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KUMPULAN MAKALAH (*PROCEEDING*)
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Keanekaragaman Hayati Indonesia dan Perannya dalam Menunjang Kemandirian Bangsa

Editor:
Dr. Alfiah Hayati
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Proceeding

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KATA PENGANTAR

Puji syukur kami panjatkan kepada Allah Subhanahu wa Ta'ala, Tuhan Semesta Alam atas karunia dan ridhoNya sehingga *Proceeding* Seminar Nasional Biodiversitas VI"Keanekaragaman Hayati Indonesia dan Perannya dalam Menunjang Kemandirian Bangsa" dapat diselesaikan dengan baik.

Proceeding ini merupakan rangkaian kegiatan Seminar Nasional Biodiversitas ke VI yang diselenggarakan pada tanggal 3 September 2016 di Departemen Biologi Universitas Airlangga. *Proceeding* ini memaparkan tentang hasil penelitian yang telah diseminarkan dan disusun berdasarkan bidang keahlian meliputi Botani, Ekologi, Mikrobiologi, dan Zoologi

Akhirnya, kami mengucapkan terima kasih kepada Kontributor artikel (peserta seminar) dan Panitia Seminar, para *Sponsorship*, dan Pimpinan, serta pihak-pihak lain yang belum kami sebut atas terselenggaranya seminar ini serta terwujudnya *proceeding* ini. Semoga Allah SWT meridhai semua langkah dan perjuangan kita, serta berkenan mencatatnya sebagai amal ibadah.Amin.

Surabaya, 3 September 2016

Panitia Seminar Nasional Biodiversitas VI

SAMBUTAN KETUA PANITIA
SEMINAR NASIONAL BIODIVERSITAS VI
FAKULTAS SAINS DAN TEKNOLOGI
UNIVERSITAS AIRLANGGA

Assalamu'alaikum warahmatullahi wabarakatuh

Puji syukur kami panjatkan ke hadirat Allah Subhanahu Wa Ta'ala Tuhan Semesta Alam, atas semua karunia yang diberikan kepada kita semua sehingga di hari yang berbahagia ini kita dapat berkumpul dalam forum Seminar Nasional Biodiversitas VI, yang diselenggarakan oleh Departemen Biologi Fakultas Sains dan Teknologi Universitas Airlangga. Ucapan terima kasih dan penghargaan yang setinggi tingginya kami sampaikan kepada yang saya hormati Dekan Fakultas Sains dan Teknologi Universitas Airlangga beserta Para Wakil Dekan, Ketua Departemen Biologi Fakultas Sains dan Teknologi Universitas Airlangga. Pembicara Utama, yang saya hormati Prof. Ir. Triwibowo Yuwono, Ph.D. Fakultas Pertanian Universitas Gadjah Mada, Prof. Ir. Tini Surtiningsih, DEA., Fakultas Sains dan Teknologi Universitas Airlangga, dan Dr. Teguh Triono, Yayasan Keanekaragaman Hayati (KEHATI) Indonesia. Terimakasih atas kehadiran dan dukungannya dalam kegiatan seminar kali ini.Terimakasih yang tak terhingga kami sampaikan kepada para pemakalah dan peserta seminar atas partisipasinya yang sangat membanggakan.

Seminar Nasional Biodiversitas VI ini merupakan sarana bagi peneliti untuk memaparkan berbagai kajian ilmiah yang terkait dengan keanekaragaman hayati Indonesia dan perannya dalam menunjang kemandirian bangsa. Panitia telah menghimpun 210 makalah dari para akademisi, peneliti, dan mahasiswa yang disajikan melalui presentasi oral dan poster. Makalah-makalah tersebut dikelompokkan menjadi empat bidang yaitu Botani, Ekologi, Mikrobiologi dan Zoologi. Peserta seminar berasal dari 53 instansi dari 19 provinsi yang tersebar di Indonesia. Propinsi Sumatera Utara (Universitas Sumatera Utara), Sumatera Barat (Universitas Andalas), Jambi (Universitas Batanghari), Jawa Barat (Universitas Padjajaran, Institut Teknologi Bandung, Puslitbio LIPI, Puslit Limnologi LIPI, Pulit Bioteknologi LIPI, Pusat Konservasi Tumbuhan Kebun Raya Bogor, Institut Pertanian Bogor, Balai Konservasi Tumbuhan Kebun Raya Cibodas, Balai Penelitian Teknologi Agroforestry), DI Yogyakarta (Universitas Muhammadiyah Yogyakarta, Balai Besar Penelitian dan Pengembangan Bioteknologi dan Pemuliaan Tanaman Hutan, Universitas gadjah Mada) Jawa Tengah (Universitas Jenderal Soedirman Purwokerto, Universitas Diponegoro, Universitas Sebelas Maret,

