

ANTIBIOFILM AND ANTIBACTERIAL ACTIVITIES OF  
MARINE SEDIMENT ACTINOMYCETES FROM BIDONG  
ISLAND, TERENGGANU

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SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
UNIVERSITI MALAYSIA TERENGGANU

2014



**ANTIBIOFILM AND ANTIBACTERIA ACTIVITIES OF MARINE  
SEDIMENT ACTINOMYCETES FROM BIDONG ISLAND TERENGGANU**

**By**

**Maizatul Azrina Binti Yaakob**

**Research Report submitted in partial fulfillment of**

**The requirements for the degree of**

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**SCHOOL OF MARINE SCIENCE AND ENVIRONMENT  
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**DECLARATION AND VERIFICATION REPORT  
FINAL YEAR RESEARCH PROJECT**

It is hereby declared and verified that this research report entitled Antibiofilm And Antibacteria Activities Of Marine Sediment Actinomycetes From Bidong Island Terengganu by (Maizatulazrina Binti Yaakob), Matric No. UK26574 have been examined and all errors identified have been corrected. This report is submitted to the School of Marine Science and Environment as partial fulfillment towards obtaining the Degree of Science (Marine Biology), School of Marine Science and Environment, Universiti Malaysia Terengganu.

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## ABBREVIATION

%	-	Percentage
Min	-	Minutes
mg	-	Milligram
μl	-	Microlit
g	-	Gram
°C	-	Degree Celsius
mm	-	Millimeter
ml	-	Milliliter
rpm	-	Rotation per minutes
SCA	-	Starch Casein Agar
PDA	-	Potato Dextrose Agar
DMSO-		Dimethyl Sulfoxide
IC <sub>50</sub>	-	50% of the minimal inhibitory concentration
MIC	-	Minimal inhibitory concentration

## ABSTRACT

The study of actinomycetes from marine sediment is essential in order to determine antibiofilm agents which ecosystem friendly. Good antibiofilm agents are which may inhibit formation of biofilm without killing the organism itself. Therefore as a preliminary investigation of this type of anti-biofilm, anti-bacteria bioassay was used to check the toxicity of the compound produces by the isolated actinomycetes. Hence, these studies were carried out to isolate Actinomycetes from marine sediment and determine their antibacterial and antibiofilm activities. Then, the potential actinomycetes strains determined by 16S rRNA and morphology studies. From the present study, different actinomycetes strains (Label Actino 1-6) were recovered from marine sediment samples collected from Bidong Island, Terengganu. These were then screened for their antibacterial activity against eight pathogenic bacteria and the preliminary screening of actinomycetes was done by cross streak method. Result indicated that three actinomycetes isolates are active against both gram positive and gram negative target bacteria (Actino 1, 2 and 3) and at least, other actinomycetes strains active against one of the test organisms; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Esherichia coli*, *Vibrio alginolyticus* and *Micrococcus* sp. Then, three active isolates were further tested to obtain minimum inhibition concentration (MIC) against selected target bacteria by utilize crude extract loaded on the sterile paper disk and the result is negative. The second part of this study is to evaluate for antibiofilm activity against *P.aeruginosa*. The results demonstrated that actino 2, 3 and 5 had higher potential to inhibit biofilm formation by *P.aeruginosa*. The IC<sub>50</sub> test for antibiofilm activities was run and then, the concentration which can inhibit biofilm formation by 50% was



0.05mg/ml for pellets actino 2, 3 and 5. Whereas, 0.06, 0.07 and 0.04mg/ml supernatant concentration for actino 2, 3 and 5 respectively. According to antibacterial and antibiofilm activities of isolated actinomycetes, actino 1, 2, 3 and 5 were selected and characterized by conventional methods which was morphology study using Bergey's Manual of Systematic Bacteriology (9<sup>th</sup> edition) and also molecular identification by 16S rRNA. The results of 16S rRNA shown that Actino 1 is identified as Bacterium CR150, Actino 2 as Bacterium GZ04, Actino 3 and actino 5 as uncultured bacterium clone. This investigation revealed that the marine actinomycetes of Bidong Island sediments are potent source of novel antibiofilm and antibacterial compounds in which Actino 5 exhibited no antibacterial activity but good inhibitor for biofilm formation.

# AKTIVITI ANTIBIOFILEM DAN ANTIBAKTERIA AKTINOMISET DARI SEDIMEN MARIN, PULAU BIDONG

## ABSTRAK

Kajian aktinomiset daripada sedimen marin adalah penting untuk menentukan agen antibiofilm yang mesra ekosistem. Ejen antibiofilm yang baik adalah yang boleh menghalang pembentukan biofilm tanpa membunuh organisma itu sendiri. Kajian awal antibiofilm dan antibakteria telah digunakan untuk memeriksa ketoksikan sebatian yang dihasilkan oleh aktinomiset. Kajian dijalankan untuk mengasingkan Actinomycetes dari sedimen marin dan menentukan aktiviti antibakteria dan antibiofilm mereka. Kemudian, aktinomiset yang berpotensi dikenalpasti menggunakan 16S rRNA dan kajian morfologi. Dari kajian ini, aktinomiset yang berbeza (Aktino 1-6) telah ditemui daripada sampel sedimen marin dari Pulau Bidong, Terengganu. Saringan awal aktiviti anti-bakteria aktinomiset terhadap lapan bakteria patogenik telah dilakukan dengan kaedah coretan silang. Keputusan menunjukkan bahawa tiga aktinomiset yang aktif terhadap kedua-dua gram positif dan negatif bakteria adalah Actino 1, 2 dan 3 dan sekurang-kurangnya, aktinomiset lain aktif terhadap salah satu daripada organisma ujian; *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Vibrio alginolyticus* dan *Micrococcus sp.* Kemudian, tiga aktinomicet sampel yang aktif telah diuji lagi untuk mendapatkan minimum konsentrasi perencat (MIC) terhadap bakteria sasaran yang dipilih dengan menggunakan ekstrak mentah yang dimuatkan pada cakera kertas steril dan hasilnya adalah negatif. Bahagian kedua kajian ini adalah untuk menilai aktiviti antibiofilm terhadap *P.aeruginosa*. Keputusan menunjukkan bahawa actino 2, 3 dan 5 mempunyai potensi tinggi untuk

menghalang pembentukan biofilm oleh *P.aeruginosa*. Ujian IC<sub>50</sub> untuk aktiviti antibiofilem telah dijalankan dan kepekatan yang menghalang pembentukan biofilm sebanyak 50% adalah 0.05mg/ml untuk pelet actino 2, 3 dan 5. Manakala, 0.06, 0.07 dan 0.04mg/ml kepekatan supernatan untuk actino 2, 3 dan 5 masing-masing. Menurut aktiviti antibakteria dan antibiofilem aktinomiset, actino 1, 2, 3 dan 5 telah dikenalpasti menggunakan kaedah konvensional iaitu kajian morfologi menggunakan Manual Bergey terhadap sistematik Bakteriologi (edisi ke 9) dan juga kaedah molekular iaitu 16S rRNA. Keputusan 16S rRNA menunjukkan bahawa Actino 1 dikenalpasti sebagai bakteria CR150, Actino 2 sebagai bakteria GZ04, Actino 3 dan actino 5 klon bakteria tidak kultur. Penyiasatan ini mendedahkan bahawa aktinomiset dari sedimen marin Pulau Bidong berpotensi penghasilan sebatian yang berguna untuk antibiofilem dan anti-bakteria. Actino 5 tidak mempamerkan sebarang aktiviti antibakteria tetapi menjadi perencat yang baik untuk aktiviti pembentukan biofilm.