Respondensi Artikel Isolation and Identification of Bacteria with Antimicrobial Activities_IOP Proceeding

Gmail **candra bawanti <bawanticandra@gmail.com>**

Abstract submission

3 messages

candra bawanti <bawanticandra@gmail.com> Tue, Aug 14, 2018 at 7:08 PM
To: embrio.ipb@gmail.com

Dear Admin

Please find as attached file, the abstract submission for Embrio International Workshop.

Awaiting for the response

Best regards

abstract Candra Bawanti.docx
15K

EMBRIO IPB <embrio.ipb@gmail.com> Tue, Aug 14, 2018 at 9:33 PM
To: bawanticandra@gmail.com

Dear Ms Candra
Thank you for submitting your abstract and filling out the online registration form. We will send you the invitation or LoA after abstract selection
[Quoted text hidden]

--

EMBRIO Management Office
FPIK International Room 4th Floor
Faculty of Fisheries and Marine Science IPB
Jl. Agatis, Kampus IPB Dramaga, Bogor 16680
Website: http://embrio.ipb.ac.id/

candra bawanti <bawanticandra@gmail.com> Wed, Aug 15, 2018 at 1:09 AM
To: EMBRIO IPB <embrio.ipb@gmail.com>

Thank you, looking forward to it.
[Quoted text hidden]

Gmail **candra bawanti <bawanticandra@gmail.com>**

! Day 2 REMINDER EMBRIO International Workshop

4 messages

EMBRIO IPB <embrio.ipb@gmail.com> Wed, Oct 10, 2018 at 7:42 AM
To: Didit Abdillah <mspdidit@gmail.com>, beginersubhan@apps.ipb.ac.id, Benaya Simeon <bsimeon@wcs.org>, Eny Budi Sri Haryani <eny.bs_haryani@yahoo.com>, Firsta Kusuma <firstakusuma13@gmail.com>, ulfahfitria@umrah.ac.id, Fitriyah Irmawati <fitriyah.irmawatisaleh@gmail.com>, fredinan@apps.ipb.ac.id, Ida Nurokhmah <ida_nurokhmah@yahoo.com>, Mukti Aprian <muktikmp@gmail.com>, Niken Dharmayanti <niken.stp@gmail.com>, r.bawole@unipa.ic.id, Rudianto Adenan <rudiantoita@gmail.com>, Siska Agustina <sagustina@wcs.org>, "Cacul .." <mycacul@gmail.com>, tutisariMSP14@gmail.com, Ulfah Mardhiah <umardhiahsir@gmail.com>, Yuanike Kaber <yuanike.kaber@gmail.com>, adinda kurnia <adindkp@gmail.com>, Aditya Hikmat Nugraha <adityahn@umrah.ac.id>, hawis@apps.ipb.ac.id, atmadipoera_itk@ipb.ac.id, Yulina Pranoto <yulinaaputt@gmail.com>, Ardiansyah Nurdin <Ardiansyahnurdin96@gmail.com>, Eko Sumartono <eko_sumartono@unib.ac.id>, aulia yopa <auliayopa@gmail.com>, Budi Prabowo <budiprabowo25@gmail.com>, charles ph simanjuntak <ichthyes@gmail.com>, Dewa Adhyatma <dewa.adhyatma@gmail.com>, Djumanto <lely4192@yahoo.com>, Eko Rini <Farasfatih08@gmail.com>, Fakhrizal Setiawan <fsetiawan@wcs.org>, Fauziyah Arifin <siti_fauziyah@yahoo.com>, Eka Dharmawan Wayan <iwayanekadharmawan@gmail.com>, i.wayan.nurjaya@apps.ipb.ac.id, marisa efanny <Efannymarisa@gmail.com>, Meitin Barataulo2n <meitinbarataulo2n@gmail.com>, meutiais@apps.ipb.ac.id, Jonson Lumban-Gaol <jonson_lumbangaol@yahoo.com>, Muhammad G Salim <ghozalys@gmail.com>, Muhammad Ichsan <michsan@wcs.org>, dati_pertami@yahoo.co.id, priska widyastuti <priskacaca@gmail.com>, Puspita Pratiwi <puspitapp68@gmail.com>, risandi@umrah.ac.id, eka_dharmawan@yahoo.com, Eko Setyobudi <setyobudi_dja@ugm.ac.id>, ratih.adharini@ugm.ac.id, totok hestirianoto <Hestirianoto@gmail.com>, tri handayani <umuamel@gmail.com>, Vanessamasoleh@yahoo.com, Vitas Atmadi Prakoso <vitas.atmadi@gmail.com>, zairion@apps.ipb.ac.id, Rary Windyaswari <windyaswari84@gmail.com>, Azwin Apriandi <azwinapriandi@gmail.com>, bustamibr@yahoo.com, franky tombokan <asinjal@yahoo.com>, Darmawan <darmawan@indo.net.id>, Dessy Aqmarina <deadessy@gmail.com>, hafinuddin@utu.ac.id, Diini 80 <diinikkp@gmail.com>, Efin Muttaqin <emuttaqin@wcs.org>, Ema Hastarini <emahastarini@gmail.com>, eni kusuma <enikusuma@yahoo.com>, Ewi Pasaribu IPB <ewi_thp2016@apps.ipb.ac.id>, dali.faiza@yahoo.co.id, fmentang@unsrat.ac.id, Ginanjar Pratama <ginanjarpratama22@gmail.com>, Grace Sanger <sanger.grace@yahoo.co.id>, hennydien@yahoo.com, ummu faqih <ummufaqih@gmail.com>, mala nurilmala <malanm28@yahoo.com>, Maretty Dolorosa <ocabutarbutar@gmail.com>, Mariyana Rustam <anarustam8@gmail.com>, masbayusy@untirta.ac.id, Meita Klara Mokoginta <meitaklara@gmail.com>, Mita Julita <tathajulitha.mj@gmail.com>, mh_ryn@yahoo.com, Mohamad Gazali <mohamadgazali@utu.ac.id>, "Mulyono S. Baskoro" <baskoro.mul@gmail.com>, mutya nanda <mutyalianda996@gmail.com>, Najmah Albaar <najmahalbaar55@gmail.com>, Naufal Fakhri <naufalfakhri17@yahoo.com>, candra bawanti <bawanticandra@gmail.com>, inun nurjanah <inun_thp10@yahoo.com>, taufik best hd <besthd22@gmail.com>, prastan fauzan <championprastan@gmail.com>, nugrahenips@ugm.ac.id, Puji Hastuti Hosta <hostapuji@gmail.com>, rahman karnila <karnilarahman@gmail.com>, rahmat wiranata <wiranatarahmat@gmail.com>, Rita Marsuci <rmarsuci@yahoo.com>, robin bahari <robinbahari@gmail.com>, antoine 60 <antoine455@gmail.com>, "Roza Yusfiandayani ." <ocha_roza@apps.ipb.ac.id>, Siti irma Rahmawati <rahmawatisitiirma@gmail.com>, tati nurhayati <nurhayati7870@yahoo.com>, Titis Panindya Murti <titispranindyamurti@gmail.com>, uju sadi <ujusadi@gmail.com>, widanarni@yahoo.com, Widya Puspantari <widya.puspantari@gmail.com>, Wiwin Kusuma Atmaja Putra <wiwin.bdp@umrah.ac.id>, yuni_ph2@yahoo.com, yuniha@ipb.ac.id, Yusro Nuri F <nurifawzya@gmail.com>, laode aslan <aslaod1966@gmail.com>, laode aslin <aslin.ode@gmail.com>, Ekowati Chasanah <ekowatichasanah@gmail.com>, 廣田主樹 <hirotakazuki.0101999@gmail.com>, iwasakikazuma629@gmai.com, Masahide Uomi <07.u.masahide@gmail.com>, 山本真生 <masaki.wakayamanit@gmail.com>, Regitri Darmawan <regitri6690@gmail.com>, Rodiah Nurbayasari <rnurbayasari@gmail.com>, seijiimoto.nitw@gmail.com
Cc: P Joana Dias <joanadias77@hotmail.com>

Dear participants,

We would like to remind you (as announced yesterday) that the place for today event is at BLST (BOGOR LIFE SCIENCE TECHNOPARK) TAMAN KENCANA, Bogor.

Google maps: https://www.google.com/maps/place/PT+Bogor+Life+Science+and+Technology,+Jl.+Taman+Kencana+No.3,+Babakan,+Central+Bogor,+Bogor+City,+West+Java+16128/@-6.5869117,106.8007907,17z/data=!4m2!3m1!1s0x2e69c44218f83b43:0x4c96fff83001f908?hl=en-US

For keynote speeches session we will use Mahoni Room and coaching session we will use Mahoni Room & Gaharu Room (for further details please kindly find in the attachment files).

Thank you.

Best Regards,
EMBRIO TEAM

2 attachments

Day 2 Schedule.pdf
667K

LIST PARTICIPANT AND ROOM DAY-2 EIW.pdf
103K

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candra bawanti <bawanticandra@gmail.com> Wed, Oct 10, 2018 at 4:16 PM
To: resmi rumenta <resmi.siregar@gmail.com>

[Quoted text hidden]

2 attachments

Day 2 Schedule.pdf
667K

LIST PARTICIPANT AND ROOM DAY-2 EIW.pdf
103K

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resmi rumenta <resmi.siregar@gmail.com> Wed, Oct 10, 2018 at 5:18 PM
To: bawanticandra <bawanticandra@gmail.com>

De, jurnal yang sudah direvisi dari EMBRIO ada ngga ya?
[Quoted text hidden]

---

candra bawanti <bawanticandra@gmail.com> Wed, Oct 10, 2018 at 5:18 PM
To: resmi rumenta <resmi.siregar@gmail.com>

Belum ada masuk di email saya bu,, email yang saya terima baru itu saja
[Quoted text hidden]

Gmail **candra bawanti <bawanticandra@gmail.com>**

(no subject)

3 messages

candra bawanti <bawanticandra@gmail.com> Fri, Sep 14, 2018 at 1:02 PM
To: EMBRIO IPB <embrio.ipb@gmail.com>
Cc: Niken Dharmayanti <niken.stp@gmail.com>, arma anti <armaanti7@gmail.com>

Dear admin

Please find as attached file, the full paper journal submission for Embrio International Workshop.

Awaiting for the response

Best regard

Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (2).docx
1231K

EMBRIO IPB <embrio.ipb@gmail.com> Fri, Sep 14, 2018 at 4:30 PM
To: candra bawanti <bawanticandra@gmail.com>

Dear Ms Candra
Thank you for submitting your full paper, we will process it and inform you soon
[Quoted text hidden]
--
EMBRIO Management Office
FPIK International Room 4th Floor
Faculty of Fisheries and Marine Science IPB
Jl. Agatis, Kampus IPB Dramaga, Bogor 16680
Website: http://embrio.ipb.ac.id/

candra bawanti <bawanticandra@gmail.com> Mon, Sep 17, 2018 at 6:46 AM
To: EMBRIO IPB <embrio.ipb@gmail.com>

Thank you for your response.
[Quoted text hidden]

Gmail **candra bawanti <bawanticandra@gmail.com>**

[EIW 2018] Reminder for IOP Publication

4 messages

EMBRIO IPB <embrio.ipb@gmail.com> Mon, Oct 15, 2018 at 1:53 PM
To: beginersubhan@apps.ipb.ac.id, Benaya Simeon <bsimeon@wcs.org>, Eny Budi Sri Haryani <eny.bs_haryani@yahoo.com>, Firsta Kusuma <firstakusuma13@gmail.com>, ulfahfitria@umrah.ac.id, Fitriyah Irmawati <fitriyah.irmawatisaleh@gmail.com>, fredinan@apps.ipb.ac.id, Ida Nurokhmah <ida_nurokhmah@yahoo.com>, Mukti Aprian <muktikmp@gmail.com>, Niken Dharmayanti <niken.stp@gmail.com>, r.bawole@unipa.ic.id, Rudianto Adenan <rudiantoita@gmail.com>, Siska Agustina <sagustina@wcs.org>, "Cacul .." <mycacul@gmail.com>, tutisariMSP14@gmail.com, Ulfah Mardhiah <umardhiahsir@gmail.com>, Yuanike Kaber <yuanike.kaber@gmail.com>, adinda kurnia <adindkp@gmail.com>, Aditya Hikmat Nugraha <adityahn@umrah.ac.id>, hawis@apps.ipb.ac.id, atmadipoera_itk@ipb.ac.id, Yulina Pranoto <yulinaaputt@gmail.com>, Aprilia Winanda <lhiaku@gmail.com>, Ardiansyah Nurdin <Ardiansyahnurdin96@gmail.com>, Eko Sumartono <eko_sumartono@unib.ac.id>, aulia yopa <auliayopa@gmail.com>, Budi Prabowo <budiprabowo25@gmail.com>, charles ph simanjuntak <ichthyes@gmail.com>, Dewa Adhyatma <dewa.adhyatma@gmail.com>, Didit Abdillah <mspdidit@gmail.com>, Djumanto <lely4192@yahoo.com>, Eko Rini <Farasfatih08@gmail.com>, Fakhrizal Setiawan <fsetiawan@wcs.org>, Fauziyah Arifin <siti_fauziyah@yahoo.com>, Eka Dharmawan Wayan <iwayanekadharmawan@gmail.com>, i.wayan.nurjaya@apps.ipb.ac.id, marisa efanny <Efannymarisa@gmail.com>, Meitin Barataulo2n <meitinbarataulo2n@gmail.com>, meutiais@apps.ipb.ac.id, Jonson Lumban-Gaol <jonson_lumbangaol@yahoo.com>, Muhammad G Salim <ghozalys@gmail.com>, Muhammad Ichsan <michsan@wcs.org>, dati_pertami@yahoo.co.id, priska widyastuti <priskacaca@gmail.com>, Puspita Pratiwi <puspitapp68@gmail.com>, risandi@umrah.ac.id, eka_dharmawan@yahoo.com, Eko Setyobudi <setyobudi_dja@ugm.ac.id>, ratih.adharini@ugm.ac.id, totok hestirianoto <Hestirianoto@gmail.com>, tri handayani <umuamel@gmail.com>, Vanessamasoleh@yahoo.com, Vitas Atmadi Prakoso <vitas.atmadi@gmail.com>, zairion@apps.ipb.ac.id, indrajaya@apps.ipb.ac.id, Rary Windyaswari <windyaswari84@gmail.com>, Asadatun Abdullah <asadatun.abdullah@gmail.com>, Azwin Apriandi <azwinapriandi@gmail.com>, bustamibr@yahoo.com, franky tombokan <asinjal@yahoo.com>, Darmawan <darmawan@indo.net.id>, Dessy Aqmarina <deadessy@gmail.com>, hafinuddin@utu.ac.id, Diini 80 <diinikkp@gmail.com>, Efin Muttaqin <emuttaqin@wcs.org>, Ema Hastarini <emahastarini@gmail.com>, eni kusuma <enikusuma@yahoo.com>, Ewi Pasaribu IPB <ewi_thp2016@apps.ipb.ac.id>, dali.faiza@yahoo.co.id, fmentang@unsrat.ac.id, Ginanjar Pratama <ginanjarpratama22@gmail.com>, Grace Sanger <sanger.grace@yahoo.co.id>, hennydien@yahoo.com, ummu faqih <ummufaqih@gmail.com>, mala nurilmala <malanm28@yahoo.com>, Maretty Dolorosa <ocabutarbutar@gmail.com>, Mariyana Rustam <anarustam8@gmail.com>, masbayusy@untirta.ac.id, Meita Klara Mokoginta <meitaklara@gmail.com>, Mita Julita <tathajulitha.mj@gmail.com>, mh_ryn@yahoo.com, Mohamad Gazali <mohamadgazali@utu.ac.id>, "Mulyono S. Baskoro" <baskoro.mul@gmail.com>, mutya nanda <mutyalianda996@gmail.com>, yuliati.sipahutar@gmail.com, Naufal Fakhri <naufalfakhri17@yahoo.com>, candra bawanti <bawanticandra@gmail.com>, inun nurjanah <inun_thp10@yahoo.com>, taufik best hd <besthd22@gmail.com>, prastan fauzan <championprastan@gmail.com>, nugrahenips@ugm.ac.id, Puji Hastuti Hosta <hostapuji@gmail.com>, rahman karnila <karnilarahman@gmail.com>, rahmat wiranata <wiranatarahmat@gmail.com>, Rita Marsuci <rmarsuci@yahoo.com>, robin bahari <robinbahari@gmail.com>, antoine 60 <antoine455@gmail.com>, "Roza Yusfiandayani ." <ocha_roza@apps.ipb.ac.id>, Siti irma Rahmawati <rahmawatisitiirma@gmail.com>, tati nurhayati <nurhayati7870@yahoo.com>, Titis Panindya Murti <titispranindyamurti@gmail.com>, uju sadi <ujusadi@gmail.com>, widanarni@yahoo.com, Widya Puspantari <widya.puspantari@gmail.com>, Wiwin Kusuma Atmaja Putra <wiwin.bdp@umrah.ac.id>, yuni_ph2@yahoo.com, yuniha@ipb.ac.id, Yusro Nuri F <nurifawzya@gmail.com>, laode aslan <aslaod1966@gmail.com>, laode aslin <aslin.ode@gmail.com>, Ekowati Chasanah <ekowatichasanah@gmail.com>

Dear participants,

Thank you for participating and contributing in the 3rd EMBRIO International Workshop (EIW) 2018.

***If** you have not submitted your full paper yet, we would like to inform you that we are eagerly waiting for your full paper to be submitted. If you would like to publish your paper in the IOP Scopus Indexed Proceeding, **please finish your payment and send your full paper as soon as possible** because the editors and reviewers need time to revise your paper.*

If you have not received your reviewed paper yet, please be patient because our reviewer still revising your paper at their best.

We attached the IOP timeline and guidelines for you to follow. Please **write your paper in english and follow the IOP guidelines.**

Thank you for your kind attention.