BPTP), Jawa Timur (IAIN tulung Agung, IKIP PGRI Madiun, Universitas PGRI Ronggolawe Tuban, Universitas Islam Lamongan, Universitas Negeri Malang, Universitas Brawijaya, Balitjestro, Universitas Muhammadiyah Malang, UPT BKT Kebun Raya Purwodadi, Universitas Negeri Surabaya, Institut Teknologi Sepuluh Nopember, Universitas Airlangga, Universitas Surabaya, Universitas PGRI Adi Buana, Universitas Tujuh Belas Agustus, Universitas Wijaya Kusuma, UPN Veteran, Universitas Negeri Jember), Bali (Balai Besar Penelitian dan Pengembangan Budidaya Laut, Balai Pengkajian Teknologi Pertanian), NTT (Universitas Muhammadiyah Kupang, Balai Taman Nasional Kelimutu), NTB (Universitas Mataram), Kalimantan Tengah (Universitas Palangka Raya), Kalimantan Selatan (Universitas Universitas lambung Mangkurat), Sulawesi Utara (Balitbang Lingkungan Hidup dan Kehutanan Manado), Sulawesi Selatan (Universitas Hasanudin), Sulawesi Tenggara (Universitas Halu Oleo), Maluku (Universitas Pattimura), Maluku Utara (Universitas Khairun Ternate), Papua (Universitas Sains dan Teknologi Jayapura), Papua Barat (Universitas Negeri Papua, Balitbang Lingkungan Hidup dan Kehutanan Manokwari, Balai Penelitian Kehutanan Manokwari). Panitia menyampaikan penghargaan atas karya ilmiah yang akan disajikan oleh pemakalah dalam forum ini.

Terimakasih dan penghargaan yang setinggi-tingginya saya sampaikan kepada Panitia Seminar Nasional Biodiversitas VI yang bekerja sama dengan sangat baik dan dengan senang hati menyumbangkan pikiran, waktu, dan tenaga untuk terlaksananya kegiatan ini. Akhirnya, saya mewakili panitia penyelenggara memohon maaf apabila dalam pelaksanaan seminar kali ini terdapat kekurangan. Semoga forum ini bermanfaat untuk pengembangan ilmu pengetahuan dan pemanfaatan biodiversitas Indonesia. Kepada peserta saya ucapkan selamat melaksanakan seminar, semoga ilmu kita semakin bertambah dan bermanfaat.

Wassalamu'alaikum warahmatullahi wabarakatuh

Ketua Panitia

Dr. Fatimah, M.Kes.

SAMBUTAN KETUA DEPARTEMEN BIOLOGI
FAKULTAS SAINS DAN TEKNOLOGI
UNIVERSITAS AIRLANGGA

Assalamu'alaikum warahmatullahi wabarakatuh

Para peserta seminar yang saya hormati, pertama-tama marilah kita panjatkan puja dan puji syukur ke hadirat Allah SWT yang telah memberi limpahan berbagai kenikmatan kepada kita sekalian, sehingga kita dapat berkumpul di forum ini. Karena kita diberikan nikmat kesehatan dan kesempatan sehingga kita dapat menyelenggarakan seminar ini. Selamat datang para peserta Seminar Nasional Biodiversitas VI di kampus Fakultas Sains dan Teknologi Universitas Airlangga.

Selanjutnya perkenankan saya menyampaikan penghargaan dan ucapan terima kasih kepada Ketua Panitia beserta seluruh jajaran kepanitiaan Seminar Nasional Biodiversitas VI yang telah mempersiapkan terselenggaranya Seminar Nasional ini. Secara khusus perkenankan saya sampaikan ucapan terima kasih kepada Bpk. Prof. Ir. Triwibowo Yuwono, Ph.D Dosen Fakultas Pertanian UGM, Teguh Triono, Ph.D Direktur Program PPB Yayasan Kehati Indonesia, dan Prof. Dr. Ir. Tini Surtiningsih, DEA Dosen Departemen Biologi Fakultas Sains dan Teknologi Universitas Airlangga yang telah berkenan menjadi pembicara kunci pada Seminar Nasional Biodiversitas VI ini.

