การพัฒนาการผลิตไวโอลาซีนจากแบคทีเรียทะเล *Pseudoalteromonas* sp. โดยเรซินดูดซับ

นายธีระ รักษาสุข

วิทยานิพนธ์ฉบับนี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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PRODUCTION DEVELOPMENT OF VIOLACEIN FROM MARINE BACTERIA *Pseudoalteromonas* sp. USING ADSORBENT RESIN

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A Thesis Submitted in Partial Fulfillment of the Requirements

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ธีระ รักษาสุข: การพัฒนาการผลิตไวโอลาซีนจากแบคทีเรียทะเล *Pseudoalteromonas* sp. โดยเรซินดูดซับ (PRODUCTION DEVELOPMENT OF VIOLACEIN FROM MARINE BACTERIA *Pseudoalteromonas* sp. USING ADSORBENT RESIN) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ.ดร. ปรีชา ภูวไพรศิริศาล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.ชุติวรรณ เดชสกุลวัฒนา, 46 หน้า.

ไวโอลาซีน เป็นอินโดล อัลคาลอยด์ ผลิตโดยแบคทีเรียหลากหลายสายพันธุ์ เนื่องจากไวโอลาซีนมีฤทธิ์ทางชีวภาพหลากหลาย เช่น ต้านเซลล์มะเร็ง ต้านจุลชีพ และต้านไวรัส ทำให้เป็นที่ต้องการในปริมาณสูง ดังนั้นกระบวนการผลิตจึงมีการพัฒนาอย่างต่อเนื่อง แต่กระบวนการผลิตเหล่านั้นถูกจำกัดด้วยการยับยั้งการเจริญเติบโตของเซลล์แบคทีเรียด้วยไวโอลาซีน ในงานวิจัยนี้จึงได้ประเมินประสิทธิภาพเรซินดูดซับ 5 ชนิด ได้แก่ HP20, XAD4, XAD7, XAD16 และ XAD1180 ในการเพิ่มผลผลิตไวโอลาซีนจากแบคทีเรียทะเล *Pseudoalteromonas* sp. RAD-5DP จากงานวิจัยพบว่าการใช้เรซินดูดซับชนิด XAD1180 แบบโปรยหว่าน ช่วยเพิ่มผลผลิตไวโอลาซีนได้สูงสุดจากกระบวนการหมัก โดยเฉพาะเมื่อเติม XAD1180 ปริมาณ 2% $w.v^{-1}$ หลังจากเพาะเลี้ยงเชื้อเป็นเวลา 16 ชั่วโมง วิธีการนี้สามารถเพิ่มผลผลิตไวโอลาซีนได้ถึง 2 เท่า เมื่อเทียบกับการเลี้ยงแบบปกติ และให้ผลผลิตไวโอลาซีนได้สูงสุดถึง 3.285 $g.L^{-1}$

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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TEERA RAKSASUK: PRODUCTION DEVELOPMENT OF VIOLACEIN FROM MARINE BACTERIA *Pseudoalteromonas* sp. USING ADSORBENT RESIN. ADVISOR: ASST. PROF. PREECHA PHUWAPRAISIRISAN, Ph.D., CO-ADVISOR: CHUTIWAN DECHASAKULWATANA, Ph.D., 46 pp.

Violacein is a violet indole alkaloid produced by several species of bacteria. Due to its multiple biological activities such as antitumor, antimicrobial and antiviral properties and need for large scale supply, effective production has been developed. However, the development has been limited due to end-product inhibition of violacein. In this study, five different adsorbent resins, namely HP20, XAD4, XAD7, XAD16 and XAD1180 were evaluated for efficiency to enhance violacein production from marine bacteria *Pseudoalteromonas* sp. RAD-5DP. Dispersion of XAD1180 enhanced the highest yield, particularly when 2% $w.v^{-1}$ XAD1180 was added to medium after 16 h of cultivation. This method could produce 2-fold increment in violacein production when compared with conventional fermentation and maximum yield of violacein was up to 3.285 $g.L^{-1}$.

Field of Study: Biotechnology Student's Signature .....

Academic Year: 2011 Advisor's Signature .....

Co-advisor's Signature .....

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CHAPTER I

INTRODUCTION

1.1 Background of Study

Natural products, or secondary metabolites, are small organic molecules produced by living organisms. These metabolites are not essential to sustain life but are thought to confer an evolutionary advantage because energy is required for the organisms to produce natural products. Natural products produced by plants, fungi, bacteria, protozoans, insects and animals have been isolated as biologically active pharmacophores. Well-known examples of valuable natural products used widely in today's medical and animal healthindustries include lovastatin (anticholesterolemic agent), cyclosporine A and tacrolimus or FK506 (immunosuppressive agents), paclitaxel and doxorubicin (antitumor agents), erythromycin (antibiotic), and amphotericin B (fungicidalagent) (Strohl, 2000).

Natural products are the most consistently successful source of drug leads. Over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives. It is, however, arguably still true: comparisons of the information presented on sources of new drugs from 1981 to 2007 indicate that almost half of the drugs approved since 1994 are based on natural products. (Harvey, 2008).

In recent, marine natural products is one of the major sources of bioactive compound because marine ecosystem has a higher diversity of living organisms compared to the land that provides numerous resources to human societies (Hill, 2010). Marine invertebrates are a diverse group having habitats in all ocean ecosystems, ranging from intertidal zone to the deep sea environments. Animals belong to marine invertebrates are composed of different taxonomic groups, which can be classified into several major phyla, namely, Porifera (sponges), Cnidaria (corals, sea anemones, hydrozoans, jellyfish), Annelida (polychaetes, marine worms), Bryozoa

(moss animals or sea mats), Mollusca (oysters, abalones, clams, mussels, squids, cuttlefish, octopuses), Arthropoda (horseshoe, lobsters, crabs, shrimps, prawns, crayfish), and Echinodermata (sea stars, sea cucumbers, sea urchin) (Thorpe, 2000).

To date, majority of marine natural products have been identified from marine invertebrates, of which sponges predominate (Lie, 2002). Sponges from phylum Porifera are the most primitive, evolutionarily ancient metazoan animals. A serious obstacle to the ultimate development of most marine natural products that are currently undergoing clinical trials or are in preclinical evaluation is the problem of supply. The concentrations of many highly active compounds in marine invertebrates are often minute, accounting for less than a millionth of the wet weight (Proksch, 2002). It is clear that large amounts of biomass of these invertebrates can never be harvested from nature without risking extinction of the respective species (Anand, 2006). Marine sponges are a rich source of structurally unique natural compounds, several of which have shown a wide variety of biological activities. It is well known that even excellent drug candidates from sponges are often not developed because those sponges are rare, difficult to collect or both. Sponge's harbor a rich diversity of microorganisms in their tissues and in some case constitute up to 40% of the biomass, e.g. the Mediterranean sponge *Aplysina aerophoba*. Numerous natural products from marine invertebrates show striking structural similarities to metabolites of microbial origin, suggesting that microorganisms are the true source of these metabolites or are intricately involved in their biosynthesis. Convincing evidence for the involvement of microorganisms in natural product synthesis has been complied for the tropical sponges *Dysidea herbacea* and *Theonella swinhoei*, in which the producing microbe is acyno bacterium in the former and a bacterium in the latter. Thus an alternative strategy targeting the microorganisms associated with sponges for the screening of bioactive natural products may prove to be an effective approach to circumvent the associated difficulties of dealing with the organism itself. (De Rosa, 2003).

In Thailand, Chutiwan Dechasakulwatana and co-worker from Institute of Marine Science Burapha University university in year 1999 reported the isolation of 468 strains of marine sponge associated bacteria collected from Gulf of Thailand. They discovered one interesting stain, *Pseudoalteromonas* sp. producing bioactive pigment named violacein.

Violacein (Figure 1.1) is a purple pigment and produced by several species of bacteria. It has been reported to have multiple biological activities such as bactericide, trypanocide, in addition, to apoptosis inducing property in cancer cell (De Carvalho, 2006). Which violacein produced by fermentation process there have tryptophan is precursor. Violacein biosynthesis is also dependent on the metabolism of carbohydrates. However, there have been few publications reporting on the bioprocess of violacein production by bacterial cultivation (Xing, 2009).