TRANSLATION:
Para peserta yang terhormat,

Terima kasih telah berpartisipasi dan berkontribusi dalam the 3rd EMBRIO International Workshop (EIW) 2018.

***Jika** Anda belum mengirimkan makalah lengkap, kami informasikan bahwa kami masih menunggu makalah lengkap Anda untuk diserahkan. Jika Anda ingin makalah tersebut diterbitkan pada IOP Scopus Indexed Proceedings, **mohon selesaikan pembayaran dan kirim makalah lengkap Anda sesegera mungkin** karena editor dan reviewer perlu waktu untuk merevisi makalah Anda.*

Jika Anda belum menerima makalah Anda yang telah di-review, harap bersabar karena reviewer kami masih merevisi dengan upaya yang terbaik.

Bersama email ini kami lampirkan timeline dan panduan IOP untuk Anda ikuti. Mohon kirimkan makalah lengkap Anda dalam bahasa Inggris dan ikuti panduan IOP.

Terima kasih atas perhatiannya.

Best regards,
EMBRIO team
--
EMBRIO Management Office
FPIK International Room 4th Floor
Faculty of Fisheries and Marine Science IPB
Jl. Agatis, Kampus IPB Dramaga, Bogor 16680
Website: http://embrio.ipb.ac.id/

2 attachments

IOP Timeline REMINDER.docx
25K

IOP Guidelines.pdf
592K

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candra bawanti <bawanticandra@gmail.com> Mon, Oct 15, 2018 at 5:00 PM
To: resmi rumenta <resmi.siregar@gmail.com>

[Quoted text hidden]

2 attachments

IOP Timeline REMINDER.docx
25K

IOP Guidelines.pdf
592K

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resmi rumenta <resmi.siregar@gmail.com> Thu, Oct 25, 2018 at 11:17 AM
To: bawanticandra <bawanticandra@gmail.com>

Dear Candra,

Bagaimana kelanjutan makalah kita de?
Apakan sudah ada kiriman makalah yang sudah di revisi oleh reviewer?
[Quoted text hidden]

---

candra bawanti <bawanticandra@gmail.com> Fri, Oct 26, 2018 at 5:49 PM
To: resmi rumenta <resmi.siregar@gmail.com>

Sampai sekarang saya belum menerima email revisi dari reviewer bu,
[Quoted text hidden]

Gmail **candra bawanti <bawanticandra@gmail.com>**

[EIW 2018] Reviewed Paper

16 messages

EMBRIO IPB <embrio.ipb@gmail.com> Mon, Nov 5, 2018 at 5:09 PM
To: candra bawanti <bawanticandra@gmail.com>

Dear participant,

Your paper has reviewed by our reviewer. Please revise your paper based on the comments given by the reviewer, and fix your paper according to the IOP guidelines format (for example, please correct your references model like instructed in the IOP guidelines). Please kindly find the files in the attachment. We hope to receive the revised paper **before the *10th of November 2018* for the IOP publication.**

Thank you.

Translation:

Makalah Anda telah ditinjau oleh reviewer kami. Mohon revisi makalah tersebut berdasarkan saran dari reviewer, dan perbaiki makalah mengikuti format IOP guidelines (sebagai contoh, mohon koreksi cara menulis sitasi dan daftar pustaka sesuai instruksi pada IOP guidelines). Makalah dan IOP guidelines telah kami lampirkan. Kami harap dapat menerima revisi makalah sebelum tanggal 10 November 2018 untuk publikasi IOP.

Terima kasih.

Best regards,
EMBRIO team

--
EMBRIO Management Office
FPIK International Room 4th Floor
Faculty of Fisheries and Marine Science IPB
Jl. Agatis, Kampus IPB Dramaga, Bogor 16680
Website: http://embrio.ipb.ac.id/

3 attachments

Candra_Antibacterial Potential of Bacteria of The Symbiont Green Algae Caulerpa racemosa Original Pulau Lima Indonesia_RN.docx
1908K

IOP Guidelines.pdf
592K

example_Taufik_2016_IOP_Conf._Ser.__Earth_Environ._Sci._37_012062.pdf
1271K

candra bawanti <bawanticandra@gmail.com> Wed, Nov 7, 2018 at 8:53 AM
To: EMBRIO IPB <embrio.ipb@gmail.com>

Dear committe,
I'm sorry, the review journal which send yesterday it's not mine. species algae of my journal is Halimeda discoidea, not Caulerpa racemosa. please check again, so that I can revised. thank you
[Quoted text hidden]

EMBRIO IPB <embrio.ipb@gmail.com> Wed, Nov 7, 2018 at 12:30 PM
To: candra bawanti <bawanticandra@gmail.com>

Dear participant,

Thank you for your information, we will check again. Please wait for our confirmation.

Best regards,

[Quoted text hidden]

---

candra bawanti <bawanticandra@gmail.com> Wed, Nov 7, 2018 at 12:57 PM
To: EMBRIO IPB <embrio.ipb@gmail.com>

Ok, thank you.
[Quoted text hidden]

EMBRIO IPB <embrio.ipb@gmail.com> Fri, Nov 9, 2018 at 11:43 AM
To: candra bawanti <bawanticandra@gmail.com>

Dear participant,

Here we attached your reviewed paper, the IOP guidelines and an example of the proper paper. Please revise your paper according to the IOP guidelines format (for example, please correct your references model like instructed in the IOP guidelines). We hope to recieve your paper as soon as possible.

Best regards,
[Quoted text hidden]

3 attachments

Candra_Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (Halimeda discoidea) from Pulau Lima, Banten Bay, Indonesia_AH.docx
1048K

IOP Guidelines.pdf
592K

example_Taufik_2016_IOP_Conf._Ser.__Earth_Environ._Sci._37_012062.pdf
1271K

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candra bawanti <bawanticandra@gmail.com> Fri, Nov 9, 2018 at 11:44 AM
To: EMBRIO IPB <embrio.ipb@gmail.com>

Thank you, I will revise that.
[Quoted text hidden]

---

candra bawanti <bawanticandra@gmail.com> Fri, Nov 9, 2018 at 11:48 AM
To: resmi rumenta <resmi.siregar@gmail.com>

Dear Mrs. Resmi
This is my review journal from reviewer, I think I can revise and send to embrio today..
Thanks
[Quoted text hidden]

3 attachments

Candra_Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (Halimeda discoidea) from Pulau Lima, Banten Bay, Indonesia_AH.docx
1048K

IOP Guidelines.pdf
592K

example_Taufik_2016_IOP_Conf._Ser.__Earth_Environ._Sci._37_012062.pdf
1271K

---

resmi rumenta <resmi.siregar@gmail.com> Fri, Nov 9, 2018 at 2:32 PM
To: bawanticandra <bawanticandra@gmail.com>

Dear Candra,

Thank you for the information and also for journal file.

My apologize, that I can't do the revision for that paper today, because I am doing the review for may research proposal. Tomorrow I have to submit it to my supervisor.
Hope i can review on Sunday.
Thank you

regards

Resmi
[Quoted text hidden]

---

candra bawanti <bawanticandra@gmail.com> Tue, Nov 13, 2018 at 7:21 PM
To: ketut andi <ketut_andi@yahoo.com>

Selamat malam bli,

Untuk kalimat dan kata yang dicoret sudah saya ganti, saya kesulitan yang diminta memperbaiki kalimatnya bli.

Terimakasih

---------- Forwarded message ---------
From: EMBRIO IPB <embrio.ipb@gmail.com>
Date: Jum, 9 Nov 2018 10.43
Subject: Re: [EIW 2018] Reviewed Paper
To: candra bawanti <bawanticandra@gmail.com>

[Quoted text hidden]

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3 attachments

Candra_Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (Halimeda discoidea) from Pulau Lima, Banten Bay, Indonesia_AH.docx
1048K

IOP Guidelines.pdf
592K

example_Taufik_2016_IOP_Conf._Ser.__Earth_Environ._Sci._37_012062.pdf
1271K

---

ketut andi <ketut_andi@yahoo.com> Wed, Nov 14, 2018 at 12:04 PM
To: candra bawanti <bawanticandra@gmail.com>

Candra,
Setelah bli lihat sekilas paper itu, banyak yang harus diperbaiki terutama yang mendasar adalah bahasa inggris dan sistem referensi nya.
Bli mohon maaf ga bisa bantu banyak karena substansi nya bli ga paham trus utk perbaiki bahasa dan reference nya harus pakai reference application karena utk jurnal internasional yang wajib itu.

Sorry ya candra

IKetut Sumandiarsa
Lecturer in the Jakarta Fisheries University
Marine and Fisheries Human Resources Development Agency
Ministry of Marine Affairs and Fisheries

[Quoted text hidden]

---

candra bawanti <bawanticandra@gmail.com> Wed, Nov 14, 2018 at 1:55 PM
To: EMBRIO IPB <embrio.ipb@gmail.com>

Dear committe,

I'm sorry to late send my paper, because I'm wait to discuss with my lecture about the revised paper.

Thank you

On Mon, Nov 5, 2018 at 4:10 PM EMBRIO IPB <embrio.ipb@gmail.com> wrote:
[Quoted text hidden]

Candra_Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (Halimeda discoidea) from Pulau Lima, Banten Bay, Indonesia_AH.docx
1229K

EMBRIO IPB <embrio.ipb@gmail.com> Mon, Nov 26, 2018 at 1:24 PM
To: candra bawanti <bawanticandra@gmail.com>

Dear participant,

Thank you for submitting your revised paper. Please revise again the citations and references format in your paper as according to the IOP guidelines and the example attached. Send it back to us as soon as possible.

Best regards,
EMBRIO team
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I have revised my paper

thanks
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EMBRIO IPB <embrio.ipb@gmail.com> Wed, Dec 5, 2018 at 2:33 PM
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Yth. Ibu Ni Pt Sri Candra Bawanti,

Mohon perbaiki penulisan sitasi (dari nama menjadi angka), sebagaimana contoh berikut. Mohon maaf karena tim editor tidak dapat memperbaiki pada bagian tersebut, selebihnya akan kami bantu dalam proses editing termasuk perbaikan tata bahasa Inggris / grammar.

Terima kasih
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candra bawanti <bawanticandra@gmail.com> Fri, Dec 21, 2018 at 5:33 PM
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Yth. EMBRIO

Selamat sore..

Saya telah memperbaiki sitasi dari nama menjadi angka sesuai yang tercantum di referensi.

Terima kasih
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EMBRIO IPB <embrio.ipb@gmail.com> Sat, Dec 22, 2018 at 6:05 PM
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Terima kasih atas revisinya, selanjutnya akan kami lanjutkan tahapan editing dan proofreading.

Best regards,
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Antibacterial ~~Potential~~ activity of ~~Bacteria of~~ The ~~Symbiont~~ Symbiotic Bacteria of Green Algae *Caulerpa racemosa* ~~Original~~ from Pulau Lima Indonesia

Abstract. The use of ~~simbion~~symbionts ~~bacteria~~ is a most efficient way to obtain ~~a~~ antibacterial ~~producer~~ bioactive compounds without ~~needing~~ extraction ~~to~~ of the host ~~of a~~ plant. *Caulerpa racemosa* or sea grapes is known to have symbiotic bacteria. This study aim~~ed~~s to examine the ~~potential~~ antibacterial activity of symbiotic bacteria ~~clusters with~~of *Caulerpa racemosa*. The research obtained ~~2~~ two isolate~~s~~ from the inside of algae that ~~are forming~~formed an obstruent zone and ~~the results~~ of ~~the~~ antagonist ~~test~~ activity against ~~bacteria with~~ *Staphylococcus aureus* and *Salmonella typhi* bacteria~~.~~ ~~isolate with the code CaD5$_1$ is a potential isolate.~~ Further analysis of the isolate coded CaD5$_1$ ~~Bacteria activities test~~ by paper disc diffusion showed the bacteria produced~~from isolate CaD5$_1$ produces~~ an ~~obsruent~~obstruent zone by 5.08 mm ~~of~~ against *Staphylococus aureus* ~~while~~ and had~~the~~ 50% ~~MIC (~~*Minimum Inhibitory Concertration* (MIC)~~)~~ ~~from isolates CaD5$_1$~~ on both pathogenic bacteria~~, is 50%. From the observation, the isolate CaD5$_1$~~.The bacteria reached ~~its~~ their optimal growth rate at ~~to~~ 16th hour ~~up~~ to 20th hour. ~~The identification of the phenotypic isolate~~The CaD5$_1$ were identified as ~~is~~ gram negative bacteria, coccus ~~shaped~~, acid intolerant ~~toward acid~~, not producing spores, not motile, and anaerobic facultative. Based on ~~the results of matching the~~these key identification properties, the isolate CaD5$_1$ ~~referring to bacteria of~~belongs to the genus *Neisseria*.

1. Introduction

Caulerpa racemosa is one type of green algae that has not yet been to maximum efficiency be misused by irresponsible despite the fact that this abundance of his riches seeking of its existence especially in Indonesia (Coppejans, 200). *Caulerpa racemosa* create~~d~~ ~~the~~ opportunity for the ~~the~~ coastal communities as vegetables or acting as a food supplement ~~the staple of food~~ .The utilization of ~~simbion~~symbiotic microorganisms has done much of the interesting as her ability to produce a compound that reason they were active , a compound of viable of it is used to for defending against outbreak of severe pest attacks and other places , according to of Baker and Cook (1974) a compound antibacterial produced by the bacterium on generally come from secondary of a metabolite that is not used for the process of growth but there is for the self defense and the face of competition from microbes belong to another in get nutrition , a habitat of , oxygen light and others .

The purpose of this research is to identify disappearing of seaweed field sample , get isolates bacteria simbion , examine the potential a compound antibacterial from bacteria

2. Research Methods

The research are consists of two phases namely research introduction and advanced research.The preliminary study consisting from the sample, isolation symbiont bacteria, selection isolates producer antibacterial compound, advanced research be undertaken by identifying isolates bacteria simbion elected. The scheme of research methodology can be seen in figure 1

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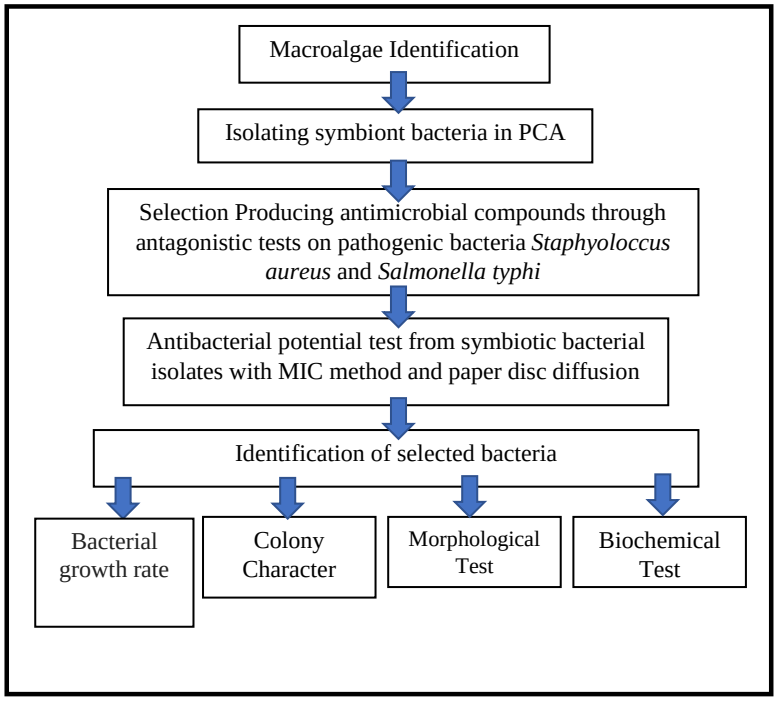
Figure 1. Scheme of Simbion *C. racemosa* Bacterial Testing Method (modification of Iyer et al., 2009)

2.1 Macroalgae Identification

Macroalgae sample identification was carried out by observing macroalgae morphology directly and



matching it with the key to determination and literature.

2.2 Insulation of symbiotic bacteria

Isolation of symbiotic bacteria is carried out by refreshing. Refreshing samples are carried out on the surface of the algae and inside of the algae (Iyer et al, 2009). Refreshing part of the algae surface aims to isolate symbiotic bacteria that are on the surface while the refresher on the inside aims to isolate symbiotic bacteria that are on the inside of the algae. Symbiotic bacterial growth is characterized by the appearance of clear zones after incubation. Colonies that produce clear zones will be isolated as symbiotic bacteria.

2.3 Selection of Symbiotic Bacteria Producing Antibacterial Compounds

To determine the ability of bacterial isolates in inhibiting the growth of pathogenic bacteria carried out qualitatively direct challenge test by spraying the isolate on the surface of the MHA (Mueller Hinton Agar) media which has been spread with the test bacteria (*Staphylococcus*

aureus and *Salmonella typhi*). Then incubated for 48 hours at a temperature of 35 ± 2° C. Isolates that form clear zones are best then inoculated to get pure culture for further testing.

2.4 Antibacterial activity test against pathogenic bacteria

Symbiotic bacteria that have been identified before, tested their antibacterial potential against pathogenic batteries of Staphylococcus aureus and Salmonella typhi using the MIC (Minimum Inhibitory Concentration) method and the diffusion method to use paper discs. Symbiotic bacterial culture in NB medium was incubated at 35 ± 2 °C for 96 hours. Separation of biomass cells with NB medium containing secondary metabolites of symbionous bacteria (supernatant) was carried out by centrifugation at a speed of 3000 rpm at 25 °C for 1 hour, the resulting supernatant was used for screening symbiotic bacteria producing antibacterial compounds.