Seminar Nasional Biodiversitas VI dengan tema "Keanekaragaman Hayati Indonesia dan Perannya dalam Menunjang Kemandirian Bangsa" tentu saja akan bermanfaat untuk memberikan pemanfaatan sumber daya alam secara berkelanjutan bagi kesejahteraan masyarakat Indonesia. Negara Indonesia tercinta ini, menduduki posisi keanekaragaman alam hayati di dunia tingkat pertama untuk tumbuh-tumbuhan palmae dan untuk jenis burung paruh bengkok, tingkat kedua untuk mamalia, dan tingkat ketiga untuk ikan tawar, tingkat keempat untuk reptil dan primata serta tingkat kelima untuk burung. Akan tetapi kita belum memiliki kemampuan sains dan teknologi untuk mengembangkannya menjadi kekuatan penggerak utama pembangunan. Kemampuan sains dan teknologi berada di negara maju yang umumnya hanya bersedia mengembangkannya di negara kita dan negara berkembang lainnya dengan prinsip hak-cipta. Ini berarti negara kita harus membeli sains dan teknologi negara maju agar dapat memanfaatkan kekayaan hayatinya sendiri.

Harapan saya dengan seminar ini paling tidak dapat sedikit demi sedikit memberikan pemahaman bagi kita, pejabat pemerintah, pengusaha, para wakil rakyat, maupun masyarakat awam untuk mengetahui apa itu kekayaan alam hayati, mengapa keanekaragamannya penting, apa kedudukan Indonesia dalam perangkat dunia dalam keunggulan keanekaragaman hayati, dan manfaatnya bagi Negara kita di masa depan.

Akhirnya saya mengucapkan terima kasih atas partisipasinya dalam Seminar Nasional Biodiversitas VI yang diselenggarakan oleh Departemen Biologi Fakultas Sains dan Teknologi Unair ini dengan harapan semoga memberikan manfaat bagi kita semua.

Wassalamu'alaikum warahmatullahi wabarakatuh

Surabaya, 3 September 2016
Ketua Departemen Biologi, Fakultas Sains dan Teknologi
Universitas Airlangga

Dr. Sucipto Hariyanto, DEA

SAMBUTAN DEKAN
FAKULTAS SAINS DAN TEKNOLOGI
UNIVERSITAS AIRLANGGA

Assalamualaikum warahmatullahi wabarakatuh

Sungguh merupakan suatu kebahagiaan tersendiri bagi kami bahwa pada tahun ini, tepatnya pada tanggal 3 September 2016 Fakultas Sains dan Teknologi (FST) telah berhasil menyelenggarakan kembali Seminar Nasional Biodiversitas VI.

Pertama kami panjatkan puji syukur kehadirat Allah SWT atas ridhonya, sehingga fakultas yang kami banggakan ini diberi cahaya dan kekuatan untuk melaksanakan seminar nasional ini.Yang kedua kami sampaikan rasa syukur dan ucapan terimakasih kepada seluruh anggota sivitas akademika khususnya Departemen Biologi dan panitia penyelenggara Seminar Nasional Biodiversitas VI, yang tetap setia mengabdi dan berkreasi dalam mengembangkan institusi dan keilmuan hingga hari yang berbahagia ini.Tanpa jerih payah dan kerja keras semua komponen, mustahil FST Universitas Airlangga mampu menuju keberhasilan.

Perkembangan informasi dan teknologi dewasa ini menuntut penguasaan ilmu yang dapat diterapkan dalam berbagai sektor kehidupan sebagai usaha untuk meningkatkan kualitas hidup manusia.Biologi adalah satu dari sekian banyak subjek keilmuan yang berkembang dengan kecepatan luar biasa khususnya temuan temuan ditingkat molekuler sampai tingkat nano science dan sudah diaplikasikan sesuai dengan kebutuhan dalam kehidupan.Ilmu Biologi terapan menjadi suatu harapan besar dalam mengiringi perkembangan teknologi *life science* dalam menunjang kemandirian bangsa. Oleh karena itu Seminar Nasional Biodiversitas VI ini mengambil tema Biodiversitas Untuk Pembangunan Berkelanjutan, dengan memfokuskan pada “Keanekaragaman Hayati Indonesia dan Perannya dalam Menunjang Kemandirian Bangsa”.

Harapan besar bagi kita semua dari hasil forum seminar seperti ini, akan memunculkan konsep-konsep baru tentang perkembangan ilmu biologi. Oleh

karena itu, setiap orang yang terlibat dalam aktivitas yang menggunakan pendekatan biologi dituntut memahami konsep-konsep dasar keilmuan itu secara seksama. Hanya dengan memahami konsep tersebut, maka manusia akan dapat menerapkan ilmu pengetahuan yang dikuasainya untuk kemaslahatan umat dalam berbagai sektor kehidupan, termasuk industri dan kedokteran.

Dalam bidang pendidikan, peserta didik perlu diberi kesempatan untuk berlatih memecahkan berbagai persoalan sebagai cara yang paling tepat untuk mempelajari konsep keilmuan. Melalui pendekatan seperti itu, maka Insya Allah generasi muda akan mempunyai kepercayaan diri yang tinggi karena potensi yang mereka miliki sebagai bekal membangun bangsa yang mandiri dan unggul.