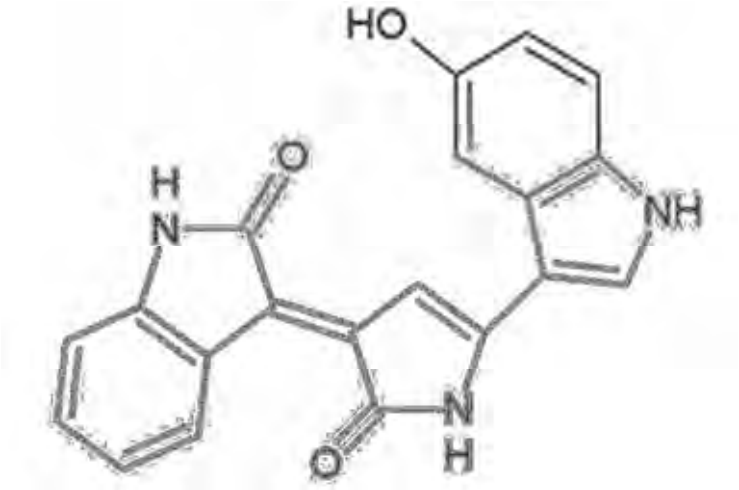

Figure 1.1 Chemical structure of violacein

1.2 Statement of Problem

Although, many report was increased violacein production with process optimization, but the result is not expected because the process was not efficiency. The problem of fermentation process for violacein production in industrial scale is low productivity of violacein production due to violacein is secondary metabolite so lead to product inhibition of bacteria cell. In addition, other problem arose from extraction and isolation process that involve the use of large amount of organic solvent and the occurrence of emulsion formed during liquid-liquid extraction (Duran, 1998)

From the above problems, in this study intend to increase productivity of violacein produced by marine bacteria *Pseudoalteromonas* sp. isolated from sponge by using absorbent

resin. This approach is based on the fact that absorbent resin specifically entraps violacein from medium while fermentation is running. A reduction in violacein from fermentation system also decreases product inhibitory effect, thus resulting in improved violacein productivity.

In addition, this method could decrease organic solvent used in extraction process and violacein can be easily recovered by soaking adsorbed resin in ethanol. The absorbent resin could be regenerate and reused in next batch fermentation.

1.3 Objectives and Scope of Study

The aim of this study is to develop fermentation system for violacein production by using absorbent resin specific to absorb violacein production for decreased end-product inhibition, and applied these in industrial scale in the future.

CHAPTER II

LITERATURE REVIEW

2.1 Marine natural product the new source of bioactive compound

More than 70% of Earth's surface is covered by oceans and marine ecosystems have the biological diversity higher than in tropical rain forests. Many marine organisms have evolved the ability to synthesize toxic compounds or to obtain them from marine microorganisms. These compounds help them deter predators, keep competitors at bay or paralyze their prey (Burkhard, 2003).

Natural products released by marine organism into the water are rapidly dilute. Therefore, the marine natural products need to be highly potent to have any effect. For this reason conduct to many researches about natural products and novel chemical from marine, with biological activities that may be useful in the quest for finding drugs with greater efficacy and specificity for the treatment of many human diseases such as anti-cancer drug Ara-C (Figure 2.1) and anti-viral drug Ara-A (Figure 2.2) (Mayer, 2000; Proksch, 2002).

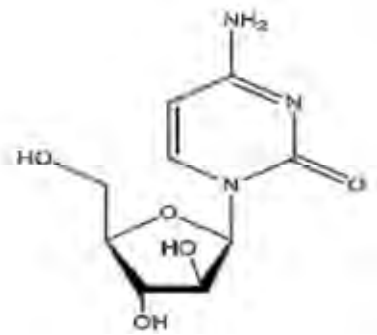

Figure 2.1 Chemical structure of Ara-C

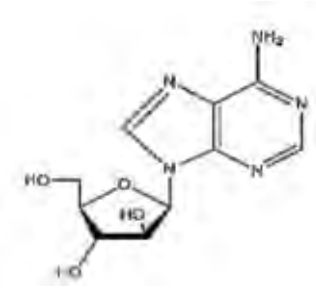

Figure 2.2 Chemical structure of Ara-A

2.1.1 Marine natural products from sponges

The marine natural product most discovered from sponge in phylum Porifera which have been used as template to develop therapeutic drugs. Spongers from phylum Porifera are the most primitive, evolutionarily ancient metazoan animals and sponge species *Spongiao fficinalis* are roasted to produce syrups for homeopathic treatments (Sipkema, 2005). Sponges are one of the richest animals with natural secondary metabolites (Belarbi, 2003). The secondary metabolites that have been isolated from sponges are in clinical trials for use as drugs and health-promoting compound (David, 2004).

Since ancient time, the human used sponge for enhancing of blood coagulation, treating wounds, bone fractures, dropsy, stomach disorders, and other infectious diseases showing that they have being continuously applying in human medicine (Gopal, 2008). For the screening of marine natural product from marine sponge there was 37 unidentified sponge species from total of 71 species have antimicrobial properties, in which samples were isolated from west coast of Baja California and Gulf of California (Rinehart, 1981).

From above topic shown sponges are very important source of marine natural product but many bioactive compounds have been discovered in sponges (Table 2.1) are only a few of these compounds have been commercialized (Faulkner, 2000). A serious obstacle to the ultimate development of most marine natural products that are currently undergoing clinical trials or that are in preclinical trial evaluation is the problem of supply (Gandhimathi, 2008) however, those obstacles may be resolved from the presence of large amounts of microorganisms within the mesophyl of many sponges which majority of marine natural products from marine sponges come from sponges associated microorganisms (symbiotic microorganism) (Hentschel, 2002;

Imhoff, 2003). If some compounds are derived from a symbiotic microorganism, culturing the microorganism could provide an improved source of the bioactive compounds.

Table 2.1 Selected sponges and their natural products showing various bioactivities (Yoo, 2001)

Sponge	Bioactive metabolites	Biological activity
Acanthella sp.	Kalihinol-A	Antibiotic
Agelas dispar	Aminozooanemonin	Antibacterial
Agelas dispar	Pyridinebetaine A	Antibacterial
Agelas mauritiana	Sceptrin	Antimicrobial
Agelas nakamurai	Ageliferine	Antibacterial
Agelas nakamurai	Debromosceptrin	Antibacterial
Agelas sp.	Agelasine	Antileukemic
Amphimedon sp.	Pyrinodemin	Cytotoxic
Aplysina aerophoba	Aeroplysinin I	Cytotoxic
Batzella sp.	Discorhabdin	Cytotoxic, enzyme inhibitor
Batzella sp.	Secobatzelline	Phosphatase inhibitor
Crella sp.	Crellastatins	Cytotoxic
Corticium sp.	Meridine	Antifungal
Cymbastela sp	Agelastatins C, D	Insecticidal
Discodermia calyx	Calyculin A	Antitumor
Discodermia kiiensis	Discodermin A	Antimicrobial
Disidea avara	Avarol	Cytotoxic
Echinoclathria sp.	Echinoclathrines	Immunosuppressive
Erylus lendenfeldi	Eryloside A	Antitumor, antifungal
Fascaplysinopsis reticulata	β-Carbolium salt	Antiparasitic
Halichondria okadai	Halichondrin B	Antitumor

2.1.2 Marine natural product from sponges associated microorganisms

Association of sponges and microbes has been highlighted in several reviews Bacteria can contribute up to 40% of the sponge biomass (equal to about 10^8 - 10^9 bacteria/g of tissue) and are probably permanently associated with the host sponge unless they are disturbed by external stress factors (Friedrich, 2001). A number of microorganism associated with sponges were found to be the sources of antibiotics and other bioactive compounds (Table 2.2) in the marine environment. It was reported that the wider biosynthetic capabilities of sponges were associated with the symbiotic microorganism (Abrell, 1997).

Table 2.2 Secondary metabolites of marine sponges associated microorganisms (Kim, 2012).