1) MIC Method (Minimum Inhibitory Concentration)

This MIC method refers to Andrews (2001) with modifications. The supernatant was diluted in an ependorf tube, supplemented with Broth Nutrient media at concentrations of 100%, 75%, 50%, and 25%. After that, 4 petri dishes were prepared for the test bacteria S. aureus and S. typhi. The surface of the cup is then divided into two equal fields, and labeled with the name of the bacteria that will be used in each area. Each dilution that has been made is inserted into petri dishes as much as 1 ml, and then added to it 9 ml of NA / Nutrient Agar. The petri dish is then shaken slowly so that the mixture is evenly mixed. And finally let it freeze. Positive control consists of 10 ml of NA and one ose of bacteria. Negative control only contains 10 ml NA. All test tubes were incubated at 35 ± 20C for 18-24 hours. Observed the results of turbidity that occurred then compared with positive and negative controls.

2) Screening symbiotic bacteria that have antibacterial potential

The supernatant was pipetted as much as 40 µl on a sterile paper disc containing a sterile watch glass and left for 1 hour in a sterile laminary flow chamber so that the supernatant was absorbed perfectly into the paper disc. The microbial suspension of S. aureus and S. typhi test which had been prepared on NB medium was planted in a pour platting manner which was 1 ml pipetted into a petri dish and added with 10 ml liquid MHA with a temperature of 40 ° C. The medium and suspension were homogenized by shaking the petri slowly to form number 8 and left for 15 minutes in the sterile laminary flow chamber to solidify. After solidifying it, 1 piece of paper disc was inoculated with supernatant, 1 paper disc containing broth nutrient as a negative control, and 1 chlorampenicol as a positive control. The inhibitory potential was measured based on the clear zones seen around the disc paper after incubation at 35 ± 2 ° C for 48 hours using a caliper.

2.4 Identification of Simbion Bacteria

Selected symbiont bacteria were then identified including observing the growth rate by spectrophotometry, and phenotopic identification of bacteria by observing cell morphology, biochemical testing and then matching the key to bacterial determination.

3. Results and Discussion

The results of the sample refresher showed two isolates producing clear zones. The two isolates were derived from the algae in the petri dish diluting parts in algae 10^{-4} and 10^{-5} with the colony code $CaD4_2$ $CaD5_1$. The number of Caulerpa racemosa symbiotic bacterial isolates obtained in

this study is not much different compared to that found in previous studies, namely Nugroho et al. (2013) obtained 3 bacterial isolates that were symbiotic with C. racemosa from Bali waters. The results of sample refresh can be seen in Figure 2

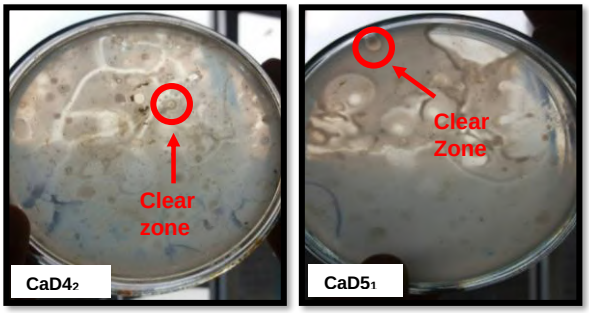


Figure 2. The isolation of *C.racemosa* symbiont bacteria on 10^{-4} and 10^{-5} dilution plates from the inside of the algae

Antagonistic test results of symbiotic bacterial isolates with pathogenic bacteria can be seen in Figure 3.

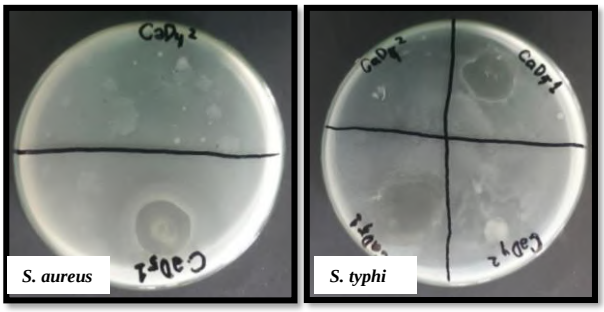


Figure 3. Antagonistic test results of symbiont bacterial isolates with *Staphylococcus aureus* and *Salmonella typhi* on PCA medium

The inhibitory zone of $CaD4_2$ isolates was challenged with *S. aureus* bacteria formed at 24 hours of observation but at 48 hours of observation the inhibition zone was lost. In contrast to $CaD5_1$ isolates that were challenged with the same pathogenic bacteria produced a wider inhibitory zone and lasted up to 48 hours of observation. None of the symbiotic bacterial isolates tested for challenge with *S. typhi* showed no growth in inhibitory zones. In general, isolates with the $CaD5_1$ code were isolates that showed the best antagonistic activity against the test bacteria when compared with the $CaD4_2$ code. Thus it can be ascertained that $CaD5_1$ isolates have antagonistic properties with pathogenic bacteria.

For the results of testing the antimicrobial activity in two methods, namely paper disc and MIC (Minimum Inhibitory Concertration) diffusion can be seen in Figures 4 and 5

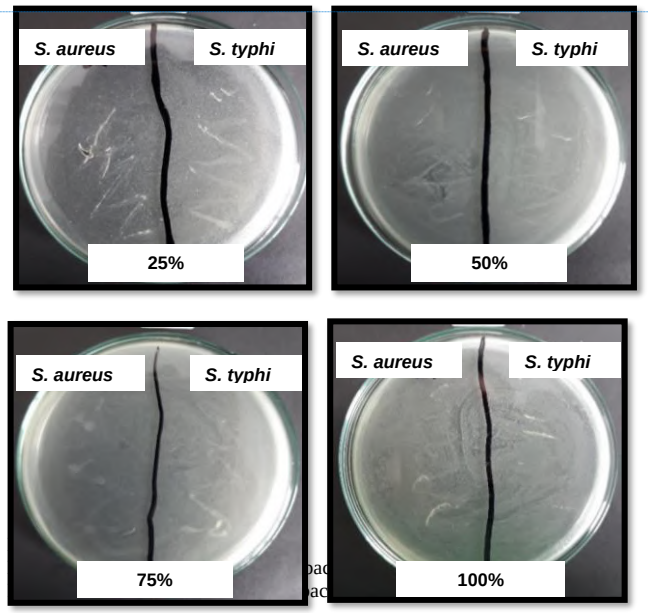


Figure 5. Results of antimicrobial tests with MIC determination

Identification of symbiont bacteria *C. racemosa* in this study included observing bacterial growth curves and phenotypic identification. Curve observation aims to determine the growth rate of symbiotic bacteria. Phenotypic identification is based on morphological observations such as cell shape and gram staining, physiological, metabolic (biochemical) or chemotaxonomic tests.

The measurement results of the bacterial growth rate of the turbidimetric method are directly proportional to the count of the cups in log cfu / ml units. The phase determination of the growth curve is based on the absorbance value curve. In the 8th hour on the growth curve shows the phase of adaptation (lag phase) that is the time needed by bacteria to adapt to their new environment. The adaptation phase lasts until 12 hours and then continues to the exponential phase (log phase) which at this time the cell will divide until the maximum number of cells is reached (a period of very rapid growth) (Pelczar and Chan, 2005). In this observation the exponential phase continues until the 16th hour. Then the optimal phase of this observation occurs from the 16th to 20th hours. The next phase is the stationary phase that occurs in the 20th to 24th hours in which the living cell of bacteria or the result of division is the same as the number of dead cells so that the number of cells lives constantly, as if there is no growth. The symbiotic bacterial death phase begins at 24 hours and so on. In certain bacteria the death phase can be seen visually by observing color changes in the media, smell, and color of mucus (Brenner, et.al 2005). CaD5$_1$ isolate growth curve can be seen in Figure 6

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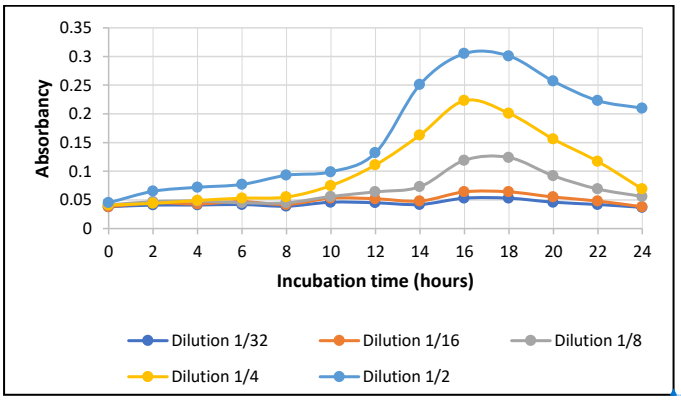

Figure 6. Growth curve of *C. racemosa* symbiotic bacteria by spectrophotometric method

From a series of phenotypic tests on $CaD5_1$ isolates, a typical character from $CaD5_1$ isolates was obtained. Characters from $CaD5_1$ isolates can be seen in the following table

Characterization	$CaD5_1$
Strain	Gram Negative
Shapes	Coccus
Acid fast	Not acid resistant
Spores	Not Spore
Motility	Negative (-)
Catalase	Negative (-)
Glucose	Positive (+)
Mannitol	Positive (-)
Gelatine	Negative (-)
Urease	Negative (-)
Citrat	Positive (+)

These characters can be used as a reference to identify further symbion bacteria. Further identification can be done using the Identification Key Table from Cowan and Steel (1974). Based on the identification key from Cowan and Steel (1974), it refers to the number 1 where the characterization indicates that the type of bacteria suspected to have similar characters is Neisseria. Specific reactions given by the bacteria tested.

4. Conclusion

The isolation results obtained two symbiotic bacterial isolates from the inside of the algae, potential isolates that can inhibit the Staphylococcus aureus test bacteria, $CaD5_1$ isolates and

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were declared bacteriocidal, based on the antibacterial activity test of selected $CaD5_1$ isolates by paper disc diffusion showed a inhibition zone of 5.08 mm while in testing the antibacterial activity with the MIC (Minimum Inhibitory Concertration) method, a minimum dose of supernatant against pathogenic bacteria was obtained as much as 50%. In observing the antibacterial growth rate it was found that bacterial growth reached the optimal phase at 16 to 20 hours. Phenotypic identification results of $CaD5_1$ isolates included in the gram negative group, the form of coccus cells, not resistant to acid, not spore forming, not motile, and Facultative anaerobes. Based on the identification key refers to the genus Neisseria.

5. Reffrence

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Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (*Halimeda discoidea*) from Pulau Lima, Banten Bay, Indonesia

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Abstract. Bacteria associated with seaweed ~~are~~ were involved in the production of metabolites associated with their host, so they tend to have nearly the same metabolites to defend themselves or as antibiotics. The aim of this study was to isolate and to identify bacteria with antimicrobial activities from the marine green algae (*Halimeda discoidea*). Laboratory analysis was isolation of bacteria, selection of bacterial with antimicrobial activities, antibacterial activity test following Priharta (2008) and Minimum Inhibitory Concentration (MIC) following Purnama (2010). ~~Our r~~Result showed that six bacterial isolates were isolated from outside and inside of the algae, which was have antimicrobial activities. One was selected for further work which was have more antimicrobial activities. The bacteria showed antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) but not against Gram-negative bacteria (*Escherichia coli*). The bacterial growth curve ~~in the spectrophotometric and TPC methods the~~ was optimum phase at the 10th hour to 16th hour. Microscopic analysis and biochemical tests showed that isolated bacteria was a rod-shaped Gram-negative, acid-free, non-endosporous, reacted positively to gelatin, citrate and carbohydrate, reacting negatively on motile, and urease. We conclude that the bacteria isolated from green algae which wa have more antimicrobial belongs to the genus *Pseudomonas guezennei*.

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Keywords : Symbiont bacteria, *Halimeda discoidea*,*Pseudomonas guezennei*.

1. Introduction

Seaweed is one of the largest producers of biomass in the marine environment. They produce various active chemical metabolites in their environment, potentially as a tool to protect themselves against other sedentary organisms. Biogenic molecules infuse unique chemical diversity in seaweed compared to other plants(Divakaran and Sreejamol, 2014). In addition to its primary economic value, the secondary metabolite content of seaweed has the potential to produce diverse bioactive metabolites as antibacterial, antiviral, antifungal and cytotastic (Zainuddin and Malina, 2009).

The inappropriate selection of antibiotics can have a negative impact, like the emergence of bacterial resistance and low antibiotic effectiveness against certain bacteria. So that further research is needed to find new antibacterials.

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Pereira and Gama (2008) reported that more than 300 secondary metabolites had been identified from green algae including the *Bryopsidales*. Green algae*Halimeda* contains epigallocatechin (a type of antioxidant) in very high amounts (28µg / g dry weight). Furthermore Yoshie *et al* (2002) *Halimeda* is also rich in minerals such as Fe, Mn, Zn and Cu. Minerals are the important component of antioxidant enzymes. Zn, Cu and Mn minerals are essential minerals for superoxide dismutase activity.

2. Materials and Methods

2.1 Material

Following material were used in the analysis include Green algae (*Halimedadiscoidea*), pure culture of *S.aureus*, pure culture of *E.coli*, aquadesh, Agar, Nutrient Broth (Oxoid), Plate Count Agar (Oxoid), Mueller Hinton Agar (Oxoid), sterile sea water, 70% alcohol , 95% alcohol, crystal violet, iodine, safranin, immersion oil, carbolfuxin dyestuff, alcoholic acid, Methylene Blue, Malachit Green solution, filter paper, tissue / cloth, cotton and paper disc.

2.2 Sampling

Samples of Green algae (*Halimeda* discoidea) was obtained from two stations (S 6º0'5.6356 and "E 106º9'15.3684) at Pulau Lima, Banten Bay. The distance from station 1 to station 2 was about 100 meters (Figure 1). Taking samples at two stations, taking supporting data such as temperature of sea water, pH, salinity and current strength.

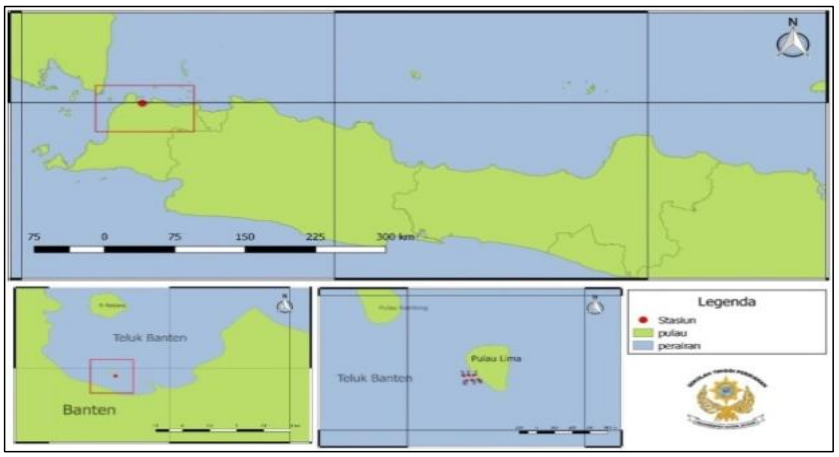

Figure 1. Sampling location

2.3 Isolation of symbiotic bacteria that produce antibacterial compounds

Isolation bacteria from surface of Algae : 15 grams of algae are rinsed with 30 ml of sterile sea water. Then the rinse water is was added 30 ml of NB medium and then shaken using a shaker at room temperature for 24 hours.

Isolation bacteria from surface of Algae : 15 ml of sterile sea water added to 15 grams of rinsed algae then crushed. The suspension was put into 30 ml of NB media and shaken using a shaker at room temperature for 24 hours.

Samples that have been refreshed on NB media were diluted into 1:10 to 1:100.000 dilution, and each dilution was grown on PCA media, following 48 hours incubation at 35° C for 48 hours. Bacteria that have a stable clear zone were isolated on strength seawater according Rippka (1998).

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2.4 Qualitative challenge test

To identify the pathogenic antimicrobial activity of isolate bacteria, qualitative challenge teset was done (Marinho *et al*, 2009 modification). One ml of test bacteria (*Staphylococcus aureus* and *Escherichia coli*) was mix into 10 ml sterile liquid Plate Count Agar and let it for about 15 minutes to become solid. Bacteria isolated from *Halimeda discoidea*. Sprayed on the surface of the media that has been spread with the test bacteria (*Staphylococcus aureus* and *Escherichia coli*). Following48 hours incubation at 35 ° C.

2.5 Antibacterial test

1) Paper disc method

The supernatant (40 µl)was placed on a sterile paper disc containing a sterile watch glass and left for 1 hour in a sterile laminary flow chamber so that the supernatant was absorbed perfectly into the paper disc.

The microbial suspension of *S. aureus*and *E. coli* test which has been prepared on NB medium is first grown in pour platting. The medium and suspension were homogenized by shaking the petri dish slowly, and left for 15 minutes in the sterile laminary flow chamber to become solid. One piece of paper disc was inoculated with supernatant, 1 paper disc containing Nutrient Broth as a negative control, and 1 piece for chloramphenicol as a positive control. The inhibitory potential was measured based on the clear zones seen around the paper disc after incubation at 35°C for 24, 48 and 72 hours (Priharta, 2008).

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2) Minimum Inhibitory Concentration (MIC)

The supernatant is diluted with 100%, 75%, 50%, and 25% of Nutrient Broth. Petri dishes were prepared for *S. aureus*and *E. coli* test bacteria (4 plates each). Each dilution (1 ml) is inserted into petri dishes, and mix with 9 ml of Nutrient Agar in the petri dish. Positive control consists of 10 ml of NA and one ose of bacteria. Negative control contains 10 ml of NA. All test tubes were incubated at 35^0C for 24 and 48 hours (Purnama, 2010).