Oleh karena itu saya berharap agar seminar nasional yang kita laksanakan pada hari ini mampu menghantarkan bangsa ini mencapai kemandirian bangsa melalui pembangunan berkelanjutan, dengan memanfaatkan keanekaragaman hayati di tanah air Indonesia tercinta ini.

Pada akhir sambutan ini sekali lagi kami ucapkan terimakasih sebesar-besarnya kepada semua yang telah ikut partisipasi dalam mensukseskan kegiatan ini, panitia, peserta, sponsor, dan semua pihak yang terkait.Semoga sukses dan lancar.

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MAKALAH UTAMA

ISOLATION AND CHARACTERIZATION OF ENDOPHYTIC BACTERIA FROM THE LEAF EXPLANTS OF *Avicennia marina* (Forsk.)

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ABSTRACT

Mangroves have important role in maintaining coastal area ecosystem as well as in protecting it from abrasion. The overexploitation of mangroves is of today's concern because it threatened the sustainability of the ecosystems. *Avicennia marina* (Forsk.) is one of mangroves that grow in Ekowisata Mangrove Wonorejo Surabaya. The previous aim of the experiment was to clone this species by plant tissue culture method in order to provide a sufficient number of seedlings for reforestation. However, there were a lot of obstacles in obtaining the sterile explants because the contaminants that live inside the leaf samples (endophytic). The isolation of endophytic bacteria was done after the surface sterilized leaf discs were cultured on MS and PCA media. The characterization was conducted biochemically based on *Bergey's manual of Determinative Bacteriology*. The results confidently showed that there were two genera of endophytic bacteria inside the leaves of *Avicennia marina* (Forsk.), i.e. *Bacillus* and *Corynebacterium*. However, there was also another genus but still not sure yet whether it was *Shigella* or *Yersinia*. The results were beneficial to further investigate the strategy to be able to obtain sterile leaf explants of *Avicennia marina* (Forsk.).

Keywords: *Avicennia marina* (Forsk.), characterization, endophytic bacteria, leaf explants.

PENDAHULUAN

Mangroves are the main vegetation live in coastal area. The importance of mangrove are to protect coastal area from destructive abrasion, to conserve flora and fauna, and as the food source for the people who live in surrounding area. Mangroves have special root system, i.e. stilt root, pneumatophores, knee root, plank root and buttress root as a mean of adaptation of these plants to their logged environment. Beside, these kinds of roots are also substantial to keep the soil solid so that the impact of abrasion could be diminished. The study of coastal zone management in Brebes Regency Coastal Area has been done by Faperi et al. (2015), showed that there was a correlation between the number of mangroves and the ecological factors. It was also informed about a decrease on mangrove number since 1983 to 2013 from 2,327 to only 243 mangrove survivals. That is why the reforestation is immensely needed to preserve the plants from extinction as well as to prevent the abrasion.

Since a mangrove tree could only produce seeds after it accomplish its vegetative phase, it needs a long of time because a mature mangrove tree could reach 20 m in height (van Steenis et al, 2013). Furthermore the seed viability is short, thus seed storage life is also short (Cousins and

Saenger, 2002). For this reason, a tissue culture method is required to produce numerous mangrove seedlings in a short time.

Avicennia marina (Forsk.) is a mangrove tree that grow in Ekowisata Mangrove Wonorejo Surabaya. This species is listed as 'least concern' species by *The IUCN Red List of Threatened Species* because its rapidity of growth and regeneration (The IUCN Red List of Threatened Species, 2016). Anyhow, the prevention act is still necessary to conserve this species from extinction since this species is vulnerable to pesticide. Furthermore, it was also mentioned that people usually exploit this species for food, fuel wood, construction material and medicine.

Obtaining a sterile explant by surface sterilization is the very first challenge in plant tissue culture works. It could be more challenging if the explants sources contain microorganisms. Without knowing what microorganisms actually are inside the explants, the surface sterilization will be more intricate. This experiment was aiming to isolate and characterize the endophytic bacteria inside the cultured leaf discs. The results will be useful to give information about alternative strategies that could be used to sterilize the leaf explants of *Avicennia marina* (Forsk.).