Group of Organisms	Compound class	Bioactivity
Fungi	Polyketides, cyclohexane, terpenes, amino acid derivatives	Cytotoxic, antioxidant, antimicrobial, kinase inhibitors
Bacteria	Proteins, peptides, propionic acid, indole, PAFA	Antimicrobial
Cyanobacteria	Ether, chlorinated metabolites, pigments	
Actinobacteria	Amino acid, polyketides, glycosides, methyl esters	Anti-inflammation, antimicrobial, antioxidant, hypocholesterolemic
Actinomycetes	Carotenoids	Antioxidant
Yeast	Indole derivatives	Radical scavengers

In Thailand of Dejsakulwatana and coworkers from Buurapa university in year 1999. They found difference 68 types of marine sponge and isolated 468 strains of bacteria associated with marine sponges. Biolagiacal activity testing indicated that most of isolated bacteria could produce several bioactive compounds, including violacein.

2.2 Violacein

Violacein [3-(1,2-dihydro-5-(5-hydroxy-1*H*-indol-3-yl)-2-oxo-3*H*-pyrrol- 3-ilydene)-1,3-dihydro-2*H*-indol-2-one] (Figure 2.3) is a purple pigment with molecular mass of 343.3.Violacein is produced by many genera of bacteria. It is insoluble in water, slightly soluble in ethanol, moderatelysoluble in dioxane and acetone, and soluble in DMSO, methanol and ethyl acetate. The UV–VIS spectrum exhibits maximum absorbance at 258, 372 and 575 nm in ethanol (Duran, 2007).

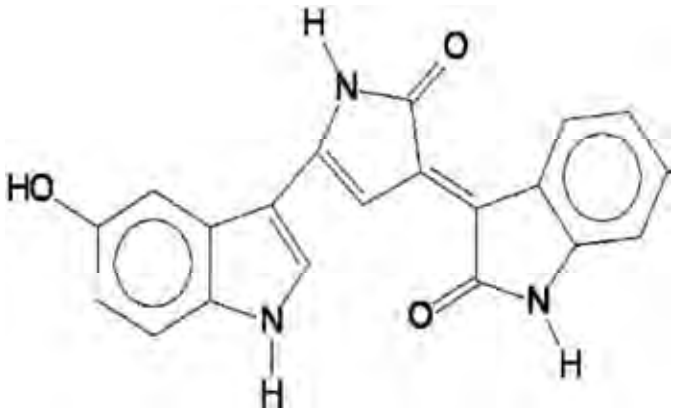

Figure 2.3 Chemical structure of violacein (Duran, 2007)

Violacein exhibits several biological activities and, at present, has gained increasing importance in industrial markets, such as in medicine, cosmetics, and textiles (Duran, 2012).

2.2.1 Violacein and its biological activities

2.2.1.1Antibacterialactivity

Violacein showed inhibitory action on the growth of Gram-positive bacteria except *Clostridium welchii*, which was moderately resistant, and there were slight inhibitions of the growth of Gram-negative bacteria, except *Neisseria meningitidis*, which was very susceptible. Then antimicrobial were test. Its conducted in 51 bacteria strains confirmed its inhibitory activity against Gram-positive bacteria and only a small effect on Gram-negative ones (De Moss, 1967; Lichstein, 1945). Further studies determination of antimicrobial activity of purified violacein from the Brazilian strain of *Chromobacterium violaceum*, indicating efficiency against both groups, with major effects on gram-positive bacteria (Duran, 1983).Violacein was produced by *Janthinobacterium lividum* exhibited antimicrobial activity against 21 bacterial strains, encompassing 18 species and ineffective on five Gram-negative strains (four species) and one strain of Bacillus. The activity was improved by increasing concentration of violacein from 2 $\mu g.mL^{-1}$ (5.8 μM) to 20 $\mu g.mL^{-1}$ (58 μM) (Naito, 1986). The mixture between violacein and deoxyviolacein isolated from the bacterium strain RT102 exhibited antibacterial effect on Gram-positive

B. *lichenniformis*, B. *subtilis*, B. *megaterium*, *Staphylococcus aureus*, and Gram-negative *Pseudomonas aeruginosa*. Violacein was able to kill bacterial cells when used at hight concentration above 15 $\mu g.ml^{-1}$ (43.7 μM). (Nakamura, 2003). Moreover, the antimycobacterial action of violacein was demonstrated by the inhibition against *Mycobacterium tuberculosis* in vitro with a minimum inhibitory concentration (MIC) of 64 $\mu g.mL^{-1}$, and a minimum bactericidal concentration (MBC) of 128 $\mu g.ml^{-1}$. Its effects were comparable with those described in the literature for pyrazinamide, a chemotherapeutic used in the treatment of tuberculosis (De Souza, 1999; Duran, 2001).

2.2.1.2 Antifungal activity

Violacein exhibited antifungal activity action on phytopathogenic fungus *Rosellinia necatrix*, the causative agent of white root rot of mulberry (*Morus* sp.) (Shirata, 1997). Violacein isolated from *Janthinobacterium lividum* exhibited also antifungal activity against with MIC (1.82 μM). *Batrachochytrium dendrobatidis* which is pathogen of amphibian and violacein (Robert, 2008).

2.2.1.3 Antiviral activity

Violacein was active against HeLa cell cultures infected with herpes simplex virus (HSV) and poliovirus. Violacein at concentration of 0.25 $\mu g.ml^{-1}$ and 0.063 $\mu g.ml^{-1}$ showed 62% and 56% of inhibitory effects aginst HSV and poliovirus respectively (May, 1991).

2.2.1.4 Antitumoral activity

Violacein exhibited antitumoral activity as the studies using a panel of tumor cell lines from the National Cancer Institute (USA). The result showed that purified violacein exhibited best activities against MOLT-4 leukemia, NCI-H460 non-small-cell lung cancer, and KM12 colon-cancer cell lines, with GI_{50} (the concentration inhibiting net cell growth by 50%) values in the range of 0.01–0.02 $\mu g.ml^{-1}$ (0.03–0.06 μM) (Melo, 2000). Moreover, violacein was able to increasing reactive oxygen species (ROS) levels in HT29 cells which ROS production could trigger of apoptosis in tumor cells, resulting in the cause of cell death.(De Carvalho, 2006).

Importantly, violacein was capable of inducing capases releasing on Ehrlich ascites tumor (EAT) cells which is the cause of apoptosis and tumor cell death [IC_{50} = 1.7 $\mu g.ml^{-1}$ (5.0 μM)]. And the first time in vito of antitumor activity of violacein. The mice treat with violacein dose of 0.1 or 1.0 $\mu g.kg^{-1}$, significantly inhibited the tumor volume and increased the lifespan of tumor-bearing mice (Bromberg, 2010).

Table 2.3 In vitro testing results of violacein in several cell lines (Duran, 2007)

Cell line	IC_{50} or GI_{50} [µg.mL^{-1} (µM)]
V79	1.7–4.1 (5.0–12)
Hum. Lymph	>3.4 (>10)
HL60	0.27 (0.8)
MOLT-4	0.01 (0.03)
NCI-H460	0.01 (0.03)
KM12	0.02 (0.06)
OCM-1	1.27 (3.69)
HT29	>3.4 (>10)
Caco-2	0.68 (2.0)
SW480	0.68 (2.0)
DLD1	0.51 (1.5)
HCT116	0.51 (1.5)
RENCA	0.2 (0.6)
EAT	1.7 (5.0)
FRhK-4	1.08 (3.14)
VERO	1.02 (2.96)
MA104	1.22 (3.55)
Hep2	1.18 (3.42)
EOMA	0.17 (0.5)

2.3.1.5 Antioxidant activity

Violacein show a significant antioxidant activity. Which the ability of violacein with b-cyclodextrin complexes prevent gastric ulceration due to protection against peroxidative damage

may be involved in such effect, together with a possible stimulatory activity of mucosal defensive mechanisms (Duran, 2003).

Sousa, and coworker in 2005 also studied the antioxidant activity of violacein on DNA. The result demonstrated that violacein prevented the strand-breaking action of methylene blue on plasmid DNA.