2.6 Identification of selected symbiont phenotypes and bacterial genotypes

1) Spectrophotometric growth curve

Stratified dilution of bacterial symbiontswas into 1/2, 1/4, 1/8, 1/16, 1/32. The wavelength is set at 686 nm. The cuvette was inserted into *Spectrophotometer*. Calculation formula with MC Farland equation (Oktafiani, 2012) as follows.

$$Y = 2.62 \times 109\ \mu - 6.39 \times 107$$

y = density (CFU / ml) and µ = adsorbs (Â)

2) TPC growth curve

One ounce of bacterial culture that had been refreshed on the PCA medium was inoculated into 13 tubes containing 9 ml of sterile Nutrient Broth and incubated. Observation of the TPC value was carried out every 2 hours during 24 hours. Bacteria was planted into Nutrient media then incubated at 35° C for 24 hours.

Observation of the colony character was carried out with Gram staining, spore staining, Z-Neelsen staining, biochemical tests including motility, gelatin, citrate, urease, and carbohydrate. Estimation of bacterial types is based on the identification key of Cowan and Steel(1993) and DNA molecular test.

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Samples of bacteria grown on PCA medium were incubated at 35°C for 3 days and then checked for purity and then used for the molecular identification process. Identification was performed using molecular analysis based on 16S rDNA fragments in bacteria. Isolation of bacterial genomic DNA was performed using PCR colony method (Packeiser et al., 2013).The supernatant was taken and used as a DNA template on PCR amplification. Symbiont bacteria species was determined by molecular testing. The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. Molecular identifica-tion was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the modi-fied GES method (Pitcher et al., 1989).The analysis of nitrogen base se-quence readings was done using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data was trimmed and assembled using the BioEdit program (BIOEDIT, 2005). Sequencing data that has been assembled in BLAST with genome data that has been registered in DNA Data Bank of Japan (DDBJ, 2017).

3. Result and Discussion

3.1 Isolation of bacteria

Of the 90 petri dishes incubated, 6 colonies shown inhibotory activity characterized by a clear zone around the colony and 3 of which originate from the outside the green algae, while the other 3 colonies are from the nside of the algae (Figure 2). Bacteria isolates from teh inside of brown algae have better antibacterial activity than bacterial isolates from its surface was reported by Sartika *et al* (2014). Symbiosis that produces secondary metabolites can be triggered because of biotic environmental barriers (Nofiani, 2005).

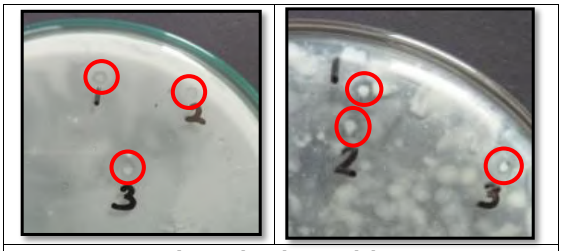

Figure 2. Colonies that shown inhibitory activity

3.2 Qualitative challenge test

Clear zone appeared at 24 hours incubation and stable until 48 hours in 37ºC. The results of the direct challenge test was known that only 1 isolate of the 6 isolates tested bacteria showed consistency of inhibitory activity against test bacteria (*S. aureus*and *E. coli*) (Figure 3). This isolate showed the best antagonistic activity against test bacteria when compared to other isolates and were chosen for further analysis.

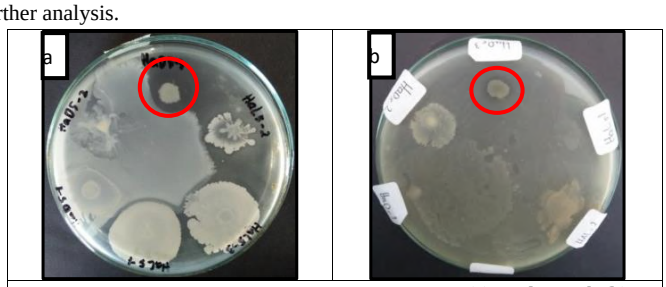

Figure 3. Antagonistic activity against *S. aureus*(a), and *E. coli* (b)

3.3 Antibacterial test

1) Paper disc method

Positive control has a wider clear zone compared to the clear zone by the supernatant in both test bacteria. Supernatant shown 6 mm in diameter clear zone and chloramphenicol as a positive control shown 17.68 mm in diameter clear zone at*Staphylococcus aureus* test bacteria. While at *Escherichia coli*, the supernatant did not shown clear zones and chloramphenicol shown 13.84 mm in diameter clear zone (Figure 4). So that the supernatant is bacteriostatic to Gram positive bacteria. The negative control did not shown clear zones two test bacteria. Lay (1994) said that chloramphenicol at a dose 0,03 mg will effectively produce a zone of inhibition of up to 18 mm so that bacteria can be said to be sensitive to antibiotics, 13-17 mm including intermediate, whereas if <12 mm is said to resistant to antibiotics.

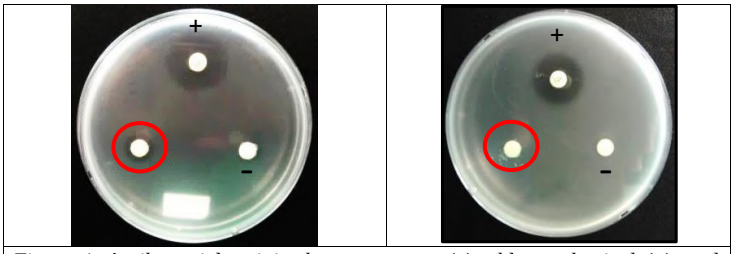

Figure 4. Antibacterial activity by supernatant (o), chloramphenicol (+), and negative control (-) against *S. aureus* (a) and against *E. coli* (b)

2) Minimum Inhibitory Concentration (MIC) test

The application of antibacterial compounds produced by selected seaweed asociated bacteria showed different antibacterial activity on both test bacteria (*S.aureus* and *E.coli*). The *S. aureus* bacteria began to be inhibited by 25% of supernatant, while *E.coli*appeared to be stunted by 75% supernatant (Figure 5). Gram negative has a thinner cell wall consisting of 10% peptidoglycan and high lipid content (11-12%). While Gram-positive bacteria have thick cell walls consisting of 60% - 100% peptidoglycan and low lipid content (1-4%) (Pelczar and Chan, 1986).

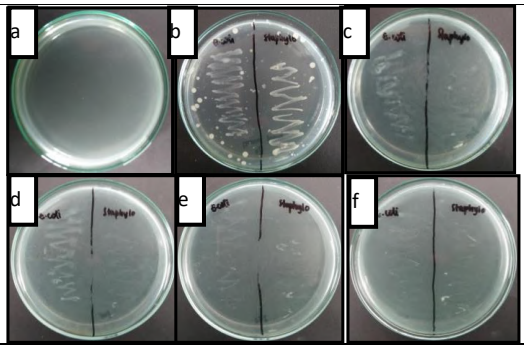

Figure 5. (MIC test results (Minimum Inhibitor Concentration), negative control (a), positive control (b), supernatant 25% (c), 50% (d), 75% (e), and 100% (f)).

The antibiotic MIC test against microbes is used to determine the sensitivity of microbes to antibiotics. MIC values are contrary to the sensitivity of the microbes tested. The lower the MIC value of an antibiotic, the greater the sensitivity of bacteria (Jawetz et al., 1996).

3.4 Identification of selected symbiont phenotypes and bacterial genotypes

1) Growth of bacteria

The lag phase or slow phase is experienced by bacterial symbiontsat 0 to 8 hours growth in both methods. This phase was time consume because thesymbiont bacteria need time for adaptation to the environment or new medium. The adaptation phase was affected by several factors, including the medium and the growth environment and the number of inoculums(Hamdiyati, 2012).

The log phase (exponential phase) of symbiont bacteriashown at 10^{th}hours of incubation for aall growth curves. In this phase, bacterial cell numbers doubles during each unit time period. This is because the bacteria have been able to adapt to the environment or medium. Generation time of most bacteria is between 20 minutes to 20 hours, but the rate of exponential growth varies between bacteria genera and also influence by culture conditions (Perry et al, 2002).In the 16^{th} to 22^{nd} hours of

incubation was the stationary phase. This phase is the phase where the number of populations that grows is the same as the number of dead cells. During stationary phase, some of the cell die and lyse. These lytic products of cells can provide nutrients for other cells, and these divided and replace the dead ones. The individual in this phase differ in certain biochemical components from cells in the exponential phase (Perry et al, 2002).

The number of cells at 24 hours incubations shows a decrease (decline or death phase). Decline or death phase is the phase when the death rate exceeds the rate of reproduction, the cell population is in the death or decline phase (Perry *et al*, 2002). Futhermore, Hamdiyati (2012) said that in the death phase, the population of microorganisms has begun to experience death becausnutrient in the medium was almost exhausted and the reserve energy in the cell was exhausted (Figure 6).

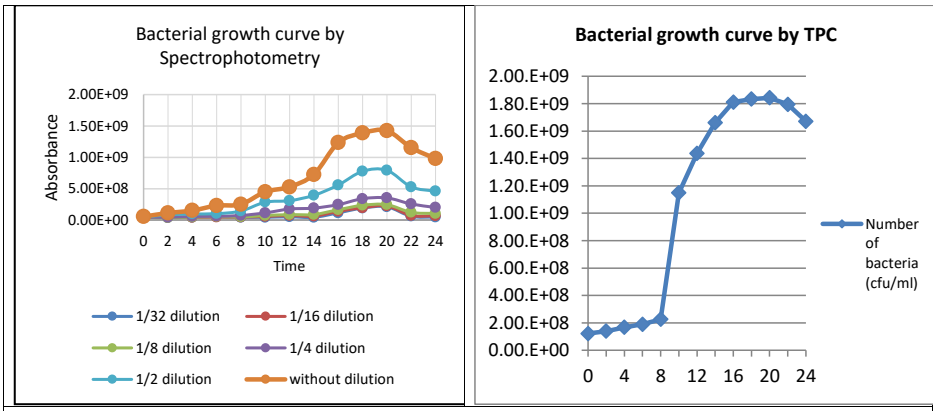

Figure 6. Growth curve of bacteria during incubation

2) Morphology and Biochemistry Test

Selected symbiont bacteria at 24 hours incubation, inoculated into the Nutrient Brothmedium and also at agar slant. In liquid (NB) media the nature of bacterial growth is on the surface, below the surface and bottom of the tube which can be seen clearly. The appearance of the medium with bacterial inoculation appears cloudy and forms a pelicle (thick membrane) on the surface of the medium. In various layers of surface can be seen various kinds of growth, in some microbes visible formation of thick pellicles on the surface layer (Lay, 1994).

The growth of symbiotic bacterial isolates was observed in order to tilt looks fertile, looks like a tree and follows the direction of the scratch. Other characteristics that can be observed were having a marginal edge, when viewed from the side it looks convex, its consistency is not like mucus but quite thick and slightly greenish in color. Microbes that thrive on the surface of a medium will look more opaque compared to infertile growth (Lay, 1994).

Gram staining shows red cells or Gram negative and rod shaped. Gram negative bacteria have thinner cell walls (lipid content greater than peptidoglycan) than cell walls of Gram positive bacteria (Figure 7a). Spore staining shows that symbiotic bacteria do not produce spores. Evidently with whole red vegetative cells without green cores (Figure 7b). As for bacteria with the ability to produce spores, vegetative cells will be red in green in the middle. Bacterial spores can be stained by heating. Heating causes the outer layer of the spore to expand, so that the dye malachite green can enterLay (1994). The Z-Neelsen staining showed that symbiotic bacteria belonged to a group of bacteria that were not acid resistant. Shown with blue bacterial cells, while if the acid resistant bacteria will be red (Figure 7c).

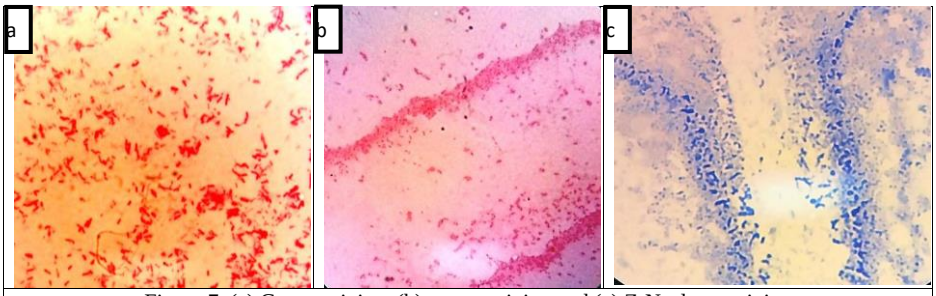

Figure 7. (a) Gram staining, (b) spore staining and (c) Z-Neelsen staining

The characteristics of known symbiont bacteria from microscopic identification and biochemical tests include the form of rods / bacilli, acid-resistant, non-spore-forming, non-motile, aerobically grown, and positive carbohydrate tests. Based on the identification key from Cowan and Steel (1993) referring to a group of bacteria that are thought to have similar characteristics, to*Pseudomonas*.

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3) 16 rDNA Molecular Examination

16S rDNA molecular examination was carried out to determine species of symbionic bacteria. Molecular identification is carried out through partial genetic analysis of 16S rDNA. PCR amplification results from 16S region of bacterial ribosome DNA. The sample PCR product was visualized using a gel documentation system measuring 1500 bp and the negative control did not contain the following DNA bands. The DNA tape used was relevant to PCR products produced around 1500 base pairs. Nitrogen base sequences sorted from symbiont bacterial isolates can be seen in Figure 8.Based on secondary data obtained from IPBCC, sequence analysis of 16S rDNA isolates from European Union-A was identified as *Pseudomonas guezennei* which had similarities in 99%, at a maximum score of 2117 (total score of 2117), demand for 100% coverage, value of E 0.0 against bacterial taxa.

TTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTTCGAAA
GGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAG
ATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCCGTAACTG
GTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG
TGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC
GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTAC
CAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAAGCCCCGGGC
TCAACCTGGGAATTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGT
GTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCAGAGAACTTT
CCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGT
GGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGG
TGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCG
GAATCGCTAGTAATCGGA

Figure 9. The sequence of nitrogen bases sequenced from symbiont bacteria
A= Adenine, T= Thiamine, G=Guanine, C=Cytosine

4. Conclution

These results demonstrated that 6 colonies shown inhibitory activity, 3 of which were originated form outside the green algae, while the other 3 colonies were from the inside of the algae.

The result of the antibacterial test known that 1 of the 6 isolates tested bacteria showed consistency of inhibitory activity against pathogenic bacteria *S.aureus* (Gram positive bacteria), mean that it was bacteriostatic to Gram-positive bacteria. Selected isolates bacteria from *Halimedadiscoidea*was Gram-negative bacteria, rod-shaped, non-spore-forming, not acid resistant and 16S rDNA examination results indicate that symbiont bacteria is a member of genus *Pseudomonas* and species *Pseudomonas guezennei*.

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Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (*Halimeda discoidea*) from Pulau Lima, Banten Bay, Indonesia[Z1]

Abstract. Bacteria associated with seaweed ~~are~~ were involved in the production of metabolites associated with their host, so they tend to have nearly the same metabolites to defend themselves or as antibiotics. The aim of this study was to isolate and to identify bacteria with antimicrobial activities from the marine green algae (*Halimeda discoidea*). Laboratory analysis was isolation of bacteria, selection of bacterial with antimicrobial activities, antibacterial activity test following Priharta (2008)[Z2] and Minimum Inhibitory Concentration (MIC) following Purnama (2010). ~~Our r~~Result showed that six bacterial isolates were isolated from outside and inside of the algae, which was have antimicrobial activities. One was selected for further work which was have more antimicrobial activities. The bacteria showed antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) but not against Gram-negative bacteria (*Escherichia coli*). The bacterial growth curve ~~in the spectrophotometric and TPC methods the~~ was optimum phase at the 10th hour to 16th hour. Microscopic analysis and biochemical tests showed that isolated bacteria was a rod-shaped Gram-negative, acid-free, non-endosporous, reacted positively to gelatin, citrate and carbohydrate, reacting negatively on motile, and urease. We conclude that the bacteria isolated from green algae which wa have more antimicrobial belongs to the genus *Pseudomonas guezennei*.[Z3]

Keywords : Symbiont bacteria, *Halimeda discoidea*,*Pseudomonas guezennei*.

1. Introduction

Seaweed is one of the largest producers of biomass in the marine environment. They produce various active chemical metabolites in their environment, potentially as a tool to protect themselves against other sedentary organisms. Biogenic molecules infuse unique chemical diversity in seaweed compared to other plants(Divakaran and Sreejamol, 2014). In addition to its primary economic value, the secondary metabolite content of seaweed has the potential to produce diverse bioactive metabolites as antibacterial, antiviral, antifungal and cytotastic (Zainuddin and Malina, 2009).

The inappropriate selection of antibiotics can have a negative impact, like the emergence of bacterial resistance and low antibiotic effectiveness against certain bacteria. So that further research is needed to find new antibacterials. [Z4]

Pereira and Gama (2008) reported that more than 300 secondary metabolites had been identified from green algae including the *Bryopsidales*. Green algae*Halimeda* contains epigallocatechin (a type of antioxidant) in very high amounts (28µg / g dry weight). Furthermore Yoshie *et al* (2002) *Halimeda* is also rich in minerals such as Fe, Mn, Zn and Cu. Minerals are the important component of antioxidant enzymes. Zn, Cu and Mn minerals are essential minerals for superoxide dismutase activity.

2. Materials and Methods

2.1 Material

Following material were used in the analysis include Green algae (*Halimedadiscoidea*), pure culture of *S.aureus*, pure culture of *E.coli*, aquadesh, Agar, Nutrient Broth (Oxoid), Plate Count Agar (Oxoid), Mueller Hinton Agar (Oxoid), sterile sea water, 70% alcohol , 95% alcohol, crystal violet, iodine, safranin, immersion oil, carbolfuxin dyestuff, alcoholic acid, Methylene Blue, Malachit Green solution, filter paper, tissue / cloth, cotton and paper disc.