METODE PENELITIAN

Leaf Sterilization

The samples source was 2 years old seedlings obtained from Ekowisata Mangrove Wonorejo Surabaya. These seedlings were transferred to a greenhouse before the isolation of the leaves. Two kinds of sterilization methods were conducted. For the first sterilization method, the leaves were firstly isolated from the plant and washed under the tap water. After that, they were soaked in a bactericidal solution (Agrept 20WP®) for 2 to 4 hours, then in a fungicidal solution (Masalgin 50WP®) for 2 to 4 hours, followed by the immersion in a 70% ethyl alcohol (EtOH) solution for 30 seconds. The next step, they were transferred into Laminar Air Flow (LAF) and underwent the subsequent sterilization methods. Inside the LAF, the leaves were soaked inside sterile distilled water for 3 times, each of which for 5 minutes. The following step was to dip the leaves inside a 4% Sodium hypochlorite (NaOCl) solution for 10 minutes followed by the soaking inside the sterile distilled water for 5 minutes and repeated 3 times. Afterwards, the leaves were dipped inside a 1.3% NaOCl solution for 15 minutes. The last step was the washing of leaves with sterile distilled water to clean them from NaOCl remaining.

The second sterilization method was just the same as the first, except it did not follow the step where the leaves were dipped inside the bactericidal and fungicidal solution.

Culture Media

Murashige and Skoog (MS) enriched with vitamins, 3% sucrose and solidified with 12 gram commercial agar was made to culture the sterile leaf discs. For isolation and characterization of endophytic bacteria, Plate Count Agar (PCA) media (Merck®) was also used. The MS media

were enriched with kanamycin and polyvinylpyrrolidone (PVP) in 5 various compositions as listed in Table 1 below. Other MS medium was also prepared for the explants underwent dipping in bactericidal and fungicidal solution for 3 hours.

Table 1. Various compositions of MS media and surface sterilization methods

Treatments	The Exposure Time of Explants' Surface Sterilization in Bactericidal and Fungicidal Solution (hour)	MS Media Composition
MS 1	2	MS
MS 2	2	MS + 100 ppm Kanamycin
MS 3	3	MS + 100 ppm Kanamycin + 100 ppm PVP*
MS 4	4	MS + 100 ppm Kanamycin + 100 ppm PVP
MS 5	4	MS + 100 ppm Kanamycin + 200 ppm PVP

*PVP stands for polyvinylpyrrolidone

Explants Culture on MS and PCA Media

The leaves were firstly cut into small discs with the size of about (1 x 1-2) cm^2. The leaf discs that underwent the first sterilization method were cultured on the MS medium, whereas those that followed the second sterilization method were cultured on the PCA medium.

Isolation and Characterization of Endophytic Microorganisms

The bacterial isolates obtained from contaminated explants cultured on MS and PCA media were then purified on the Nutrient Agar (NA) medium by using four quadrant streak method. This method was repeated 5 times until the single colony was achieved. The single colonies were then characterized morphologically including the size, the color, the form, the margin, the elevation, Gram type and the present or absent of spore. Afterwards, they were characterized biochemically based on *Bergey's Manual of Determinative Bacteriology.* The characterization process were carbohydrate fermentation test, Voges-Proskauer test, oxidase test, catalase test, sulphur indole motility test, citrate utilization test, urease test, starch hydrolysis test and nitrate reduction test.

HASIL DAN PEMBAHASAN

The Growth of Endophytic Bacteria on Various MS Media

Table 2 showed the occurrence of contamination on explants cultured on various MS media. The starting dates of the bacterial growth were vary, from day 5 to day 9 of the culture. The results

also showed that 87% to 100% of cultured explants were contaminated in within 10 to 22 days. Figure 1 showed the leaf discs culture of *Avicennia marina* (Forsk.) on MS 3 medium.

Table 2. Contamination and browning percentage of explants cultured on various MS media

Treatment	Number of Cultured Explants	First Day of Contamination (day)	Last Day of Contamination (day)	Contamination Percentage (%)*	Browning percentage (%)*
MS 1	30	5	14	30/30 (100)	0/30 (0)
MS 2	24	7	16	23/24 (96)	1/24 (4)
MS 3	32	9	22	28/32 (88)	4/32 (12)
MS 4	30	5	10	26/30 (87)	0/30 (0)
MS 5	30	8	14	29/30 (97)	30/30 (100)

*Observed at last day of contamination

Table 2 informs us that the contamination decreased from 100% on MS 1 to 96% on MS 2 as the adding of 100 ppm kanamycin to the medium. The longer the exposure time in bactericidal and fungicidal solution (3-4 hours) seemed to be more effective to decrease the contamination (MS 3 and MS 4), but the adding of 200 ppm PVP to the media increased the contamination percentage (MS 5).