2.2.2 Biosynthesis and optimization method of violacein production

Since 1882, when violacein produce by *Chromobacterium violaceum* was first reported, the biological of violacein (Boisbaudran, 1882). The violacein biosynthesis pathway has been under study since then. In early studies, violacein production from *C. violaceum* was increased when passage oxygen in culturing broth. This result shows that violacein production is a respiratory production (Friedheim, 1936). Although *C. violaceum* is able to grow under both aerobic and anaerobic conditions, violacein production occurs only in the presence of oxygen (De Moss, 1959). Violacein production is involved in the regulation of tryptophan production, which at high concentrations is toxic to microorganism (De Moss, 1967). Moreover, violacein production is non-growth associated production because violacein production was stopped when *C. violaceum* grown on medium broth. Showing that violacein is not involved for growth and survival of *C. violaceum* (Sivendra, 1975; Duran, 1980).

Many review shows the studies of biosynthesis pathway of violacein production on the role of tryptophan and other indole derivatives in the stimulation of violacein biosynthesis (De Moss, 1960; Hoshino, 1987a,b; Hoshino, 1990; Duran, 1994). Momen, (2000) grew C. *violaceum* for produce violacein production by the medium containing mixture of [2-12C], [indole-3-13C] and [indole-2-13C] tryptophan, and found that all the carbon, hydrogen and nitrogen atoms of the violacein molecule come from tryptophan. This show that tryptophan appears to be the only precursor molecule in violacein biosynthesis.

In general, in the violacein-production field was the most studied from *C. violaceum.* However, violacein production also produced by other bacteria, such as *Janthinobacterium lividum (*Nakamura, 2003*), Alteromonas luteoviolacea* (Laatsch, 1984*), Pseudoalteromonas luteoviolacea* (Yada, 2008*) and Duganella* sp. B2. (Wang, 2008).

There are many reviews about optimization method for increasing violacein production (Table 2.4). The violacein and biofilm production from *Janthinobacterium lividum*was increased when using glycerol as carbon source instead of glucose as carbon source. This shows that violacein and biofilm production is controlled by the carbon source, being inhibited by glucose and enhanced by glycerol (Pantanella, 2007).

Violacein production from bacterium *Pseudoalteromonas luteoviolacea*, isolated from the surface of the marine sponge *Acanthella cavernosa*, was relatively higher at static conditions than under agitation. (Yang, 2007).

Janthinobacterium lividum XT1 strain isolated in China was cultivated at temperature lower than 20^0C due to the requirements for cell growth and violacein production. Sucrose and casein were the carbon and nitrogen sources, respectively (Lu, 2009).

In recently review, *Duganella* sp. B2 was isolated and optimized to produce a good yield (1.62 g/L) of crude violacein (Wang, 2009).

Table 2.4 Violacein production from various microorganisms (Duran, 2012)

Strain	Comments	Yields
Psychotropic bacterium RT102	Close to *J. lividum*	20^0C; 30 h; pH 6.0
		3.5 g. L^{-1}
Janthinobacterium lividum		25^0C
strain DSM1522		0.016 g.L^{-1}
J. lividum S9601		No data were found
Marine sediment bacterium		25^0C; 96 h
Pseudoalteromonas sp.		0.52 g.L^{-1}
Psychrotrophic bacterium, XT1	Close to *J. lividum*	15^0C pH 8
		0.8 g.L^{-1}
C. *violaceum*	Formerly *Bacillus violaceum*	27^0C, 144 h pH 6.2
		0.025 g.L^{-1}
Alteromonas luteoviolacea	Marine bacteria	22^0C; 72 h
		0.002 g.L^{-1}

Table 2.4 Violacein production from various microorganisms (continue)

Strain	Comments	Yields
C. *violaceum* B78	From Amazon River, Manaus, Brazil	28^0C; 24 h; pH 7.2 Liquid fermentation 3.4 $g.L^{-1}$
C. *violaceum* ATCC 553		30^0C; 72 h; pH 7.2 0.002 $g.L^{-1}$
C. *violaceum* CCT 3496		30^0C;36 h; pH 7.2 0.43 $g.L^{-1}$
Pseudoalteromonas luteoviolacea	From sponge A. cavernosa	20^0C; 240 h 0.013 $g.L^{-1}$
*Duganella*sp. B2	From China	25^0C; 40 h; pH 8.4 1.62 $g.L^{-1}$
C. *violaceum* CCT 3496		30^0C; 24 h Semisolid fermentation 2.0 $g.L^{1}$
C. *violaceum* B78	From Amazon River, Manaus, Brazil	32^0C; 36 h; pH 6.8; Semisolid fermentation 2.6 $g.L^{-1}$

2.3 Improvement of secondary metabolite production from fermentation process using adsorbent resin

The data from Table 2.5, the violacein production has fewer yields because violacein is a secondary metabolite. Which the accumulation of secondary metabolite production in the fermentation broth inhibits further production; this effect is call end-product inhibition.

However, adsorbent resin have been used successfully to reduce end-product inhibition and thus, to improve overall process efficiency.

Adsorbent resins are highly porous structures whose internal surfaces can absorb and then desorb a wide variety of different species depending on the environment in which they are used. For example, in polar solvents such as water, adsorbent resins exhibit non-polar or hydrophobic behavior and so can absorb organic species that are sparingly soluble. This hydrophobicity is most pronounced with the styrenic adsorbents (dipole moment = 0.4 debye). In non-polar solvents, such as hydrocarbons, etc. most adsorbents exhibit slightly polar or hydrophilic properties and so will adsorb species with some degree of polarity. This polarity is most pronounced with the acrylic adsorbents (dipole moment = 1.83 debye) and the phenolic adsorbents (dipole moment = 1.63 debye).The adsorption of a particular species can also depend upon its similarity to a particular adsorbent resins on the basis that "like attracts like" (Zhang, 2006) Adsorbent resins are widely used for the recovery of organic compounds and antibiotics from liquid (Arrigo, 2007; Dominguez, 2011). From describe reason adsorbent resins have been used to reduce end-product inhibition of secondary metabolites from fermentation process, which the example reviews about this was showed below.

The presence of XAD7 in culturing medium was increased thiophenes production from hairy root cell of *Tagete spatula*to 40% when compare with simple batch fermentation. From this can be explained by XAD7 was decreased feed-back inhibition effect of thiophenes production by, XAD7 can be adsorb thiophenes in medium broth (Buitelaar, 1993).

The fermentation process for prodigiosin production using HP-20 was investigated to increase the productivity of prodigiosin, which this productivity was increased to 31% when compared with conventional fermentation. The observed results can be explained by the reduction of end product inhibition from medium broth (Kim, 1999).

In this study, fermentation with adsorbent resin was investigated to increase violacein production in fermentation process, and optimal conditions for using adsorbent resin were investigated.

CHAPTER III

MATERIALS AND METHODS

3.1 Microorganism

Pure culture of *Pseudoalteromonas* sp. RAD-5D was isolated from marine sponge which identified by Institute of Marine Science, Burapha University.

This bacteria was stored in slant tube (5 ^{0}C) of modifier Zobell agar (MZA) containing peptone (1 g), yeast extract (1 g), phytone (0.5 g), sodium thiosulphate (0.2 g), agar (20 g), sodium sulfite (0.05 g), Fe-citrate (1 mL) in sea water (900 ml) and distilled water (100 mL). Medium was adjusted to pH 7.6 before autoclaving.

3.2 Growth profile and determination of violacein production

Cell grown in the modified Zobell broth (MZB) was monitored by viable count method. Sub- culture of *Pseudoalteromonas* sp. RAD-5DP (5 mL) was inoculated into MZB (50 mL) in Erlenmeyer flask and the culture were shaken at 100 rpm (IKA® HS 260 basic) and 30°C. The supernatant (2 mL) was sampled at start time and every 12 h. The one milliliter of sample was diluted in 0.85 % w.v^{-1} saline before spreading a sample on MZA surface. Another portion of sample was diluted by methanol (1 mL), and violacein concentration in resulting solution was determined using UV-VIS spectrophotometer (Hewlett Packard G1103A, Germany) at 570 nm.

The growth profile and violacein production were obtained by plotting the logarithm data of colony forming unit per milliliter (cfu.mL^{-1}) and violacein production (mg.mL^{-1}) versus culture time (h).