2.2 Sampling

Samples of Green algae (*Halimeda* discoidea) was obtained from two stations (S 6º0'5.6356 and "E 106º9'15.3684) at Pulau Lima, Banten Bay. The distance from station 1 to station 2 was about 100 meters (Figure 1). Taking samples at two stations, taking supporting data such as temperature of sea water, pH, salinity and current strength.

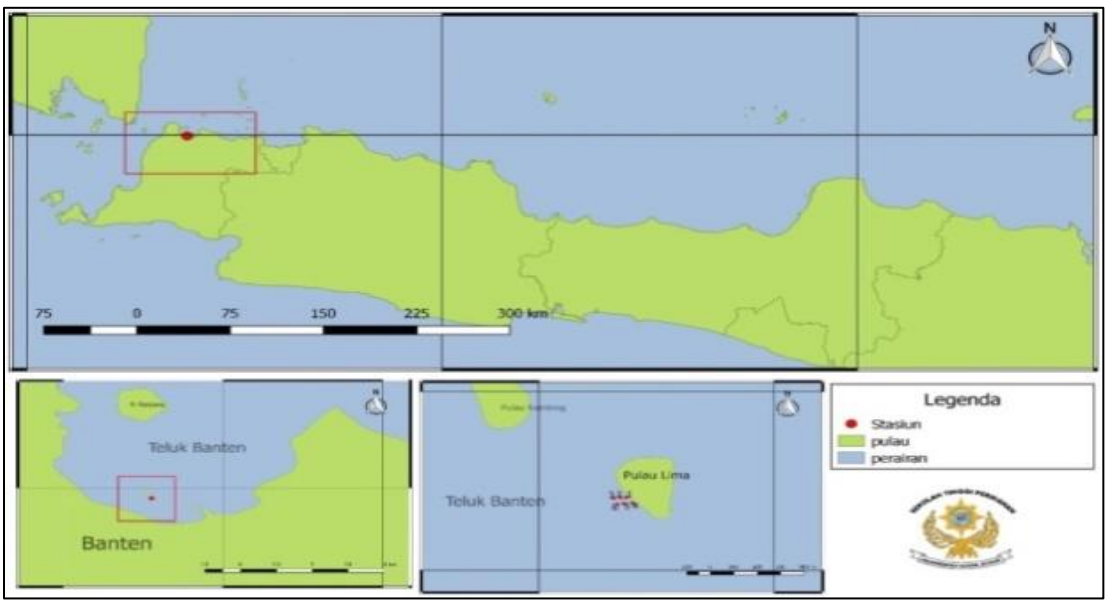


Figure 1. Sampling location

2.3 Isolation of symbiotic bacteria that produce antibacterial compounds

Isolation bacteria from surface of Algae : 15 grams of algae are rinsed with 30 ml of sterile sea water. Then the rinse water is was added 30 ml of NB medium and then shaken using a shaker at room temperature for 24 hours.

Isolation bacteria from surface of Algae : 15 ml of sterile sea water added to 15 grams of rinsed algae then crushed. The suspension was put into 30 ml of NB media and shaken using a shaker at room temperature for 24 hours.

Samples that have been refreshed on NB media were diluted into 1:10 to 1:100.000 dilution, and each dilution was grown on PCA media, following 48 hours incubation at 35° C for 48 hours. Bacteria that have a stable clear zone were isolated on strength seawater according Rippka (1998).

[Z5]*2.4 Qualitative challenge test*

To identify the pathogenic antimicrobial activity of isolate bacteria, qualitative challenge teset was done (Marinho *et al*, 2009 modification). One ml of test bacteria (*Staphylococcus aureus* and *Escherichia coli*) was mix into 10 ml sterile liquid Plate Count Agar and let it for about 15 minutes to become solid. Bacteria isolated from *Halimeda discoidea*. Sprayed on the surface of the media that has been spread with the test bacteria (*Staphylococcus aureus* and *Escherichia coli*). Following48 hours incubation at 35 ° C.

2.5 Antibacterial test

1) Paper disc method

The supernatant (40 µl)was placed on a sterile paper disc containing a sterile watch glass and left for 1 hour in a sterile laminary flow chamber so that the supernatant was absorbed perfectly into the paper disc.

The microbial suspension of *S. aureus*and *E. coli* test which has been prepared on NB medium is first grown in pour platting. The medium and suspension were homogenized by shaking the petri dish slowly, and left for 15 minutes in the sterile laminary flow chamber to become solid. One piece of paper disc was inoculated with supernatant, 1 paper disc containing Nutrient Broth as a negative control, and 1 piece for chloramphenicol as a positive control. The inhibitory potential was measured based on the clear zones seen around the paper disc after incubation at 35°C for 24, 48 and 72 hours (Priharta, 2008).

[Z6]*2) Minimum Inhibitory Concentration (MIC)*

The supernatant is diluted with 100%, 75%, 50%, and 25% of Nutrient Broth. Petri dishes were prepared for *S. aureus*and *E. coli* test bacteria (4 plates each). Each dilution (1 ml) is inserted into petri dishes, and mix with 9 ml of Nutrient Agar in the petri dish. Positive control consists of 10 ml of NA and one ose of bacteria. Negative control contains 10 ml of NA. All test tubes were incubated at 35^0C for 24 and 48 hours (Purnama, 2010).

2.6 Identification of selected symbiont phenotypes and bacterial genotypes

1) Spectrophotometric growth curve

Stratified dilution of bacterial symbiontswas into 1/2, 1/4, 1/8, 1/16, 1/32. The wavelength is set at 686 nm. The cuvette was inserted into *Spectrophotometer*. Calculation formula with MC Farland equation (Oktafiani, 2012) as follows.

$$Y = 2.62 \times 109\ \mu - 6.39 \times 107$$

y = density (CFU / ml) and µ = adsorbs (Â)

2) TPC growth curve

One ounce of bacterial culture that had been refreshed on the PCA medium was inoculated into 13 tubes containing 9 ml of sterile Nutrient Broth and incubated. Observation of the TPC value was carried out every 2 hours during 24 hours. Bacteria was planted into Nutrient media then incubated at 35° C for 24 hours.

Observation of the colony character was carried out with Gram staining, spore staining, Z-Neelsen staining, biochemical tests including motility, gelatin, citrate, urease, and carbohydrate. Estimation of bacterial types is based on the identification key of Cowan and Steel(1993) and DNA molecular test.[Z7]

Samples of bacteria grown on PCA medium were incubated at 35°C for 3 days and then checked for purity and then used for the molecular identification process. Identification was performed using molecular analysis based on 16S rDNA fragments in bacteria. Isolation of bacterial genomic DNA was performed using PCR colony method (Packeiser et al., 2013).The supernatant was taken and used as a DNA template on PCR amplification. Symbiont bacteria species was determined by molecular testing. The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. Molecular identifica-tion was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the modi-fied GES method (Pitcher et al., 1989).The analysis of nitrogen base se-quence readings was done using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data was trimmed and assembled using the BioEdit program (BIOEDIT, 2005). Sequencing data that has been assembled in BLAST with genome data that has been registered in DNA Data Bank of Japan (DDBJ, 2017).

3. Result and Discussion

3.1 Isolation of bacteria

Of the 90 petri dishes incubated, 6 colonies shown inhibotory activity characterized by a clear zone around the colony and 3 of which originate from the outside the green algae, while the other 3 colonies are from the nside of the algae (Figure 2). Bacteria isolates from teh inside of brown algae have better antibacterial activity than bacterial isolates from its surface was reported by Sartika *et al* (2014). Symbiosis that produces secondary metabolites can be triggered because of biotic environmental barriers (Nofiani, 2005).

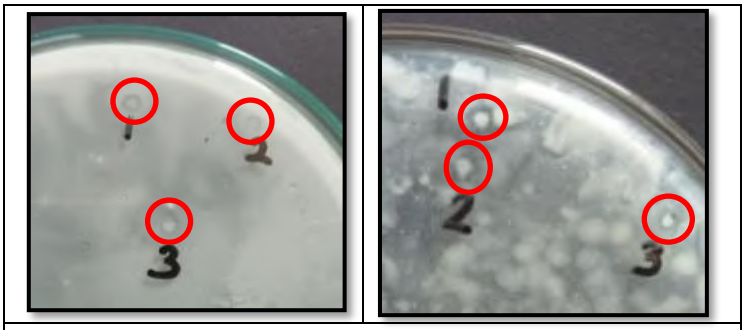


Figure 2. Colonies that shown inhibitory activity

3.2 Qualitative challenge test

Clear zone appeared at 24 hours incubation and stable until 48 hours in 37ºC. The results of the direct challenge test was known that only 1 isolate of the 6 isolates tested bacteria showed consistency of inhibitory activity against test bacteria (*S. aureus*and *E. coli*) (Figure 3). This isolate showed the best antagonistic activity against test bacteria when compared to other isolates and were chosen for further analysis.

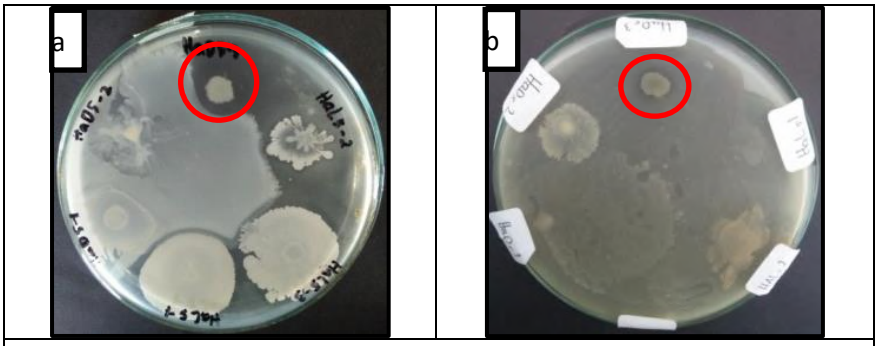
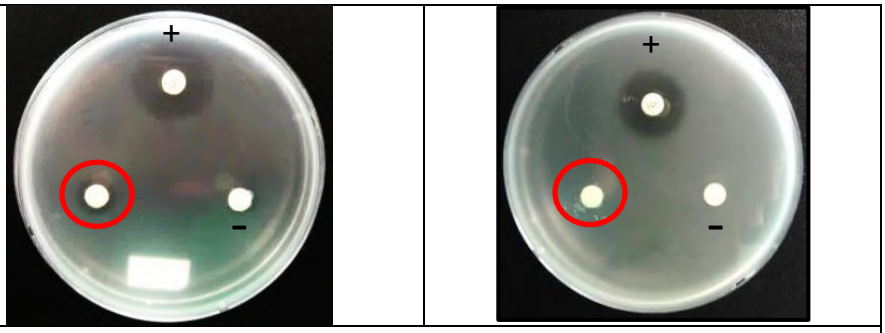


Figure 3. Antagonistic activity against *S. aureus*(a), and *E. coli* (b)

3.3 Antibacterial test

1) Paper disc method

Positive control has a wider clear zone compared to the clear zone by the supernatant in both test bacteria. Supernatant shown 6 mm in diameter clear zone and chloramphenicol as a positive control shown 17.68 mm in diameter clear zone at*Staphylococcus aureus* test bacteria. While at *Escherichia coli*, the supernatant did not shown clear zones and chloramphenicol shown 13.84 mm in diameter clear zone (Figure 4). So that the supernatant is bacteriostatic to Gram positive bacteria. The negative control did not shown clear zones two test bacteria. Lay (1994) said that chloramphenicol at a dose 0,03 mg will effectively produce a zone of inhibition of up to 18 mm so that bacteria can be said to be sensitive to antibiotics, 13-17 mm including intermediate, whereas if <12 mm is said to resistant to antibiotics.



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2) Minimum Inhibitory Concentration (MIC) test

The application of antibacterial compounds produced by selected seaweed asociated bacteria showed different antibacterial activity on both test bacteria (*S.aureus* and *E.coli*). The *S. aureus* bacteria began to be inhibited by 25% of supernatant, while *E.coli*appeared to be stunted by 75% supernatant (Figure 5). Gram negative has a thinner cell wall consisting of 10% peptidoglycan and high lipid content (11-12%). While Gram-positive bacteria have thick cell walls consisting of 60% - 100% peptidoglycan and low lipid content (1-4%) (Pelczar and Chan, 1986).

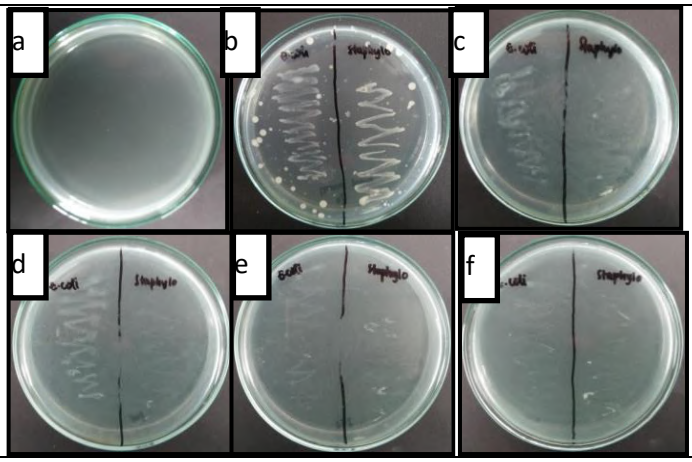

Figure 5. (MIC test results (Minimum Inhibitor Concentration), negative control (a), positive control (b), supernatant 25% (c), 50% (d), 75% (e), and 100% (f)).

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3.4 Identification of selected symbiont phenotypes and bacterial genotypes

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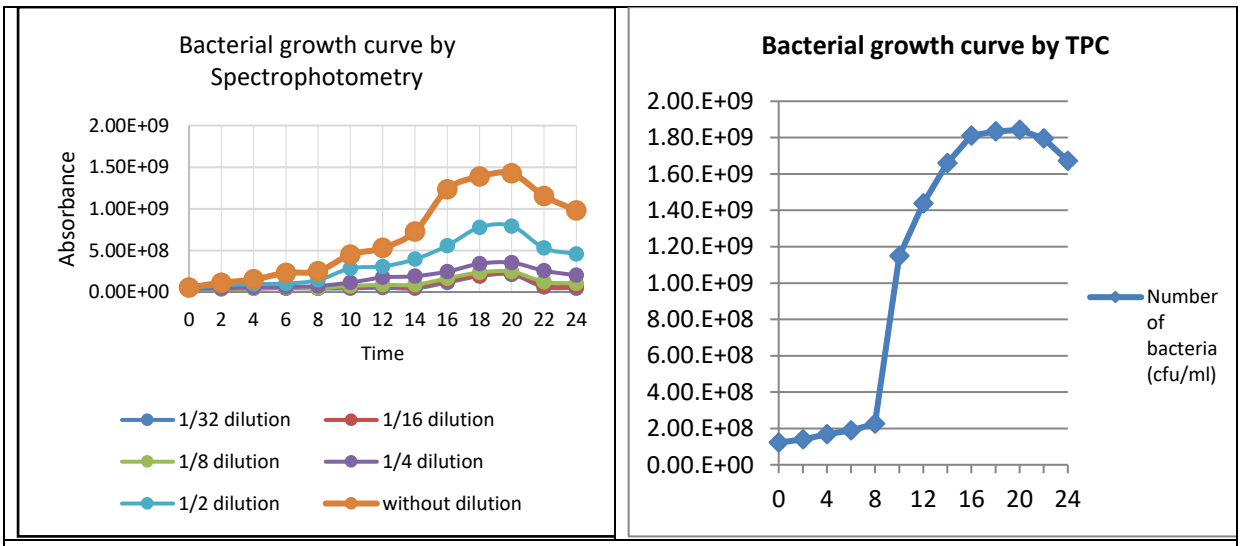

Figure 6. Growth curve of bacteria during incubation

2) Morphology and Biochemistry Test

Selected symbiont bacteria at 24 hours incubation, inoculated into the Nutrient Brothmedium and also at agar slant. In liquid (NB) media the nature of bacterial growth is on the surface, below the surface and bottom of the tube which can be seen clearly. The appearance of the medium with bacterial inoculation appears cloudy and forms a pelicle (thick membrane) on the surface of the medium. In various layers of surface can be seen various kinds of growth, in some microbes visible formation of thick pellicles on the surface layer (Lay, 1994).

The growth of symbiotic bacterial isolates was observed in order to tilt looks fertile, looks like a tree and follows the direction of the scratch. Other characteristics that can be observed were having a marginal edge, when viewed from the side it looks convex, its consistency is not like mucus but quite thick and slightly greenish in color. Microbes that thrive on the surface of a medium will look more opaque compared to infertile growth (Lay, 1994).

Gram staining shows red cells or Gram negative and rod shaped. Gram negative bacteria have thinner cell walls (lipid content greater than peptidoglycan) than cell walls of Gram positive bacteria (Figure 7a). Spore staining shows that symbiotic bacteria do not produce spores. Evidently with whole red vegetative cells without green cores (Figure 7b). As for bacteria with the ability to produce spores, vegetative cells will be red in green in the middle. Bacterial spores can be stained by heating. Heating causes the outer layer of the spore to expand, so that the dye malachite green can enterLay (1994). The Z-Neelsen staining showed that symbiotic bacteria belonged to a group of bacteria that were not acid resistant. Shown with blue bacterial cells, while if the acid resistant bacteria will be red (Figure 7c).