The bactericidal solution contained streptomycin sulphate, an antibiotic that active to inhibit the growth of Gram-negative bacteria. Kanamycin which was added to the MS medium is broad-spectrum antibiotic used to treat Gram-negative and Gram-positive bacterial infection (Gourevitch et al, 1958). Both kanamycin and streptomycin sulphate were effective in eliminating contaminating bacteria on the culture of *Guadua angustifolia* Kunth. (Nadha et al, 2012). Savitri (2014) successfully obtained 95-98% *Phaleria macrocarpa* leaf discs' survivals after being exposed to streptomycine sulphate solution for 2 to 26 hours followed by soaking them inside NaOCl solution.

Polyvinylpyrrolidone (PVP) is a clarifying and stabilizing agent (FAO, 1986) used as browning control (Reutle and Natter, 1994) or preventing browning either by rinsing the explants or incorporating into the media (Sathyanarayana and Varghese, 2007). However, the present of PVP in this experiment did not help to prevent the browning of the explants except those cultured on MS 4.

Since the contamination and the browning were still in high percentage, this experiment was ended. We presumed that they were endophytic bacteria because the contaminants started to grow at least at day 5 and appeared from the explants surface. The next experiment was conducted to observe the contaminants, so we could find the best methods of surface sterilization.

The Growth of Endophytic Bacteria on MS and PCA Media

The sterile leaf discs, obtained from those which were exposed to bactericidal and fungicidal solution followed by several sterilization by using NaOCl, cultured on MS medium (without the adding of kanamycin and PVP) showed contamination after one week. The contaminants seemed to be bacteria because of their macroscopic appearance on the MS medium. Figure 1A shows the contamination on MS medium after one week. The PCA medium was prepared to compare the growth of endophytic bacteria in both media. Without sterilize the explants by using bactericidal and fungicidal solution, we tried to isolate fungus that might be also live endophytically. In addition, with the help of nutrients contained in the PCA medium, the fungus might be grow. Yet the result showed that the contaminants on PCA medium were also bacteria. The contamination started at day 4, as shown on Figure 1B. The earlier time of contamination was probably because the explants have not exposed to bactericidal solution, not as the same as those cultured on MS medium. We cultured 5 sterile leaf discs in each culture bottle contained 25 ml solid media. This culture was repeated 5 times both on MS and PCA media. We found 10 contaminated explants from the MS medium, whereas from the PCA medium we observed 15 contaminated explants. The bacterial isolation was then conducted for each of the contaminated explants.

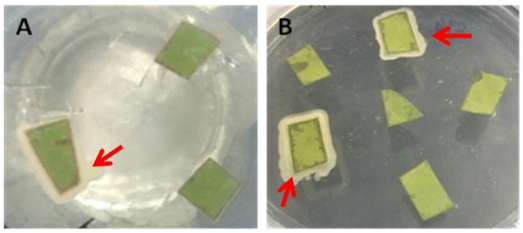

Figure 1. Explants contamination prior to endophytic bacteria. A, Leaf discs underwent sterilization with bactericidal, fungicidal and NaOCl solution, cultured on MS basal medium; B, Leaf discs underwent sterilization with NaOCl but without bactericidal and fungicidal solution, cultured on PCA medium.
Red arrow shows the appearance of contaminants at the leaf discs' edges.

Isolation and Characterization of Morphological Properties of Endophytic Bacteria

All of 25 isolates (10 isolates were obtained from MS medium and 15 from PCA medium) were purified on NA medium to obtain single colonies. Table 3 shows the morphological characteristic of bacterial isolates which were isolated from MS medium.

Table 3. Morphological characteristics of bacterial isolates from MS medium

Isolates Name	Isolate Width*	Isolate Color	Isolate Form	Isolate Margin	Isolate Elevation	Gram Type	Spore Forming	Isolate Shape
M1	Big	Translucent, Dull	Circular	Entire	Convex	Positive	Present	Rod
M2	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
M3	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
M4	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
M5	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
M6	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
M7	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
M8	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
M9	Big	Translucent, Dull	Circular	Entire	Convex	Positive	Present	Rod
M10	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod

*small (≤ 0.5μm), medium (0.5μm – 1μm), big (≥ 1μm)

Based on Table 3, we assume that isolate M1 and M9 are the same bacteria because of the exact similarities of morphological appearances, whereas isolate M2, M4, M6 and M7 are also the same colony. The same assumption also applies to isolate M3, M5, M8 and M10 that they are actually the same species. So now we have 3 groups of bacterial isolates from contaminated explants cultured on MS Medium.

In addition, the information about morphological entities of bacterial isolates that were isolated from PCA media is as shown on Table 4.