3.3 Preparation of adsorbent resins

Resins HP20, XAD-4, XAD-7, XAD-16, and XAD-1180 (Amberlite® Sigma-Aldrich Corp. USA) having different characteristics and properties (Table 3.1) as adsorbent resins were used in this study. Prior to use, these resins were soaked in distilled water and methanol for 24 h then drying in hot air at 60^0C after the resins were sterilized by autoclaving (Hirayama manufacturing Corp. Japan HVE-50) at 121^0C for 15 min.

Table 3.1 Typical physical properties of adsorbent resins

Resin type	Function group	Pore radian (A^0)	Surface area ($m^2.g^{-1}$)	Porosity ($mL.g^{-1}$)	Particle size (mm)	Density ($g.mL^{-1}$)
HP-20	SDVB	260	500	1.30	0.25-0.85	1.01
XAD-4	SDVB	50	725	0.98	0.24-0.84	1.08
XAD-7	acrylic	90	450	1.14	0.24-0.84	1.24
XAD-16	SDVB	100	600	1.82	0.24-0.84	1.08
XAD-1180	SDVB	300	800	1.68	0.24-0.84	1.04

3.4 Adsorption efficient of violacein on adsorbent resins

3.4.1 Adsorbtion capacity

The adsorption capacity of violacein to each resin were evaluated with 0.5 g (Weight, W) various adsorbent resins in each flask. Crude extract of violacein (0.3023 g) was added to distilled water (20 mL, V_0) and final concentration of violacein was 1.21 $mg.mL^{-1}$ (initial concentration, C_0). The violacein solution (3 mL) was transferred into each flask containing 0.5 g

of resin. The solution and resin were allowed to contact for 1 h at 30 ^{0}C under agitation 130 rpm. After adsorbtion time, the violacein concentration in supernatant (C_e , mg.mL^{-1}resin) was determined by UV-VIS spectrophotometer at 570 nm. The adsorption capacity (Q_a, mg.g^{-1}resin) was calculated according to the following expression.

$$Q_a = \frac{V_0(C_0 - C_e)}{W}$$

3.4.2 Desorption capacity

From the previous experiment in 3.4.1 the desorption capacity (Q_d, mg.g^{-1} resin) was determined by separating the solution from resin by filtration with plankton net (100 μm). The resin was eluted with 5 ml methanol (V_d, total volume of solution after elution with methanol). The violacein concentration in methanol after elution (C_d, mg.ml^{-1}) was measured by UV-VIS spectroscopy at 570 nm. The adsorption capacity was calculated according to the following expression

$$Q_d = \frac{V_d C_d}{W}$$

3.4.3 Desorption Ratio

Desorption Ratio was determined by the ratio between desorption capacity and adsorption capacity ($Q_d.Q_a^{-1}$) or calculated according to the following expression.

$$\text{Desorption ratio} = \frac{\text{Desorption capacity}}{\text{Adsorption capacity}}, \quad \left(\frac{V_d C_d}{V_0(C_0 - C_e)} \right)$$

3.5 Factors effecting violacein production in the presence of adsorbent resins

For all of fermentation were studied in Erlenmeyer flasks with various types of adsorbent resins (HP20, XAD4, XAD7, XAD16 and XAD1180). There were carried out in a 2 L Erlenmeyer flask with 1 L (MZB) working volume and fermentation at 30^0C for 48 h with an inoculum size of 10% ($v.v^{-1}$) on an orbital shaker at 100 rpm. After 48 h, various types of adsorbent resins were dispersed into medium broth and continue fermentation until 72 h.

Then cell, resin and medium will be separated by filtration. The plankton net 100 μm was used for resin separation out from cell and medium. After that, cell and medium will be separated from the fermented water by centrifugation (Hettich EBA-20, Germany) at 8500 rpm for 15 minutes and will be filtrated by the filter 0.45 μm. After the three fractions has been achieved.

The each fraction was extracted violacein production. The violacein production attached to cell extract by: the solution of methanol: chloroform (2:1) was added into cell pellet and break cell with ultrasonic (Bandelin UW2200, United instrument Co., Ltd. Germany Berlin). Then cell residue was removed by centrifugation at 5000 rpm, 11 min and the solvent was removed by evaporation (Buchi Rotavapor R-114) the result of dry crude extract of violacein.

The violacein production attached to adsorbent resins extracted by: the methanol (50 $mL.g^{-1}$) was added into adsorbent resin for 24 h. The solution was evaporated under reduced pressure until violacein was dried.

The violacein production in medium was extracted with ethyl acetate with ratio 1:5 between ethyl acetate and medium in separation funnel. The ethyl acetate layer was contained violacein which removed of ethyl acetate by evaporation in reduced pressure. The result of dry crude extract of violacein was dissolved with methanol, and this solution was adjusted the volume to 25 mL in volumetric flask. Which concentration of violacein production was

determined using UV-VIS spectrophotometer at 570 nm. The production of violacein was calculated from the standard curve obtained using purified violacein. Which amount of violacein production in each fraction and total production was reported in yield of violacein production ($g.L^{-1}$).

3.6 Fermentation with an adsorbent resin in various times

Study of effect of time to violacein production by all culturing flasks was added 2% w/v of adsorbent resin at 0, 8, 16, 24 and 32 hours after inoculum [10% ($v.v^{-1}$)] in 50 mL of MZB medium. The individual flask cultures were then growth for 48 h at 30 ^{0}C with shaking (100 rpm). After 48 h the fraction of cell, resin and medium will be separated and determination of violacein production as described in 3.5.

3.7 Effect of the amount of XAD1180 on fermentation for violacein production

The bacterial culture was grown in MZB (50 mL) under the same condition in experiment 3.6. Six groups of experiments were performed, where XAD1180 was added at the concentration of 2, 4, 8, 10, 15 and 20%w/v, respectively. After 48 h, violacein production in each fraction (cell, medium and resin) was determined as described in 3.5.

3.8 Fermentation with a bag packed adsorbent and dispersed resin

Adsorbent resin XAD 1180 was added with resin packed in plankton net bag. Adsorbent resin XAD 1180 (optimum concentration in 3.7) was contained into plankton net bag. The resin bag was added to the MZB with an inoculum size of 10% ($v.v^{-1}$) at optimum time (3.6). All

cultures flasks were incubated at 30 ^{0}C in 48 h with shaking (100 rpm). Violacein production was determined as described in 3.5, and the result was reported in total yield of violacein production.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Violacein production profile during cultivation of *Pseudoalteromonas* sp. RAD-5DP in medium broth (ZMA)

Time courses for growth of *Pseudoalteromonas* sp. RAD-5DP and production of violacein are shown in Figure 4.1. Bacterial growth reached a stationary phase by 24 h and the growth was declined after 48 h. Maximum viable cell count was measured at 48 h. Production of pigment observed after 6-7 hours of inoculation. The production of violacein increased rapidly after 12 h and static after 24 h. This result indicates that cell growth was inhibited by high concentration of violacein.