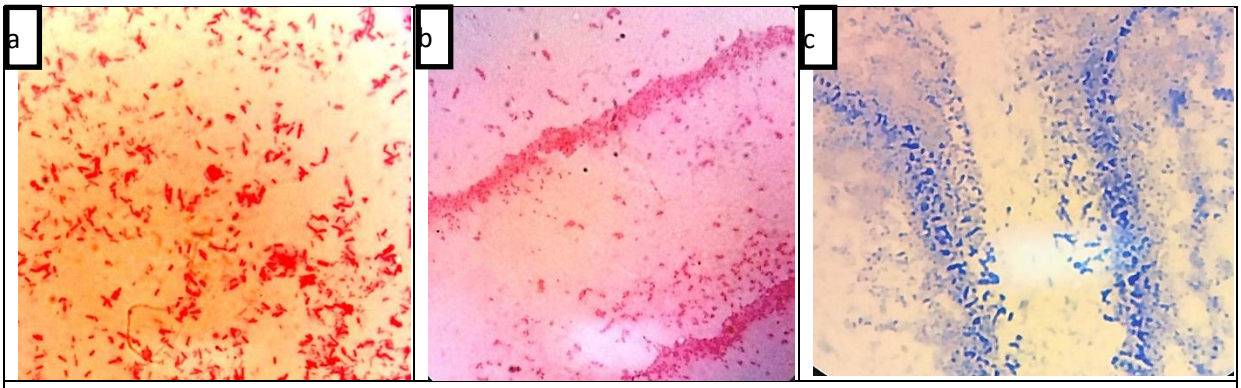

Figure 7. (a) Gram staining, (b) spore staining and (c) Z-Neelsen staining

The characteristics of known symbiont bacteria from microscopic identification and biochemical tests include the form of rods / bacilli, acid-resistant, non-spore-forming, non-motile, aerobically grown, and positive carbohydrate tests. Based on the identification key from Cowan and Steel (1993) referring to a group of bacteria that are thought to have similar characteristics, to*Pseudomonas*. [Z8]

3) 16 rDNA Molecular Examination

16S rDNA molecular examination was carried out to determine species of symbionic bacteria. Molecular identification is carried out through partial genetic analysis of 16S rDNA. PCR amplification results from 16S region of bacterial ribosome DNA. The sample PCR product was visualized using a gel documentation system measuring 1500 bp and the negative control did not contain the following DNA bands. The DNA tape used was relevant to PCR products produced around 1500 base pairs. Nitrogen base sequences sorted from symbiont bacterial isolates can be seen in Figure 8.Based on secondary data obtained from IPBCC, sequence analysis of 16S rDNA isolates from European Union-A was identified as *Pseudomonas guezennei* which had similarities in 99%, at a maximum score of 2117 (total score of 2117), demand for 100% coverage, value of E 0.0 against bacterial taxa.

TTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTTCGAAA
GGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAG
ATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCCGTAACTG
GTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG
TGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC
GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTAC
CAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAAGCCCCGGGC
TCAACCTGGGAATTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGT
GTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCAGAGAACTTT
CCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGT
GGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGG
TGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCG
GAATCGCTAGTAATCGGA

Figure 9. The sequence of nitrogen bases sequenced from symbiont bacteria
A= Adenine, T= Thiamine, G=Guanine, C=Cytosine

4. Conclution

These results demonstrated that 6 colonies shown inhibitory activity, 3 of which were originated form outside the green algae, while the other 3 colonies were from the inside of the algae.

The result of the antibacterial test known that 1 of the 6 isolates tested bacteria showed consistency of inhibitory activity against pathogenic bacteria *S.aureus* (Gram positive bacteria), mean that it was bacteriostatic to Gram-positive bacteria. Selected isolates bacteria from *Halimedadiscoidea*was Gram-negative bacteria, rod-shaped, non-spore-forming, not acid resistant and 16S rDNA examination results indicate that symbiont bacteria is a member of genus *Pseudomonas* and species *Pseudomonas guezennei*.

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Isolation and Identification of Bacteria with Antimicrobial Activities from Green Algae (*Halimeda discoidea*) from Pulau Lima, Banten Bay, Indonesia

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Abstract. Bacteria associated with seaweed were involved in the production of metabolites associated with their host, so they tend to have nearly the same metabolites to defend themselves or as antibiotics. The aim of this study was to isolate and to identify bacteria with antimicrobial activities from the marine green algae (*Halimeda discoidea*). Laboratory analysis was isolation of bacteria, selection of bacterial with antimicrobial activities, antibacterial activity test and Minimum Inhibitory Concentration (MIC). Result showed that six bacterial isolates were isolated from outside and inside of the algae, which was have antimicrobial activities. One was selected for further work which was have more antimicrobial activities. The bacteria showed antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) but not against Gram-negative bacteria (*Escherichia coli*). The bacterial growth curve was optimum phase at the 10th hour to 16th hour. Microscopic analysis and biochemical tests showed that isolated bacteria was a rod-shaped Gram-negative, acid-free, non-endosporous, reacted positively to gelatin, citrate and carbohydrate, reacting negatively on motile, and urease. We conclude that the bacteria isolated from green algae which was have more antimicrobial belongs to the genus *Pseudomonas guezennei*.
Keywords : Symbiont bacteria, *Halimeda discoidea*, *Pseudomonas guezennei*.

1. Introduction

Seaweed is one of the largest producers of biomass in the marine environment. They produce various active chemical metabolites in their environment, potentially as a tool to protect themselves against other sedentary organisms. Biogenic molecules infuse unique chemical diversity in seaweed compared to other plants [3]. In addition to its primary economic value, the secondary metabolite content of seaweed has the potential to produce diverse bioactive metabolites as antibacterial, antiviral, antifungal and cytotastic [19].

Antibiotics are drugs that are common in medicine.The inappropriate selection of antibiotics can have a negative impact, like the emergence of bacterial resistance and low antibiotic effectiveness against certain bacteria. So that further research is needed to find new antibacterials.

More than 300 secondary metabolites had been identified from green algae including the *Bryopsidales* [11]. Green algae *Halimeda* contains epigallocatechin (a type of antioxidant) in very high amounts (28µg/g dry weight). Furthermore *Halimeda* is also rich in minerals such as Fe, Mn, Zn and Cu. Minerals are the important component of antioxidant enzymes. Zn, Cu and Mn minerals are essential minerals for superoxide dismutase activity [18].

2. Materials and Methods

2.1. Material

Following material were used in the analysis include Green algae (*Halimedadiscoidea*), pure culture of *S.aureus*, pure culture of *E.coli*, aquadesh, Agar, Nutrient Broth (Oxoid), Plate Count Agar (Oxoid), Mueller Hinton Agar (Oxoid), sterile sea water, 70% alcohol , 95% alcohol, crystal violet, iodine, safranin, immersion oil, carbolfuxin dyestuff, alcoholic acid, Methylene Blue, Malachit Green solution, filter paper, tissue / cloth, cotton and paper disc.

2.2. Sampling

Samples of Green algae (*Halimeda* discoidea) was obtained from two stations (S 6º0'5.6356 and "E 106º9'15.3684) at Pulau Lima, Banten Bay. The distance from station 1 to station 2 was about 100 meters (Figure 1). Taking samples at two stations, taking supporting data such as temperature of sea water, pH, salinity and current strength.

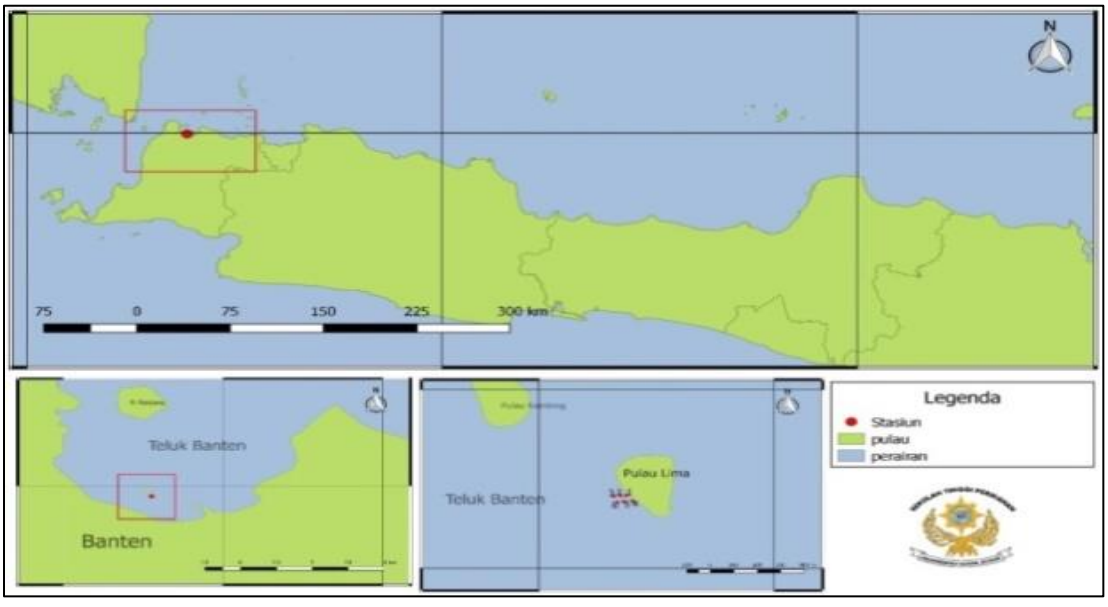

Figure 1. Sampling location

2.3. Isolation of symbiotic bacteria that produce antibacterial compounds

Isolation bacteria from surface of Algae : 15 grams of algae are rinsed with 30 ml of sterile sea water. Then the rinse water is was added 30 ml of NB medium and then shaken using a shaker at room temperature for 24 hours. Isolation bacteria from surface of Algae : 15 ml of sterile sea water added to

15 grams of rinsed algae then crushed. The suspension was put into 30 ml of NB media and shaken using a shaker at room temperature for 24 hours. Samples that have been refreshed on NB media were diluted into 1:10 to 1:100.000 dilution, and each dilution was grown on PCA media, following 48 hours incubation at 35° C for 48 hours. Bacteria that have a stable clear zone were isolated on strength seawater [16].

2.4. Qualitative challenge test

To identify the pathogenic antimicrobial activity of isolate bacteria, qualitative challenge teset was done [7]. One ml of test bacteria (*Staphylococcus aureus* and *Escherichia coli*) was mix into 10 ml sterile liquid Plate Count Agar and let it for about 15 minutes to become solid. Bacteria isolated from *Halimeda discoidea*. Sprayed on the surface of the media that has been spread with the test bacteria (*Staphylococcus aureus* and *Escherichia coli*). Following 48 hours incubation at 35 ° C.

2.5. Antibacterial test

2.5.1. Paper disc method

The supernatant (40 µl)was placed on a sterile paper disc containing a sterile watch glass and left for 1 hour in a sterile laminary flow chamber so that the supernatant was absorbed perfectly into the paper disc. The microbial suspension of *S. aureus* and *E. coli* test which has been prepared on NB medium is first grown in pour platting. The medium and suspension were homogenized by shaking the petri dish slowly, and left for 15 minutes in the sterile laminary flow chamber to become solid. One piece of paper disc was inoculated with supernatant, 1 paper disc containing Nutrient Broth as a negative control, and 1 piece for chloramphenicol as a positive control. The inhibitory potential was measured based on the clear zones seen around the paper disc after incubation at 35°C for 24, 48 and 72 hours [14].

2.5.2. Minimum Inhibitory Concentration (MIC)

The supernatant is diluted with 100%, 75%, 50%, and 25% of Nutrient Broth. Petri dishes were prepared for *S. aureus*and *E. coli* test bacteria (4 plates each). Each dilution (1 ml) is inserted into petri dishes, and mix with 9 ml of Nutrient Agar in the petri dish. Positive control consists of 10 ml of NA and one ose of bacteria. Negative control contains 10 ml of NA. All test tubes were incubated at 35^0C for 24 and 48 hours [15].

2.6. Identification of selected symbiont phenotypes and bacterial genotypes

2.6.1. Spectrophotometric growth curve

Stratified dilution of bacterial symbiontswas into 1/2, 1/4, 1/8, 1/16, 1/32. The wavelength is set at 686 nm. The cuvette was inserted into *Spectrophotometer*. Calculation formula with MC Farland equation as follows.

$$Y = 2.62 \times 109\ \mu - 6.39 \times 107$$

y = density (CFU / ml) and µ = adsorbs (Â)

2.6.2. TPC growth curve

One ounce of bacterial culture that had been refreshed on the PCA medium was inoculated into 13 tubes containing 9 ml of sterile Nutrient Broth and incubated. Observation of the TPC value was carried out every 2 hours during 24 hours. Bacteria was planted into Nutrient media then incubated at 35^{o} C for 24 hours. Observation of the colony character was carried out with Gram staining, spore staining, Z-Neelsen staining, biochemical tests including motility, gelatin, citrate, urease, and carbohydrate. Estimation of bacterial types is based on the identification key [2] and DNA molecular test.

Samples of bacteria grown on PCA medium were incubated at 35°C for 3 days and then checked for purity and then used for the molecular identification process. Identification was performed using molecular analysis based on 16S rDNA fragments in bacteria. Isolation of bacterial genomic DNA was performed using PCR colony method [9].The supernatant was taken and used as a DNA template on PCR amplification. Symbiont bacteria species was determined by molecular testing. The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. Molecular identifica-tion was done through partial genetic analysis of 16S rDNA. DNA extraction was performed using the modi-fied GES method [13].The analysis of nitrogen base se-quence readings was done using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data was trimmed and assembled using the BioEdit program. Sequencing data that has been assembled in BLAST with genome data that has been registered in DNA Data Bank of Japan [4].

3. Result and Discussion

3.1. Isolation of bacteria

Of the 90 petri dishes incubated, 6 colonies shown inhibotory activity characterized by a clear zone around the colony and 3 of which originate from the outside the green algae, while the other 3 colonies are from the nside of the algae (Figure 2). Bacteria isolates from teh inside of brown algae have better antibacterial activity than bacterial isolates from its surface was reported by [16]. Symbiosis that produces secondary metabolites can be triggered because of biotic environmental barriers [8].

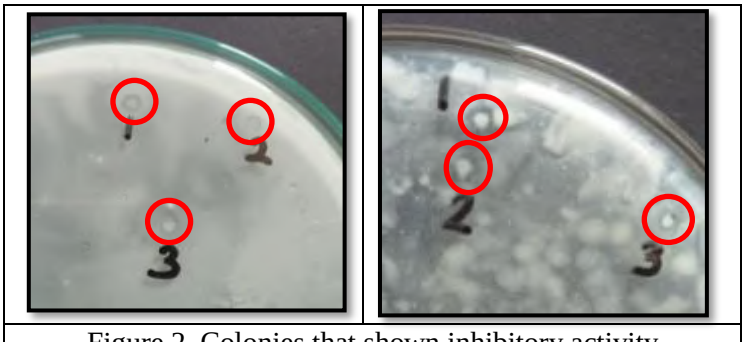

Figure 2. Colonies that shown inhibitory activity

3.2. Qualitative challenge test

Clear zone appeared at 24 hours incubation and stable until 48 hours in 37ºC. The results of the direct challenge test was known that only 1 isolate of the 6 isolates tested bacteria showed consistency of inhibitory activity against test bacteria (*S. aureus*and *E. coli*) (Figure 3). This isolate showed the best

antagonistic activity against test bacteria when compared to other isolates and were chosen for further analysis.

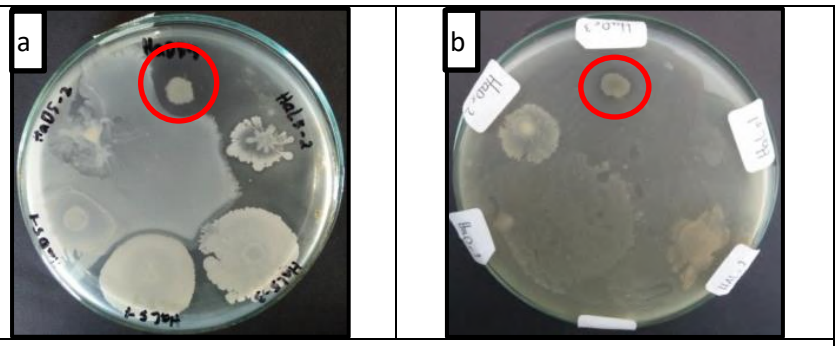

Figure 3. Antagonistic activity against *S. aureus*(a), and *E. coli* (b)

3.3. Antibacterial test

3.3.1. Paper disc method

Positive control has a wider clear zone compared to the clear zone by the supernatant in both test bacteria. Supernatant shown 6 mm in diameter clear zone and chloramphenicol as a positive control shown 17.68 mm in diameter clear zone at*Staphylococcus aureus* test bacteria. While at *Escherichia coli*, the supernatant did not shown clear zones and chloramphenicol shown 13.84 mm in diameter clear zone (Figure 4). So that the supernatant is bacteriostatic to Gram positive bacteria. The negative control did not shown clear zones two test bacteria. Chloramphenicol at a dose 0,03 mg will effectively produce a zone of inhibition of up to 18 mm so that bacteria can be said to be sensitive to antibiotics, 13-17 mm including intermediate, whereas if <12 mm is said to resistant to antibiotics [6].

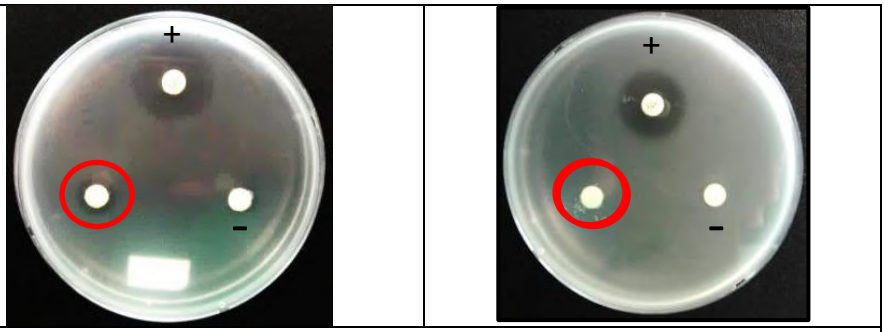

Figure 4. Antibacterial activity by supernatant (o), chloramphenicol (+), and negative control (-) against *S. aureus* (a) and against *E. coli* (b)

3.3.2. Minimum Inhibitory Concentration (MIC) test

The application of antibacterial compounds produced by selected seaweed asociated bacteria showed different antibacterial activity on both test bacteria (*S.aureus* and *E.coli*). The *S. aureus* bacteria began to be inhibited by 25% of supernatant, while *E.coli*appeared to be stunted by 75% supernatant (Figure 5). Gram negative has a thinner cell wall consisting of 10% peptidoglycan and high lipid content (11-12%). While Gram-positive bacteria have thick cell walls consisting of 60% -100% peptidoglycan and low lipid content (1-4%) [10].