Table 4. Morphological characteristics of bacterial isolates from PCA medium

Isolate Name	Isolate Width*	Isolate Color	Isolate Form	Isolate Margin	Isolate Elevation	Gram Type	Spore Forming	Isolate Shape
P1	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
P2	Big	Translucent, Dull	Circular	Entire	Convex	Positive	Present	Rod
P3	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
P4	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
P5	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
P6	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
P7	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
P8	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
P9	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
P10	Big	Translucent, Dull	Circular	Entire	Convex	Positive	Present	Rod
P11	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
P12	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
P13	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod
P14	Small	Translucent, Dull	Circular	Entire	Convex	Positive	Absent	Rod
P15	Medium	White, Dull	Circular	Entire	Convex	Negative	Absent	Rod

*small (≤ 0.5µm), medium (0.5µm – 1µm), big (≥ 1µm)

As there also are the similarities between some isolates, so we supposed that isolate P1, P4, P7, P8, P11 and P14 were one species, whereas isolate P2 and P10 were the same colony, and also isolate P3, P5, P6, P9, P12, P13 and P15 were the same. This means that we now have 3 groups of isolates from contaminated leaf discs cultured on PCA medium.

The Results of Biochemical Characterization of Endophytic Bacteria

Each of the 25 isolates were then followed the biochemical test based on *Bergey's manual of Determinative Bacteriology.* The results are as shown on Table 5 below.

Table 5. Biochemical characteristic of bacterial isolates

Bacterial Isolate from MS Medium (isolate number)	Bacterial Isolate from PCA Medium (isolate number)	Gram Type	Spore Forming	Biochemical Test*	Result	Suspected Species
M2, M4, M6, M7	P1, P4, P7, P8, P11, P14	Positive	Absent	[1]Catalase	Positive	*Corynebacterium kutscheri*
				[2]Starch hydrolysis	Positive	
M1, M9	P2, P10	Positive	Present	[1]Starch hydrolysis	Positive	*Bacillus megaterium*
				[2]Voges-Proskauer	Negative	
				[3]Swollen cell	Negative	
				[4]Citrate utilization	Positive	
M3, M5, M8, M10	P3, P5, P6, P9, P12, P13, P15	Negative	Absent	[1]Oxidase	Negative	*Shigella sonnei* or *Yersinia pestis*
				[2]Lactose fermentation	Negative	
				[3]Indole	Negative	
				[4]Urease	Negative	
				[5]Motility	Non motile	
				[6]Ornithine decarboxylase	Do not know	

*The biochemical test was conducted respectively from step 1 to 2 and so on.

The biochemical test informs us that there were exactly 3 same groups of isolates found in sterile leaf discs of *Avicennia marina* Forsk. isolated from both, MS and PCA media. They are *Corynebacterium kutscheri* and *Bacillus megaterium*, and one other suspected species that still need further test to determine whether it is a *Shigella sonnei* or *Yersinia pestis*. Isolate M2, M4, M6, M7, P1, P4, P7, P8, P11, and P14 are characterized as catalase positive and starch hydrolysis positive. It means that it can produce catalase and enzyme that catalyzes starch. Isolate M1, M9, P2, and P10 could hydrolyze starch but showed negative result towards Voger-Proskauer test, which implied that these isolates did not produce acetoin. Swollen cell test showed that these microbes had no swollen cell, while in citrate-utilization test, they showed positive result. Isolate M3, M5, M8, M10, P3, P5, P6, P9, P12, P13, and P15 were negative toward oxidase test, denoted that these isolates did not have cytochrome c. They were also negative toward lactose fermentation test, which counted that they could not ferment lactose as their carbon source. Even more, they showed negative results toward indole and urease test, which implied that they did not have triptophanase enzyme to produce indole from tryptophan and urease enzyme to split urea into smaller molecules.

Ornithine decarboxylase test is used to determine whether a bacterium can use the amino acid ornithine as a source of energy. The bacterium which positive to this test has ornithine decarboxylase enzyme that can help the catabolism of ornithine. The positive result of this test

will be indicated by the color change from yellow to purple. Unfortunately we did not have ornithine in our laboratory so we could not conduct this test.

Bacillus megaterium has been reported as endophyte in several plant hosts. In 1995, McInroy & Kloepper successfully isolated *Bacillus megaterium* from the stems of sweet corn. Araújo et al (2001) isolated several bacteria and fungi including *Bacillus megaterium* from leaf tissues of citrus. From the root-nodules of *Medicago sativa*, Khalifa and Almalki (2015) isolated *Bacillus megaterium* BMN1, which later known that it had a potential to produce indole acetic acid and acetoin. To date, we have not found yet the report that explained about the presence of *Bacillus megaterium* in mangrove's tissues. Yet other species such as *Bacillus tequilensis* and *Bacillus subtilis* have been isolated from mangroves *Ceriops decandra* and *Bruguiera cylindrica* respectively (Eldeen et al, 2015).

