions than under dark conditions.

1.1.2 Phototrophic bacteria and their role

Phototrophic bacteria are widely distributed in nature and play a role in carbon dioxide assimilation and nitrogen fixation. Moreover, phototrophic bacteria have been found to contribute greatly to the purification of the environment. In large amounts, the bacteria have been used as feed for small animals, fish and shellfish. The bacteria are also beneficial to rice cultivation (Michiharu *et al.*, 2001). It also found that phototrophic bacteria play a major role in the natural purifying process of various kinds of waste water containing high organic concentration. Based on the theory of purification, various experiments and pilot plant tests were carried out. The commercialization was made about 20 years ago and many purification treatment plants are being operated at present.

In particular, it was found that anoxygenic phototrophic bacteria are very useful for growth of brine shrimp, one of the most important salt water plankton, which is used as feed for fish and shellfishes and for collection of their eggs. Thus, a favorable outlook for completely artificial fish culture, which thus far was considered in feasible, has now been formed. Further, the fact was also identified that fry of fishes (loach, goldfish, carp, ark shell, sweet fish, etc.) are direct predators of phototrophic bacteria soon after hatching, resulting in an increase in weight and survival rate more than two fold within 2 to 4 weeks after hatching (nearly no death was noted in some experiments) (Michiharu *et al.*, 2001)

Further, purple sulfur-and green sulfur-bacteria are phototrophic bacteria that use hydrogen sulfide, which is highly toxic, and the activities of these phototrophs converts H_2S into nonpoisonous sulfur compounds (Michiharu *et al.*, 2001). Therefore, sulfur phototrophs are utilized for cleansing of wastewaters high in sulfide or to clean the bottom environment containing high level of sludge. For example, in the culture pond for eels, abnormal death was noted from the deteriorating water quality in winter, whereas treatment with phototrophic sulfur bacteria made the environment suitable for continued growth.

Special attention has been paid to the use of phototrophic bacteria in the culture of prawn. 50% of the prawn consumed in Japan is cultured in such large water tanks (prawns amounting to 20000 million Yen per month are consumed domestically), while another 50% depends on importation. Thus, prawns worth 100 million Yen per annum are

cultured in one of such water tanks (more than 100 tanks are operating in Japan). In the past prawns in such culture tanks were frequently affected by gill disease causing great economic damage; however this has now been completely prevented by supplementing the tanks with anoxygenic phototrophic bacteria (Michiharu *et al.*, 2001). In addition, such affects have also been displayed in suppression of virus diseases noted on others including swellfish.

1.2 Objectives

Major objective in this study is to isolate indigenous phototrophic bacteria from various sources and observe the uses of bacteria as aquaculture feed.

1.2.1 Specific objectives are as follows:

1. To isolate the bacteria from native environment.
2. To study the morphological characteristics of the bacteria.
3. To mass culture of the bacteria.
4. To observe the suitability of bacteria to be use as aquaculture feed supplement.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Phototrophic bacteria, including oxygenic and anoxygenic phototrophic bacteria, can transform light energy into metabolically useful chemical energy by chlorophyll or bacteriochlorophyll mediated process (Imhoff, 1999). Major differences between oxygenic and anoxygenic phototrophic bacteria relate to their photosynthetic bacteria and structure and complexity of the photosynthetic apparatus. Photosynthesis in anoxygenic phototrophic bacteria depends on oxygen deficient conditions, because synthesis of photosynthetic pigment is repressed by oxygen (bacteria like *Erythrobacter longus* are expections in this rule); in contrast to photosynthesis in plants and cyanobacteria (including *Prochloron* and related forms), oxygen is not produced. Unlike the cyanobacteria and eukaryotic algae, anoxygenic phototrophic bacteria are unable to use water as an electron donor (Imhoff, 1999). Most characteristically, sulfide and other reduced reduced sulfur compounds, but also hydrogen and a number of small organic molecules, are used as photosynthetic electron donors. (Anoxygenic photosynthesis with

sulfide, an inhibitor of photosystem II, as electron donor is also carried out by some cyanobacteria using photosystem I only).

As a consequence, the ecological niches of anoxygenic phototrophic bacteria are anoxic parts of waters and sediments, which receive light of sufficient quantity and quality to allow phototrophic development. Representatives of this group are widely distributed in nature and found in freshwater, marine and hypersaline environments, hot springs, and artic lakes, as well as elsewhere. They live in all kinds stagnant water bodies, in lakes, waste water ponds, coastal lagoons, stratified lakes and other aquatic habitats but also in marine coastal sediments, in moist soils, and in paddy fields (Imhoff, 1999).

The anoxygenic phototrophic bacteria are an extremely heterogeneous eubacterial group on the basis of both structural and physiological properties. They are treated taxonomically in a number of well distinct families and groups and also appear to be phylogenetically quite diverse. The various species of these bacteria contain several type of bacteriochlorophylls and a variety of carotenoids as pigments, which function in the transformation of light in to chemical energy and give the cell cultures a distinct coloration varying with the pigment content from various shades of green, yellowish green, brownish green, brown, brownish red, red, pink, purple, and purple-violet to even blue (carotenoidless mutants of some species containing bacteriochlorophyll a) (Imhoff, 1999).

REFERENCES

Alexander J.B.Zehnder, 1988. Biology of anaerobic microorganisms. A John Wiley & Sons Inc. New York.