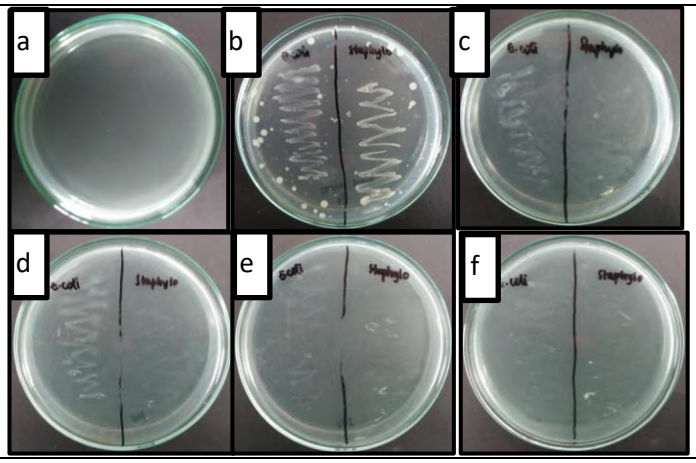

Figure 5. (MIC test results (Minimum Inhibitor Concentration), negative control (a), positive control (b), supernatant 25% (c), 50% (d), 75% (e), and 100% (f)).

The antibiotic MIC test against microbes is used to determine the sensitivity of microbes to antibiotics. MIC values are contrary to the sensitivity of the microbes tested. The lower the MIC value of an antibiotic, the greater the sensitivity of bacteria [5].

3.4. Identification of selected symbiont phenotypes and bacterial genotypes

3.4.1. Growth of bacteria

The lag phase or slow phase is experienced by bacterial symbiontsat 0 to 8 hours growth in both methods. This phase was time consume because thesymbiont bacteria need time for adaptation to the environment or new medium. The adaptation phase was affected by several factors, including the medium and the growth environment and the number of inoculums.

The log phase (exponential phase) of symbiont bacteriashown at 10^{th}hours of incubation for aall growth curves. In this phase, bacterial cell numbers doubles during each unit time period. This is because the bacteria have been able to adapt to the environment or medium. Generation time of most bacteria is between 20 minutes to 20 hours, but the rate of exponential growth varies between bacteria genera and also influence by culture conditions [12]. In the 16^{th} to 22^{nd} hours of incubation was the stationary phase. This phase is the phase where the number of populations that grows is the same as the number of dead cells. During stationary phase, some of the cell die and lyse. These lytic products of cells can provide nutrients for other cells, and these divided and replace the dead ones. The individual in this phase differ in certain biochemical components from cells in the exponential phase [12].

The number of cells at 24 hours incubations shows a decrease (decline or death phase). Decline or death phase is the phase when the death rate exceeds the rate of reproduction, the cell population is in the death or decline phase [12]. In the death phase, the population of microorganisms has begun to experience death because nutrient in the medium was almost exhausted and the reserve energy in the cell was exhausted (Figure 6).

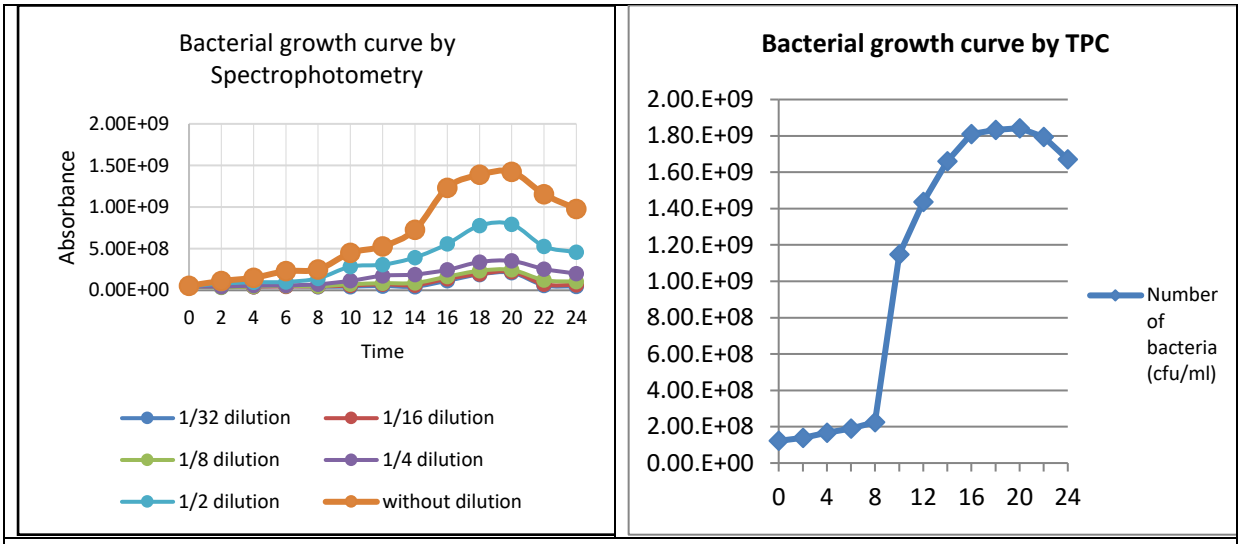

Figure 6. Growth curve of bacteria during incubation

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Selected symbiont bacteria at 24 hours incubation, inoculated into the Nutrient Brothmedium and also at agar slant. In liquid (NB) media the nature of bacterial growth is on the surface, below the surface and bottom of the tube which can be seen clearly. The appearance of the medium with bacterial inoculation appears cloudy and forms a pelicle (thick membrane) on the surface of the medium. In various layers of surface can be seen various kinds of growth, in some microbes visible formation of thick pellicles on the surface layer [6].

The growth of symbiotic bacterial isolates was observed in order to tilt looks fertile, looks like a tree and follows the direction of the scratch. Other characteristics that can be observed were having a marginal edge, when viewed from the side it looks convex, its consistency is not like mucus but quite thick and slightly greenish in color. Microbes that thrive on the surface of a medium will look more opaque compared to infertile growth [6].

Gram staining shows red cells or Gram negative and rod shaped. Gram negative bacteria have thinner cell walls (lipid content greater than peptidoglycan) than cell walls of Gram positive bacteria (Figure 7a). Spore staining shows that symbiotic bacteria do not produce spores. Evidently with whole red vegetative cells without green cores (Figure 7b). As for bacteria with the ability to produce spores, vegetative cells will be red in green in the middle. Bacterial spores can be stained by heating. Heating causes the outer layer of the spore to expand, so that the dye malachite green can enter [6]. The Z-Neelsen staining showed that symbiotic bacteria belonged to a group of bacteria that were not acid resistant. Shown with blue bacterial cells, while if the acid resistant bacteria will be red (Figure 7c).

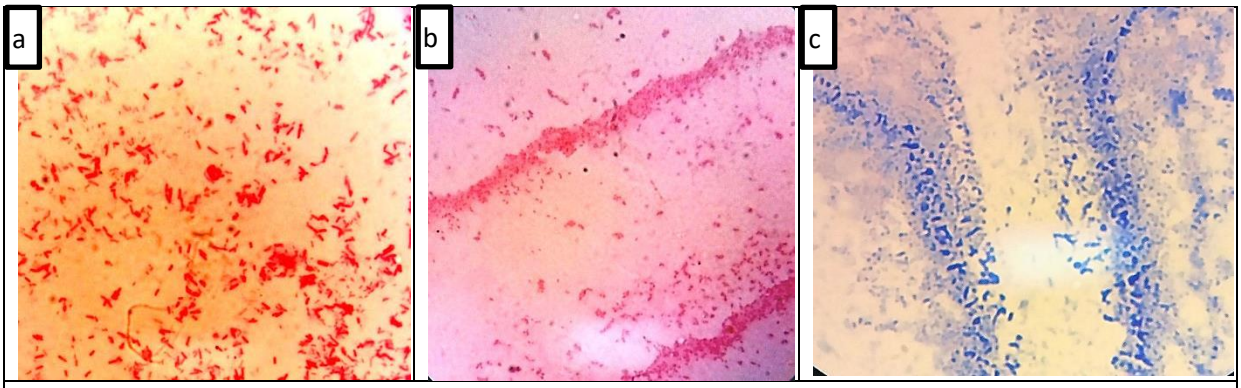

Figure 7. (a) Gram staining, (b) spore staining and (c) Z-Neelsen staining

The characteristics of known symbiont bacteria from microscopic identification include the form of rods / bacilli, acid-resistant, non-spore-forming. Biochemical tests include symbiont bacteria is non-motile, aerobically grown, and positive carbohydrate tests. Based on the identification key from Cowan and Steel (1993) referring to a group of bacteria that are thought to have similar characteristics, to *Pseudomonas*.

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TTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTTCGAAA
GGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAG
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TGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC
GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTAC
CAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTA
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CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCAGAGAACTTT
CCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGT
GGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGG
TGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCG
GAATCGCTAGTAATCGGA

Figure 9. The sequence of nitrogen bases sequenced from symbiont bacteria
A= Adenine, T= Thiamine, G=Guanine, C=Cytosine

4. Conclution

These results demonstrated that 6 colonies shown inhibitory activity, 3 of which were originated form outside the green algae, while the other 3 colonies were from the inside of the algae. The result of the antibacterial test known that 1 of the 6 isolates tested bacteria showed consistency of inhibitory activity against pathogenic bacteria *S.aureus* (Gram positive bacteria), mean that it was bacteriostatic to Gram-positive bacteria. Selected isolates bacteria from *Halimeda discoidea* was Gram-negative bacteria, rod-shaped, non-spore-forming, not acid resistant and 16S rDNA examination results indicate that symbiont bacteria is a member of genus *Pseudomonas* and species *Pseudomonas guezennei*.

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Isolation and identification of bacteria with antimicrobial activities from green algae (*Halimeda discoidea*) from Pulau Lima, Banten Bay, Indonesia

Isolation and identification of bacteria with antimicrobial activities from green algae (*Halimeda discoidea*) from Pulau Lima, Banten Bay, Indonesia

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Abstract. Bacteria associated with seaweed were involved in the production of metabolites associated with their host, so they tend to have nearly the same metabolites to defend themselves or as antibiotics. The aim of this study was to isolate and to identify bacteria with antimicrobial activities from the marine green algae (*Halimeda discoidea*). Laboratory analysis was for the isolation of bacteria, selection of bacteria with antimicrobial activities, antibacterial activity tests and Minimum Inhibitory Concentration (MIC). Results showed that six bacterial isolates were isolated from outside and inside of the algae, which have antimicrobial activities. One was selected for further work which was have more antimicrobial activities. The bacteria showed antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) but not against Gram-negative bacteria (*Escherichia coli*). The bacterial growth curve was at an optimum phase at the 10th to 16th hour. Microscopic analysis and biochemical tests showed that isolated bacteria were a rod-shaped Gram-negative, acid-free, non-endosporous, reacted positively to gelatin, citrate and carbohydrate, reacting negatively on motile, and urease. We conclude that the bacteria isolated from green algae which had more antimicrobial properties belongs to the genus *Pseudomonas guezennei*.

Keywords: *Halimeda discoidea, Pseudomonas guezennei,* symbiont bacteria

1. Introduction

Seaweed is one of the largest producers of biomass in the marine environment. They produce various active chemical metabolites in their environment, potentially as a tool to protect themselves against other sedentary organisms. Biogenic molecules infuse unique chemical diversity in seaweed compared to other plants [1]. In addition to its primary economic value, the secondary metabolite content of seaweed has the potential to produce diverse bioactive metabolites as antibacterial, antiviral, antifungal and cytostatic [2].

Antibiotics are drugs that are common in medicine. The inappropriate selection of antibiotics can have a negative impact, like the emergence of bacterial resistance and low antibiotic effectiveness against certain bacteria. Further research is needed to find new antibacterial metabolites.

More than 300 secondary metabolites had been identified from green algae including the *Bryopsidales* [3]. Green algae *Halimeda* contains epigallocatechin (a type of antioxidant) in very high amounts (28µg/g dry weight). Furthermore, *Halimeda* is also rich in minerals such as Fe, Mn, Zn and Cu. Minerals are an important component of antioxidant enzymes. Zn, Cu and Mn minerals are essential minerals for superoxide dismutase activity [4].

2. Materials and Methods

2.1. Materials

The following material were used in the analysis include Green algae (*Halimeda discoidea*), pure culture of *S. aureus*, pure culture of *E. coli*, aquades, Agar, Nutrient Broth (Oxoid), Plate Count Agar (Oxoid), Mueller Hinton Agar (Oxoid), sterile sea water, 70% alcohol, 95% alcohol, crystal violet, iodine, safranin, immersion oil, carbolfuxin dyestuff, alcoholic acid, Methylene Blue, Malachit Green solution, filter paper, tissue / cloth, cotton and paper disc.

2.2. Sampling

Samples of Green algae (*Halimeda discoidea*) were obtained from two stations (S 6º0'5.6356 and "E 106º9'15.3684) at Pulau Lima, Banten Bay. The distance from station 1 to station 2 was about 100 meters (figure 1). Taking samples at two stations, taking supporting data such as temperature of sea water, pH, salinity and current strength.

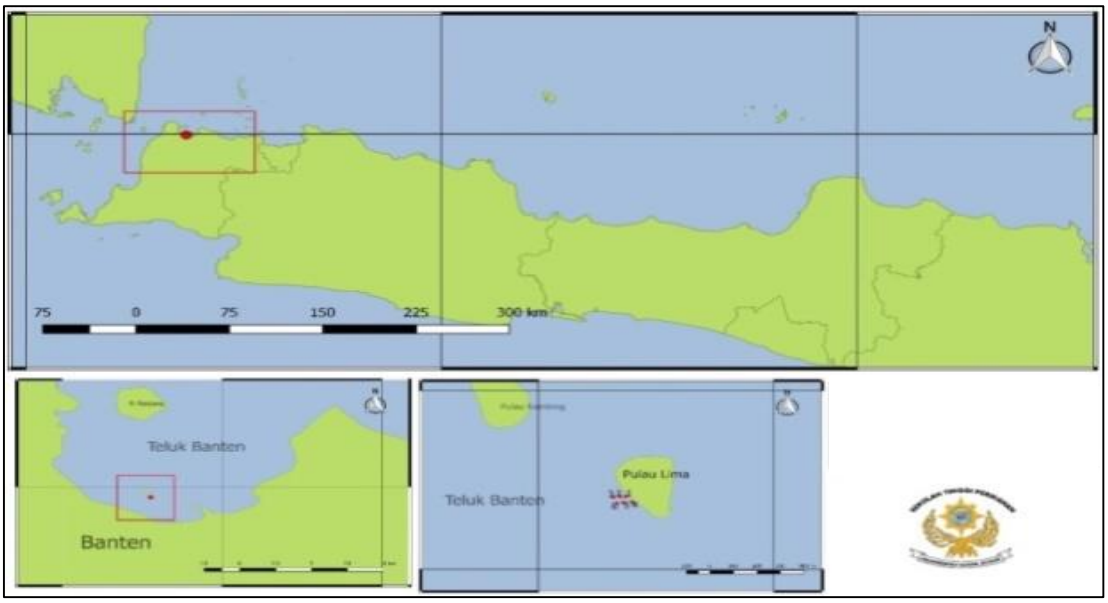

Figure 1. Sampling location, ● = station, ▭ = island, ▭ = sea.

2.3. Isolation of symbiotic bacteria that produce antibacterial compounds

Isolation bacteria from the surface of algae : 15 grams of algae is rinsed with 30 ml of sterile sea water. Then the rinse water was added to 30 ml of NB medium and then shaken using a shaker at room temperature for 24 hours. Isolation bacteria from the surface of algae : 15 ml of sterile sea water was added to 15 grams of rinsed algae and then crushed. The suspension was put into 30 ml of NB media and shaken using a shaker at room temperature for 24 hours. Samples that have been refreshed on NB media were diluted into 1:10 to 1:100.000 dilution, and each dilution was grown on PCA media, following 48 hours of incubation at 35° C for 48 hours. Bacteria that have a stable clear zone were isolated on strength seawater [5].

2.4. Qualitative challenge test

To identify the pathogenic antimicrobial activity of isolate bacteria, qualitative challenge tests were done [6]. One ml of test bacteria (*Staphylococcus aureus* and *Escherichia coli*) was mixed into 10 ml sterile liquid Plate Count Agar (PCA) and was let to stand for about 15 minutes to become solid. Bacteria was isolated from *Halimeda discoidea*. Sprayed on the surface of the media that had been

spread with the test bacteria (*Staphylococcus aureus* and *Escherichia coli*). Following 48 hours of incubation at 35°C.

2.5. Antibacterial test

2.5.1. Paper disc method. The supernatant (40 µl) was placed on a sterile paper disc containing a sterile watch glass and left for 1 hour in a sterile laminary flow chamber so that the supernatant was absorbed perfectly into the paper disc. The microbial suspension of *S. aureus* and *E. coli* test which have been prepared on NB medium was grown in pour platting. The medium and suspension were homogenized by shaking the petri dish slowly, and left for 15 minutes in the sterile laminary flow chamber to become solid. One piece of paper disc was inoculated with supernatant, 1 paper disc containing Nutrient Broth as a negative control, and 1 piece for chloramphenicol as a positive control. The inhibitory potential was measured based on the clear zones seen around the paper disc after incubation at 35°C for 24, 48 and 72 hours [7].