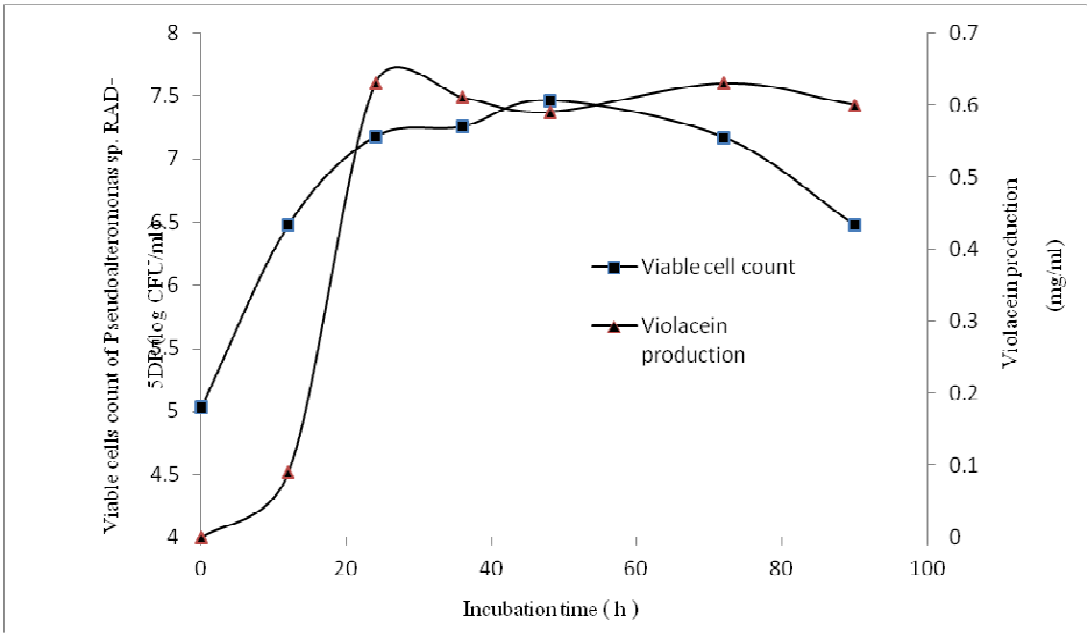

Figure 4.1 Time courses for growth of *Pseudoalteromonas* sp. RAD-5DP and the production of violacein

4.2 Adsorbent resins efficient for violacein adsorption

Five adsorbent resins (XAD4, XAD7, XAD16, XAD1180 and HP20) were tested to violacein adsorption efficiently. There are 3 parameters be use when choosing an efficient adsorbent including the adsorption capacity, desorption capacity and desorption ratio. In Table 4.1, the adsorption capacities of adsorbent resins, XAD4, XAD7, XAD16, XAD1180 and HP20 to the violacein were 4.15, 5.14, 4.60, 3.74 and 4.5 $mg_{violacein} .g^{-1}_{resin}$ respectively. The desorption capacities of adsorbent resins, XAD4, XAD7, XAD16, XAD1180 and HP20 to violacein were 0.29, 0.67, 0.33, 0.63 and 0.37 $mg_{violacein} .g^{-1}_{resin}$ respectively .

Moreover, desorption ratio is the most important parameter which is the ratio between adsorption capacity and desorption capacity. Desorption ratio of adsorbent resins, XAD4, XAD7, XAD16, XAD1180 and HP20 to the violacein were 6.99, 13.04, 7.17, 16.84 and 8.13 respectively. As the results from the experiment, XAD7 has presented the high values for both adsorption capacity and desorption capacity. However, the XAD1180 is prove to have the highest desorption ratio value which verify that the XAD1180 is the most effective adsorbent resins in this experiment.

Table 4.1 Adsorption capacity, desorption capacity and desorption ratio of violacein on various resins

Adsorbent resin	**Adsorption capacity** ($mg_{violacein} .g^{-1}_{resin}$)	**Desorption capacity** ($mg_{violacein} .g^{-1}_{resin}$)	**Desorption ratio (x100)**
XAD4	4.15	0.29	6.99
XAD7	5.14	0.67	13.04
XAD16	4.60	0.33	7.17
XAD1180	3.74	0.63	16.84
HP20	4.55	0.37	8.13

4.3 Production of violacein with various adsorbent resins

In order to investigate the relationship between resin type and violacein production, culture broth (1L) was added with five different adsorbent resins (XAD4, XAD7, XAD16, XAD1180 and HP20). After 24 h inoculation, violacein production in cell, resin and medium was determined using the method previously described.

From Table 4.2, the violacein production was increased by adding adsorbent resins XAD16 and XAD1180, except for XAD4, XAD7 and HP-20. Generally, violacein recovered in medium was at least 2-fold of that remained in cell; whereas violacein found in medium and cell of control were not significantly different. The observed results can be explained by the following reasons.

In case of adding XAD7, violacein recovered in medium broth was 0.1173 $g.L^{-1}$, which was comparable to that (0.1402 $g.L^{-1}$) of control. However, violacein secreted by *Psedoalteromonas* sp. was poorly adsorbed by XAD7. This may be caused by acrylic functional group of this resin, which produces weak affinity between resin and violacein. Therefore, high concentration of violacein gradually increased in medium broth resulted in end product inhibition and low total yield, respectively.

In case of adding XAD4, total production of violacein was improved by 8% when compared with that using XAD7. This result was linked to violacein adsorbed on XAD4, which was 2.5-fold of that adsorbed on XAD7. The elevated amount of violcein adsorbed on XAD4 was due to SDVB functional group of this resin, which produces strong affinity between resin and violacein. This reason can be confirmed by reported of Jin (2008), which they demonstrate that non-polarity adsorbent resin (XDA-1 and AB-8) in general have good affinity for weak polarity molecules (*N*-(*p*-coumaroyl) serotonin and *N*-feruloylserotonin). Which polarity of these molecules was determined by their indole ring, amide and phenolic hydroxyl group. However, total production of violacein was lower than that of control.

In case of adding XAD16, the concentration of recovered violacein in this resin was 2.0-fold higher than that in XAD4 although resin materials are identical. This result can be explained by higher by pore radian of XAD16 (100 ^{0}A) compared with XAD4 (50 ^{0}A). Which this may be

correlated with the compound easily accessible large pore radian of resin particle and the hard to absorption in the small pore radian of resin (Dutta, 1999). In addition, the resin with lager pore radian has more absorption affinity may be the compound can be easily absorbed to the deep of resin pore. Therefore, the total of violacein production was increased by 14% compared with control.

In case of adding XAD1180, similar results were observed with total production of violacein up to 29% compared with control and highest violacein adsorbed among resins used. This result was possibly due to pore radian of XAD1180 (300^0A), which was the largest in this experiment, and larger surface area (800 $m^2.g^{-1}$). However, the addition of HP-20 did not help to improve total violacein production compared with control. This result could be explained by the lowest density of HP-20 (1.01 $g.mL^{-1}$), thus preventing

the contact of resin to medium broth. From these results and previous experiment (4.2), XAD1180 was proved to be the most efficient resin in improved production of violacein in this research.

This study is the first report of using adsorbent resin in fermentation process for violacein production. The reports of violacein product from other bacteria include *Chromobacterium violaceum* (Duran, 2012). XAD1180 was also used to adsorb crocin, a carotinoid from *Gardenia jasminoides*. It showed promising adsorption capacity and selectivity towards crocin, in addition to HP-20, HPD-100A and AB-8. Yang suggested that adsorption mechanism on XAD1180 surface processed through physical rather than chemical mean, therefore facilitating desorption process and enhancing product recovery (Yang, 2009)

Table 4.2 Violacein production in the batch fermentation with various adsorbent resins

Resin type	Growth (O.D. 660 nm)	Extracted yield ($g.L^{-1}$) of violacein from			Total yield of violacein ($g.L^{-1}$)
		Broth	Cell	Resin	
Control	0.420	0.0781	0.0621	-	0.1402
HP-20	0.441	0.0633	0.0704	0.0150	0.1487
XAD-4	0.453	0.0494	0.0273	0.0533	0.1300
XAD-7	0.330	0.0691	0.0259	0.0223	0.1173
XAD-16	0.575	0.0440	0.0181	0.1070	0.1689
XAD-1180	0.618	0.0510	0.0246	0.1151	0.1907