Azad, S.A., Chong, V.C.,Vikineswary, S.2002. Phototrophic bacteria as feed supplement for rearing *Penaeus monodon*. World Aquaculture Society **33**:2.

Azad,S.A., Chong,V.C., Vikineswary,S &Ramachandran K.B[2],2001.Potential of waste grown phototrophic bacteria as a feed ingredient in aquafeeds. Jurnal Biosains **12**:2.

Bollag,D.M.&Edestein,S.T.(1993).Protein Methods.Wiley-Liss,Inc.New York.

Donald M.Atlas.1996.Handbook of Microbial Media.CRC Press,United Staes.

Dow, C.S.(1982). Experiments with photosynthetic bacteria. Pp 408-422.In: Primrose,S.B. and Wardlaw, A.C.(eds.).Source book of experiment for the teaching of microbiology. Academic Press. New York.

Gest,H.,and J.L.Favinger.1983. *Heliobacterium chlorum*, an anoxygenic brownish-green photosynthetic bacterium containing a "new" form of bacteriochlorophyll. Arch.Microbiol.**136**:11-16.

Gest,H.1993.*Photosynthetic and quasi-photosynthetic bacteria.FEMS Microbiology Letters* **112**:1-6.

Getha,K.,Chong,V.C.& Vikineswary,S.1998.Potential use of the phototrophic bacterium, *Rhodopseudomonas palustris*, as an aquaculture feed. *Asian Fisheries Science* **10**,223-232.

Imhoff,J.F.,1999.*Photosynthetic Prokaryotes.*Plenum Press, New York, 53-92.

Imhoff,J.F.1999.Taxonomy,phylogeny,and general ecology of anoxygenic phototrophic bacteria.In:*Photosynthetic procaryotes* (eds.N.H.Mann and N.G.Carr),pp.53-92.Plenum Press, New York.

Imhoff,J.F.1984.Quinones of phototrophic purple bacteria.FEMS Microbiol.Lett.**25**:85-89.

Imhoff,J.F & H.G.Truper,1991. A handbook on the Biology of Bacteria: Ecophysiology,Isolation, Identification and Application. Springer Verlas, New York.

Intriago,P.,1992.The regulation of fatty acid biosynthesis in some estuarine strains of *flexibacter*.J.Gen. Microbiol. **138**:109-114.

Jensen,S.L&Jensen,A.1971.Quantitative determination of carotenoids in photosynthetic tissues.Pp.586-602.In:Pietro,A.S(ed.) Methods in *Enzymology*,Volume23,Photosynthesis.Academic Press,New York.USA.

John S.Lucas & Paul.C.Southgate.2003. Aquaculture farming aquatic animals and plants.Iowa State Press,USA.

Kobayashi,M.and M.Kondo.1984.The role of phototrophic bacteria in nature and their utilization.Proceedings of the Third International Symposium on our Environment, Singapore.

Kobayashi,M.and S.Kurata.1978.The mass culture and cell utilization of photosynthetic bacteria. *Process Biochemistry* **13**:27-30.

Michiharu Kobayashi. 1975. Role of Photosynthetic Bacteria in Foul Water Purification. *Progress in water Technology* 7,309-315.

Michiharu, K&Michihiko[2], 2001.Roles of phototrophic bacteria and their utilization. In: Photosynthetic microorganisms in environment biotechnology. Springer. Verlas.

Pfennig,N.,and H.G.Truper.1983.Taxonomy of phototrophic green and purple bacteria: a review.Ann.Micribiol.(Paris)B134:9-20.

Pillay.T.V.R.1990.Aquaculture principles and practices.Blackwell Publishing,United Kingdom.

Prasertsan,P.,Choorit,W.& Suwanno,S.1993.Isolation, identification and growth conditions of photosynthetic bacteria found in seafood processing wastewater. *World Journal of Microbiology and Biotechnology* 9,590-592.

Rehana Abidi,2003. Use of probiotics in larval rearing of new candidate species. *National Bureau of Fish Genetic Resources* Vol.III No.2.

Sasaki,K.,N.Noparatnaraporn and S.Nagai.1991. Use of photosynthetic bacteria for the production of SCP and chemicals from agroindustrial wastes.In:*Bioconversion of waste materials to industrial products* (ed.A.M.Martin),pp.225-264.Elsevier Applied Science, London.

Sasikala,C.H and Ramana,V.C.H.1995. Advance in Applied Microbiology Volume 41. Acedemic Press, New York.

Sawada,H.,Parr,R.C.&Rogers,P.L.(1997).Photosynthetic bacteria in waste treatment.Journal of *Fermentation Technology*,55(4):326-336.

Schmidt, K (1978). Biosynthesis of carotenoids. Pp 729-750. In:(Layton,R.K. and Sistrom, W.R.(eds.). The photosynthetic bacteria. Plenum Press.New York.

Shipman,R.H., Fan,L.T. & Kao, L.C. 1977.*Advance in Applied Microbiology*. Kansas State University, Kansas.

Sojka,G.A,Freeze,H.H&Gest,H.(1970).Quantitative estimation of bacteriochlorophyll in situ.*Achieves of Biochemistry and Biophysics*,136:578-580.

Sunita,M.& Chanchal Mitra,K. 1993. Photoproduction of hydrogen by photosynthetic bacteria from sewage and waste water. *J. Biosci* **18**,155-160.

Tacon,A.G.J.1990.Standard methods for the nutrition and feeding of farmed fish and shrimp. Argent Laboratories Press, Washington.