2.5.2. Minimum Inhibitory Concentration (MIC). The supernatant is diluted with 100%, 75%, 50%, and 25% of Nutrient Broth. Petri dishes were prepared for *S. aureus* and *E. coli* test bacteria (4 plates each). Each dilution (1 ml) is inserted into petri dishes, and mix with 9 ml of Nutrient Agar in the petri dish. Positive control consists of 10 ml of NA and one ose of bacteria. Negative control contains 10 ml of NA. All test tubes were incubated at 35^0C for 24 and 48 hours [8].

2.6. Identification of selected symbiont phenotypes and bacterial genotypes

2.6.1. Spectrophotometric growth curve. Stratified dilution of bacterial symbionts was into 1/2, 1/4, 1/8, 1/16, 1/32. The wavelength is set at 686 nm. The cuvette was inserted into a *Spectrophotometer*. The calculation formula with MC Farland equation is as follows.

$$Y = 2.62 \text{ x } 109 \text{ µ} - 6.39 \text{ x } 107$$

y = density (CFU / ml) and µ = adsorbs (Â)

2.6.2. TPC growth curve. One ounce of bacterial culture that had been refreshed on the PCA medium was inoculated into 13 tubes containing 9 ml of sterile Nutrient Broth and incubated. Observation of the TPC value was carried out every 2 hours during a 24 hour period. Bacteria was planted into the Nutrient media and was then incubated at 35^o C for 24 hours. Observation of the colony character was carried out with Gram staining, spore staining, Z-Neelsen staining, biochemical tests including motility, gelatin, citrate, urease, and carbohydrate. Estimation of bacterial types is based on the identification key [9] and DNA molecular test.

Samples of bacteria grown on PCA medium were incubated at 35°C for 3 days and then checked for purity and then used for the molecular identification process. Identification was performed using molecular analysis based on 16S rDNA fragments in bacteria. Isolation of bacterial genomic DNA was performed using PCR colony method [10]. The supernatant was taken and used as a DNA template on PCR amplification. Symbiont bacteria species was determined by molecular testing. The DNA of the symbiont bacteria isolates was amplified using primers 9F and 1541R. The DNA bands used were relevant to the resulting PCR product of about 1400 base pairs. Molecular identification was done through the partial genetic analysis of 16S rDNA. DNA extraction was performed using the modified GES method [11]. The analysis of nitrogen base sequence readings was done using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The next sequenced raw data was trimmed and assembled using the BioEdit program. Sequencing data that has been assembled in BLAST with genome data that has been registered in DNA Data Bank of Japan [12].

3. Results and Discussion

3.1. Isolation of bacteria

Of the 90 petri dishes incubated, 6 colonies shown inhibitory activity characterized by a clear zone around the colony and 3 of which originate from the outside the green algae, while the other 3 colonies are from the inside of the algae (figure 2). Bacteria isolates from the inside of brown algae have better antibacterial activity than bacterial isolates from its surface [5]. Symbiosis that produces secondary metabolites can be triggered because of biotic environmental barriers [13].

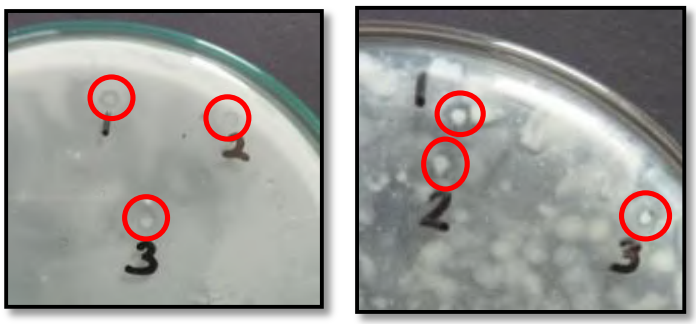

Figure 2. Colonies that shown inhibitory activity.

3.2. Qualitative challenge test

Clear zones appeared at 24 hours of incubation and was stable until 48 hours at a temperature of 37ºC. The results of the direct challenge test were known that only 1 isolate of the 6 isolates tested bacteria showed consistency of inhibitory activity against test bacteria (*S. aureus* and *E. coli*) (figure 3). This isolate showed the best antagonistic activity against test bacteria when compared to other isolates and was chosen for further analysis.

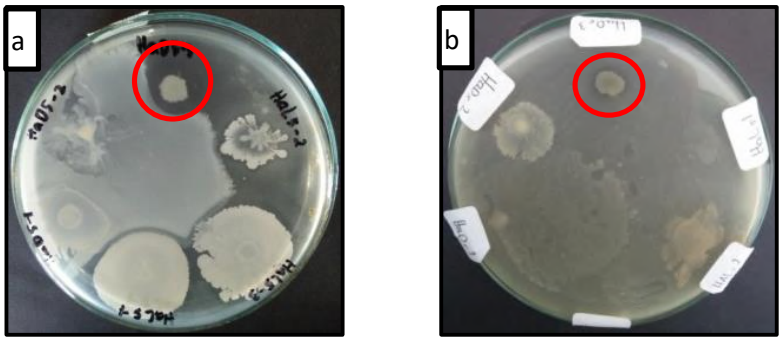

Figure 3. Antagonistic activity against *S. aureus*(a), and *E. coli* (b).

3.3. Antibacterial test

3.3.1. Paper disc method. Positive control had a wider clear zone compared to the clear zone by the supernatant in both test bacteria. Supernatant showed a 6 mm in diameter clear zone and chloramphenicol as a positive control showed a 17.68 mm in diameter clear zone in *Staphylococcus aureus* test bacteria. While in *Escherichia coli*, the supernatant did not shown clear zones and chloramphenicol showed a 13.84 mm in diameter clear zone (figure 4). So that the supernatant was bacteriostatic to Gram positive bacteria. The negative control did not show clear zones for the two test bacteria. Chloramphenicol at a dose of 0.03 mg will effectively produce a zone of inhibition of up to 18 mm so that bacteria can be said to be sensitive to antibiotics, 13-17 mm including intermediate, whereas if <12 mm is said to resistant to antibiotics [14].

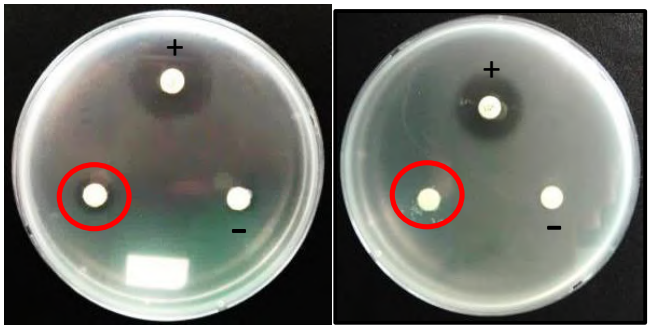

Figure 4. Antibacterial activity by supernatant (o), chloramphenicol (+), and negative control (-) against *S. aureus* (a) and against *E. coli* (b).

3.3.2. Minimum Inhibitory Concentration (MIC) test. The application of antibacterial compounds produced by selected seaweed associated bacteria showed different antibacterial activity on both test bacteria (*S.aureus* and *E.coli*). The *S. aureus* bacteria began to be inhibited by 25% of supernatant, while *E.coli* appeared to be stunted by 75% supernatant (figure 5). Gram negative has a thinner cell wall consisting of 10% peptidoglycan and high lipid content (11-12%). While Gram-positive bacteria have thick cell walls consisting of 60% -100% peptidoglycan and low lipid content (1-4%) [15].

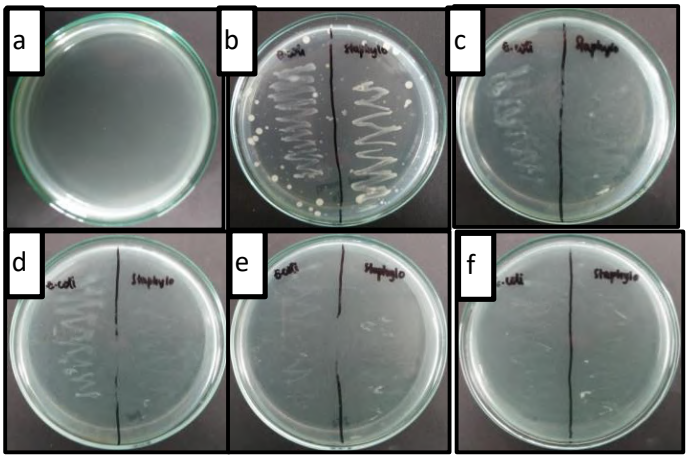

Figure 5. (MIC test results (Minimum Inhibitor Concentration), negative control (a), positive control (b), supernatant 25% (c), 50% (d), 75% (e), and 100% (f)).

The antibiotic MIC test against microbes was used to determine the sensitivity of microbes to antibiotics. MIC values are contrary to the sensitivity of the microbes tested. The lower the MIC value of an antibiotic, the greater the sensitivity of bacteria [16].

3.4. Identification of selected symbiont phenotypes and bacterial genotypes

3.4.1. Growth of bacteria. The lag phase or slow phase is experienced by bacterial symbionts at 0 to 8 hours of growth in both methods. This phase was time consuming because the symbiont bacteria needed time for adaptation to the environment or new medium. The adaptation phase was affected by several factors, including the medium and the growth environment and the number of inoculums.

The log phase (exponential phase) of symbiont bacteria was shown at 10^{th}hours of incubation for all growth curves. In this phase, bacterial cell numbers double during each unit time period. This is because the bacteria have been able to adapt to the environment or medium. Generation time of most bacteria is between 20 minutes to 20 hours, but the rate of exponential growth varies between bacteria genera and was also influenced by culture conditions [17]. In the 16^{th} to 22^{nd} hours of incubation there was the stationary phase. This phase is the phase where the number of populations that grow is the same as the number of dead cells. During stationary phase, some of the cell die and lyse. These lytic products of cells can provide nutrients for other cells, and these divided and replace the dead ones.

The individual in this phase differ in certain biochemical components from cells in the exponential phase [17].

The number of cells at 24 hours of incubations shows a decrease (decline or death phase). Decline or death phase is the phase when the death rate exceeds the rate of reproduction, the cell population is in the death or decline phase [17]. In the death phase, the population of microorganisms has begun to experience death because nutrient in the medium was almost exhausted and the reserve energy in the cell was exhausted (figure 6).

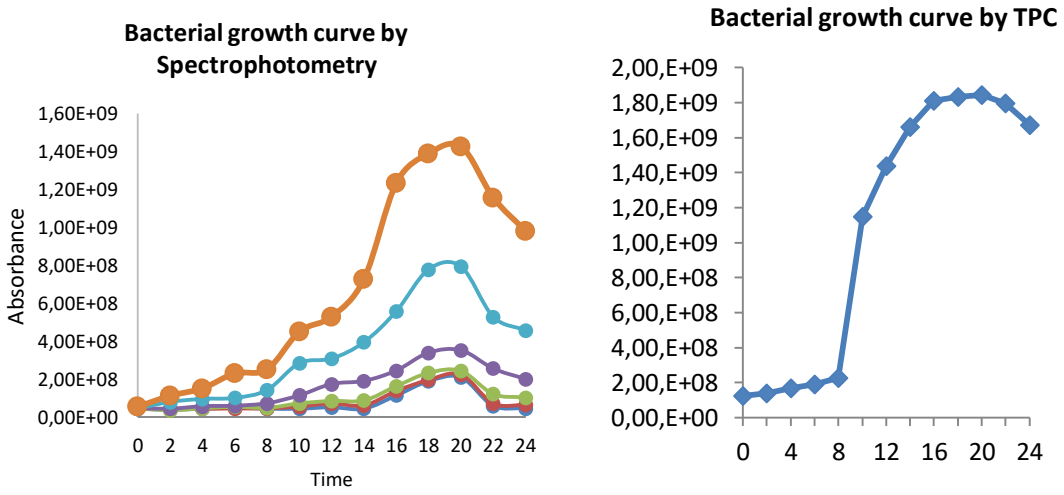

Figure 6. Growth curve of bacteria during incubation. Left: Bacterial growth curve by Spectrophotometry, $^{1}/_{32}$ dilution (—●—); $^{1}/_{16}$ dilution (—●—); $^{1}/_{8}$ dilution (—●—); ¼ dilution (—●—); ½ dilution (—●—); without dilution (—●—). Right: Bacterial growth curve by TPC (CFU/mL).

3.4.2. Morphology and Biochemistry Test. Selected symbiont bacteria at 24 hours of incubation, inoculated into the Nutrient Broth medium and also at agar slant. In liquid (NB) media the nature of bacterial growth is on the surface, below the surface and bottom of the tube which can be seen clearly. The appearance of the medium with bacterial inoculation appears cloudy and forms a pellicle (thick membrane) on the surface of the medium. In various layers of surface there can be seen various kinds of growth, in some microbes visible formation of thick pellicles on the surface layer [14].

The growth of symbiotic bacterial isolates was observed in order to tilt looks fertile, looks like a tree and follows the direction of the scratch. Other characteristics that can be observed were having a marginal edge, when viewed from the side it looks convex, its consistency is not like mucus but quite thick and slightly greenish in color. Microbes that thrive on the surface of a medium will look more opaque compared to infertile growth [14].

Gram staining shows red cells or Gram negative and rod shaped. Gram negative bacteria have thinner cell walls (lipid content greater than peptidoglycan) than cell walls of Gram positive bacteria (figure 7a). Spore staining shows that symbiotic bacteria do not produce spores. Evidently with whole red vegetative cells without green cores (figure 7b). As for bacteria with the ability to produce spores, vegetative cells will be red and green in the middle. Bacterial spores can be stained by heating. Heating causes the outer layer of the spore to expand, so that the dye malachite green can enter [14]. The Z-Neelsen staining showed that symbiotic bacteria belonged to a group of bacteria that were not acid resistant shown with blue bacterial cells, while the acid resistant bacteria will be red (figure 7c).

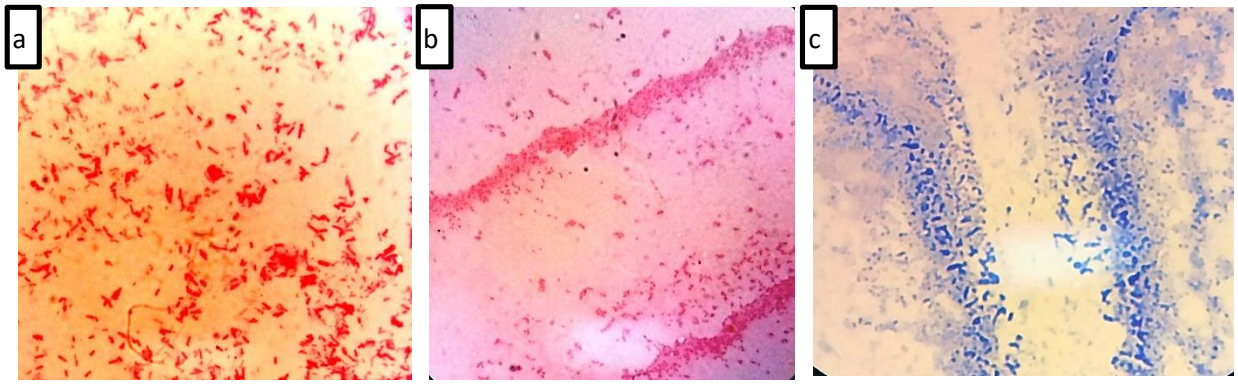

Figure 7. (a) Gram staining, (b) spore staining and (c) Z-Neelsen staining.

The characteristics of known symbiont bacteria from microscopic identification include the form of rods / bacilli, acid-resistant, non-spore-forming. Biochemical tests include symbiont bacteria is non-motile, aerobically grown, and positive carbohydrate tests. Based on the identification key from Cowan and Steel (1993) referring to a group of bacteria that are thought to have similar characteristics, to *Pseudomonas*.

3.4.3. Sixteen rDNA Molecular Examination. 16S rDNA molecular examination was carried out to determine species of symbionic bacteria. Molecular identification is carried out through partial genetic analysis of 16S rDNA. PCR amplification results from 16S region of bacterial ribosome DNA. The sample PCR product was visualized using a gel documentation system measuring 1500 bp and the negative control did not contain the following DNA bands. The DNA tape used was relevant to PCR products produced around 1500 base pairs. Nitrogen base sequences sorted from symbiont bacterial isolates can be seen in figure 8. Based on secondary data obtained from IPBCC, sequence analysis of 16S rDNA isolates from European Union-A was identified as *Pseudomonas guezennei* which had similarities in 99%, at a maximum score of 2117 (total score of 2117), demand for 100% coverage, value of E 0.0 against bacterial taxa.

TTCAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATAACGTTTCGAAA
GGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAG
ATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCCCACCAAGGCGACGATCCGTAACTG
GTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG
TGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTC
GGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTAC
CAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTA
ATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAAGCCCCGGGC
TCAACCTGGGAATTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGT
GTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGA
CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGCAGAGAACTTT
CCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACCTCGGGT
GGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATG
GCCCTTACGGCCAGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGG
TGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCG
GAATCGCTAGTAATCGGA

Figure 8. The sequence of nitrogen bases sequenced from symbiont bacteria, A = adenine, T = thiamine, G = guanine, C = cytosine.

4. Conclusion

These results demonstrated that 6 colonies showed inhibitory activity, 3 of which were originated form outside the green algae, while the other 3 colonies were from the inside of the algae. The results of the antibacterial test showed that 1 of the 6 isolates tested bacteria showed consistency of inhibitory activity against pathogenic bacteria *S.aureus* (Gram positive bacteria), meaning that it was bacteriostatic to Gram-positive bacteria. Selected isolates bacteria from *Halimeda discoidea* was Gram-negative bacteria, rod-shaped, non-spore-forming, not acid resistant and 16S rDNA examination results indicate that symbiont bacteria is a member of genus *Pseudomonas* and species *Pseudomonas guezennei*.

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