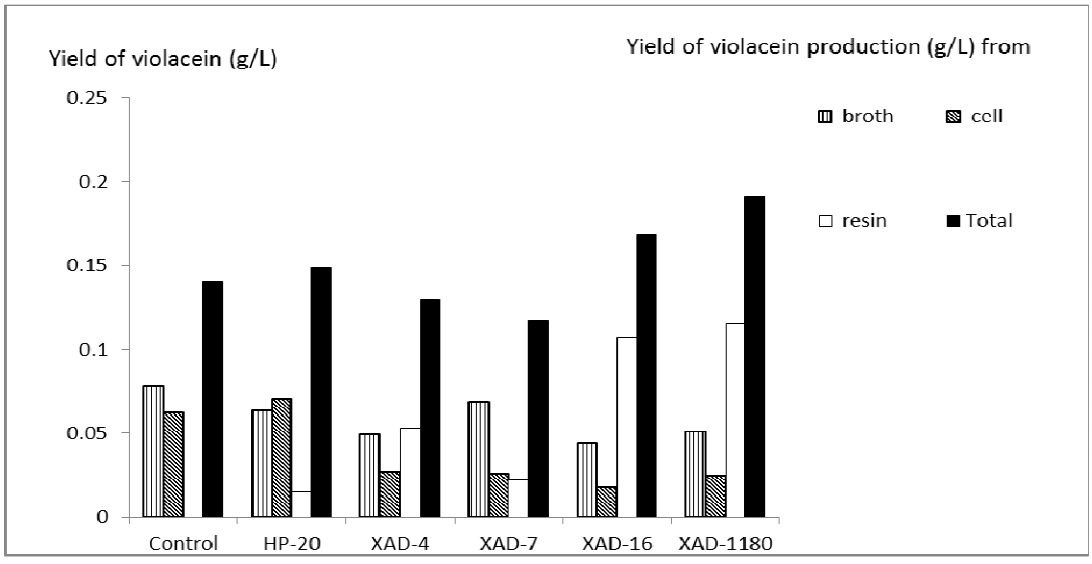

Figure 4.2 Violacein production in the batch fermentation with various adsorbent resins

4.4 Production of violacein with adsorbent resin XAD1180 in various times

To optimize violacein production in the presence of XAD1180, time of resin addition was investigated. The sterilized XAD1180 (2% $w.v^{-1}$) was added to medium culture broth at 0, 8,

16, 24 and 36 h after inoculation, and all cultures were carried out until 48 h. Table 4.3 shows that violacein production was increased from 15-47 %, compared with control, when resin was added into medium broth at 0 to 24 h. Interestingly, the highest violacein production (47%) was detected when resin was added at 16 h, which is the late log phase of bacterial growth. However, the violacein production was gradually dropped after 16 h resin addition, and the lowest violacein production was detected when resin was added at 36 h, which is lower than that of control. This result similar with the review of Kim (1999), the maximal prodigiosin production (40% increment) from *Serratia* sp. KH-95 was detected when HP-20 was added in medium broth at 10 h, which at 10 h is the production phase of bacterial. From this result, resin was added at 16 h (log phase or nearly production phase) is the optimum time to enhance yield of violacein.

Table 4.3 Violacein production when addition of adsorbent resins XAD1180 in various times

Addition time (h)	Extracted yield ($g.L^{-1}$) of violacein from			Total yield of violacein ($g.L^{-1}$)
	Broth	Cell	Resin	
Control	0.160	0.298	0	0.458
0	0.142	0.104	0.282	0.528
8	0.122	0.118	0.346	0.586
16	0.208	0.132	0.334	0.674
24	0.180	0.148	0.258	0.586
36	0.062	0.120	0.258	0.440

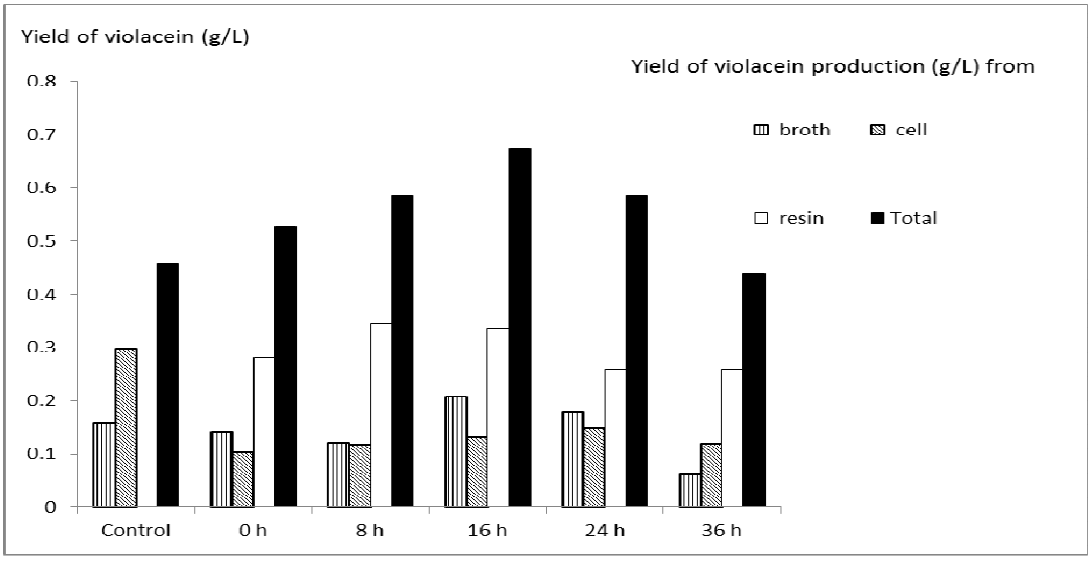

Figure 4.3 Violacein production when addition of XAD1180 in various times

4.5 Effect of the amount of XAD1180 on fermentation for violacein production

The different six amount (2, 4, 8, 10 15 and 20 %w.v^{-1}) of XAD1180 was added to culture medium broth at 16 h after inoculation, and all of these were incubated until 48 h. From the result in Table 4.4, the yield of violacein was conversely related to the amount of violacein added. The highest violacein production was detected in culture broth with 2 and 4 % w.v^{-1} XAD1180 added. However, violacein production was significantly dropped when culture broth was added XAD1180 more than 4 %w.v^{-1}; this may be caused by excess resin blocking oxygen dissolved into medium broth, , and the essentially components in medium broth has been removed by higher concentration of resin, which was cause of lowered cell growth led to low yield of violacein. (Chiang, 2007).

In conclusion, 2%w.v^{-1} XAD1180 was suitable for violacein production due to effective productivity and economic reason.

Table 4.4 Violacein production when addition of adsorbent resin XAD1180 in various concentration

Adsorbent resin concentration (% w.v^{-1})	Extracted yield (g.L^{-1}) of violacein from			Total yield of violacein (g.L^{-1})
	Broth	Cell	Resin	
Control	0.226	1.186	0	1.412
2	0.386	1.330	1.160	2.876
4	0.310	1.306	1.214	2.830
8	0.242	0.682	1.140	2.064
10	0.256	0.597	1.254	2.107
15	0.398	0.678	1.006	2.082
20	0.178	0.172	0.878	1.228

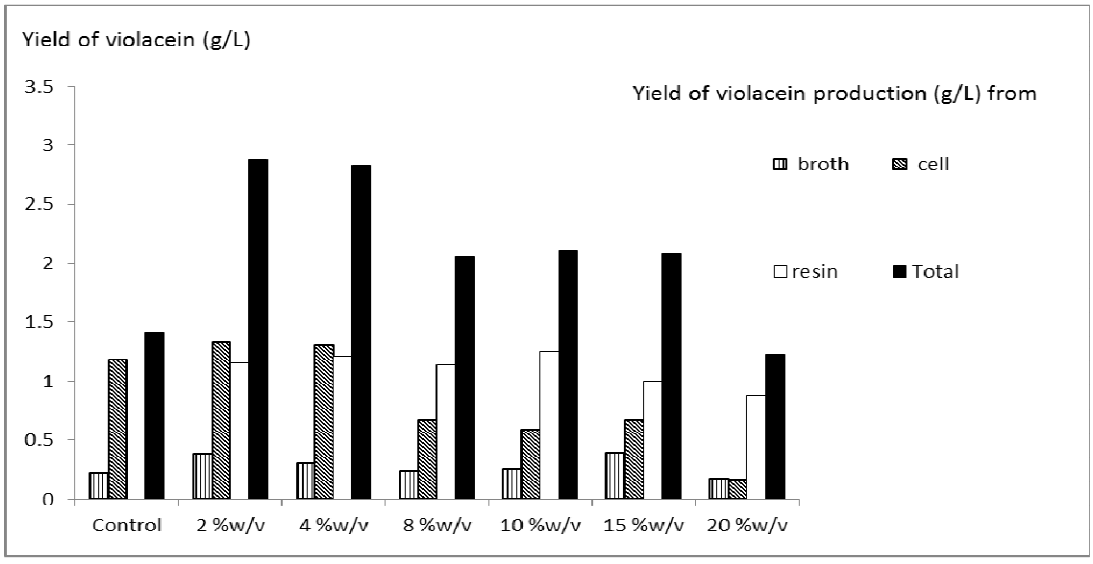

Figure 4.4 Violacein production when addition of XAD1180 in various concentrations

4.6 Comparison of violacein production between dispersed resin and bag packed resin

After optimal conditions of violacein production were obtained, we next developed how to dispersed resin in culture broth effectively. In fact, after fermentation the resin was first removed from supernatant by filtration using plankton net. To facilitate large scale production, the filtration process would be replaced by a method easily to handle. We design plankton net bag containing resin, which was immerged into instead of dispersed.

XAD-1180 (2% $w.v^{-1}$) in dispersed form and bag packed form was added into the medium broth. The result of violacein production of this experiment as shown in table 4.5, the violacein production with addition of dispersed resin in fermentation was increased to 1.16-fold when compared with control, but it using resin in packed bag form the production of violacein was decreased to 1.15-fold. The result can be explained by the following reasons. Firstly, the resin in dispersed form has surface area to contact with violacein production in broth higher than resin in bag packed form. Secondly, some violacein production can be absorbed by plankton net bag. Which it cannot eluted by methanol because violacein production permanent absorb on plankton net bag. From this result, it was proved that the use of dispersed resin form have been higher efficiency than resin in form bag packed if culturing bacterial for violacein production in Erlenmeyer flask.

Table 4.5 Violacein production when addition of dispersed resin and bag packed resin

Type of resin used	Total yield of violacein ($g.L^{-1}$)
Control	2.822
Dispersed resin	3.285
Bag packed resin	2.452

CHAPTER V

CONCLUSION

This research was carried out to improve violacein production from marine bacteria *Pseudoalteromonas sp.* RAD-5DP using adsorbent resin.

In the experiment, we found that *Pseudoalteromonas* sp. RAD-5DP started to produce violacein at an incubation time of 12 h. The end-product inhibition was observed when maximum violacein production was appeared at an incubation time of 24 h.

To apply resins suitable for violacein production, desorption ratios of different adsorbent resins, namely HP20, XAD4, XAD7, XAD16 and XAD1180, were determined. Adsorbent resins used in this experiment, could be classified into two groups: polyacrylic resin (XAD7) and polystyrene divinyl benzene (HP20, XAD4, XAD16 and XAD1180). XAD1180 had the highest desorption ratio of 16.84 towards pure violacein. In addition, cultivation of *Pseudoalteromonas* sp. in the presence of XAD1180 also produced highest yield of violacein (1.5-fold compared with control). The result proved that XAD1180 was the most suitable resin in improving violacein production.

Several important parameters for enrichment of violacein production such as, addition time and concentration of resin used were optimized. For optimum conditions, we found that addition of 2% $w.v^{-1}$ XAD1180 at an incubation time of 16 h could generate violacein 2-fold compared with control. In addition, the fermentation with resin dispersed (XAD1180) into medium broth gave better yield (3.285 $g.L^{-1}$) of violacein than that packed in plankton net bag (2.452 $g.L^{-1}$).

In summary, this is the first report of using adsorbent resin in violacein production from marine bacteria *Pseudoalteromonas* sp.. The addition of adsorbent resin clearly enhanced violacein production with maximum yield of 3.285 $g.L^{-1}$. Previously, violacein had been produced by Psychotropic bacterium RT102 (Nakamura, 2003) and *Chromobacterium violaceum* B78 (Riveros. 1989) up to 3.4 and 3.5 $g.L^{-1}$, respectively. In addition to comparable yield produce by *Pseudoalteromonas* sp., our experiment has advantage over above reports in that

Pseudoalteromonas sp. is a non-pathogenic bacteria and cultivation temperature range (28-32 ^{0}C) is suitable for producing violacein in Thailand.

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APPENDIX

APPENDIX

Culturing media

1. **Modified marine Zobell broth**

Peptone	1 g
Yeast extract	1 g
Phytone	0.5 g
Sodium thiosulphate	0.2 g
Sodium sulphate	0.05 g
Fe-citrate	1 mL

These components were added in 1 L of mixer solution between sea water (90% v/v) and distilled water (10% v/v), and adjust pH to 7.6. The medium was sterilized by autoclave at 121 ^{0}C 15 min.

2. **Modified marine Zobell agar**

Peptone	1 g
Yeast extract	1 g
Phytone	0.5 g
Sodium thiosulphate	0.2 g
Sodium sulphate	0.05 g
Fe-citrate	1 mL

These components were added in 1 L of mixer solution between sea water (90% v/v) and distilled water (10% v/v). Agar 15 g was melted into the medium and the medium was adjusted pH to 7.6. The medium was sterilized by autoclave at 121 ^{0}C 15 min.

Preparing of absorbent resin

Absorbent resin was immersed into distilled water for overnight, and the resin was soaked in 95% (v/v) methanol. The solvent was removed by pipette, and the resin was dried using hot air oven at 50 ^{0}C until the resin was dried.

Calibration curve of violacein

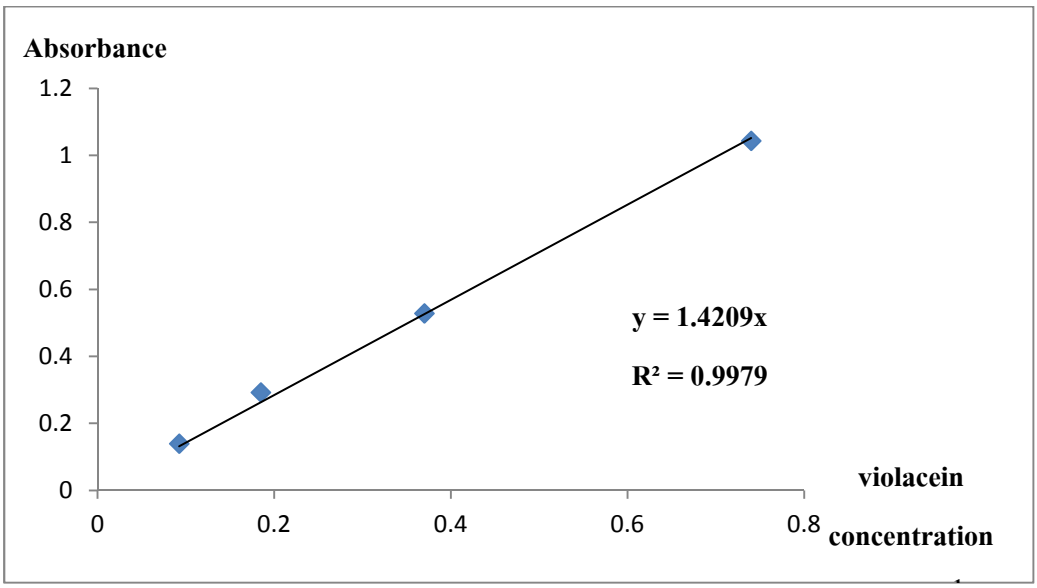

Figure I Calibration curve of violacein

Growth profile and determination of violacein production from *Pseudoalteromonas* sp. RAD-5DP in medium broth (ZMA)

Table I Result of growth profile and determination of violacein production from *Pseudoalteromonas* sp. RAD-5DP

Culturing time (h)	**Bacterial growth (log CFU.mL^{-1})**	**Violacein production**
0	5.03	0
12	6.48	0.09
24	7.18	0.63
36	7.25	0.61
48	7.46	0.59
72	7.17	0.63
90	6.48	0.60

BIOGRAPHY

Mr. Teera Raksasuk was born on 24 August 1986. He finished high school at Prachinratsadornumrong in 2003 and a graduate of Chulalongkorn University and has him B.Sc in Microbiology in 2007. Since 2009 he was studied in M.Sc. at biotechnology program faculty of science Chulalongkorn University.

Publication related with this thesis

1. Raksasuk , T., Dechasakulwattana, C. and Phuwapraisirisan, P. (2011). Production development of violacein from marine bacteria isolated from sponge using adsorbent resins. The 23rd Annual Meeting of the Thai Society for Biotechnology "Systems Biotechnology: Quality & Success", February 1-2, Bangkok, Thailand.