Corynebacterium kutscheri is common in animal but never known as a living thing inside the plant. It is a pathogen that cause pneumonia in rats (Giddens Jr. et al, 1968). Later in 2007, Holmes and Korman reported that this bacterium is the normal microorganism live in the oral cavity of healthy mice and rats. It caused swelling and erythema in the patient's finger which had been bitten by rat. Nevertheless, there is another species related to *C. kutscheri* that live inside the plant body. *Corynebacterium xerosis* is one of endophytic bacteria isolated from *Mentha rotundifolia* L. (Abla et al, 2014). Furthermore, El-Banna (2006) reported that both *C. kutscheri* and *C. xerosis* were isolated from the soil sample. Through this finding, we probably could assume that the soil where the *A. marina* lives also contained *C. kutscheri*, thus it could penetrate to the plant's leaves via the root. However further investigation must be conducted to prove this assumption.

Both *Shigella sonnei* and *Yersinia pestis* are species belong to Enterobacteriaceae. Although *Shigella sonnei* is actually the common microbe found in the gastrointestinal tract as the causal agent of shigellosis disease (Boumghar-Bourtchai et al, 2008), a *Shigella sonnei*'s strain has successfully isolated from *Aloe vera*'s roots, stems and leaves (Akinsanya et al, 2015). *Yersinia pestis* is known as pathogen in human, as the causal agent of pneumonic plague (Treille & Yersin, 1894 in Rasmussen et al, 2015). Until now, there is no study has been reported about the involvement of this bacterium as an endophyte. However there are some reports have informed about the occurence of Enterobacteriaceae as endophytic agents. For example *Enterobacter* spp. has been found in maize (McInroy & Kloepper, 1995) and *E. sakazakii* was found as an endophyte in soybean (Kuklinsky-Sobral, 2004). There were also some other genera like *Erwinia* sp. in soybean (Kuklinsky-Sobral, 2004), *E. coli* in lettuce (Ingham et al, 2005) and *Klebsiella oxycota* in soybean (Kuklinsky-Sobral, 2004). The implications of this Enterobacteriaceae family usually have a close relation with the poor sanitation (Musgrove et al, 2009). Thus, the plants that are colonized by Enterobacteriaceae probably have been exposed to human and animal waste. In this case, as reported by Husain (2014), that people who live next to Jagir river have the

habitual to dispose their human waste into this river. Jagir river flows into the Ekowisata Mangrove Surabaya. So, it is a high possibility that the seawater of Ekowisata Mangrove Surabaya, which is the place where our explants have been collected, is also contaminated with this human waste.

Although no exact species has been informed, Gayathri et el (2010) have reported that mangrove plants, such as *Rhizophora apiculata, Avicennia marina* and three other species, were the host of 72 bacterial endophytes. They isolated 5 pigmented and 12 non-pigmented colonies from the sterile leaf extract of *A. marina* alone. In vitro propagation of *A. marina* has been done before by Cousins and Saenger in 2002. They used the first binodal stem explants and sterilized them with 0.1% mercuric chloride ($HgCl_2$) solution. Yet the surface sterilization study was reported to be difficult with the yield was only less than 10%. The use of 0.1% $HgCl_2$ for surface sterilization was also applied by Vartak and Shindikar (2008) to obtain sterile hypocotyl segments of *Bruguiera cylindrica* L. $HgCl_2$ is hazardous as it the cause of cancer when inhaled or absorbed through the skin (NJ Health, 2009). In the contrary, this substance is the most suitable agent to provide the highest survival from runner tips and nodal segments of strawberry (Jan et al, 2013) and also from nodal and leaf segments of *Morinda citrifolia* (Elakkuvan and Manivannam, 2015).

The further investigation that should be conducted to produce sterile leaf explants is to apply antibiotics into the MS medium prior to explants culture. Vancomycin can be used against *Corynebacterium* as this bacterium is sensitive to vancomycin (Soriano et al, 1995). *Bacillus* can be diminished by the addition of penicillin and vancomycin (Hitchins and Slepecky, 1968) to the culture medium. The recommended antibiotics for *Shigella* are ampicillin and cephalexin (McIver et al, 2002). While for *Yersinia*, as suggested by Bottone (1997), the use of ceftriaxone and chloramphenicol is preferable to stop this microbe's growth. Yet the optimal concentration of these antibiotics must be sought to ensure their efficacy in eliminating these endophytes from *Avicennia marina*'s leaf explants.

DNA sequencing is probably needed to confirm the validity of the biochemical test. In addition to produce sterile leaf explants, this technique is beneficial as the new information of endophytic bacterial diversity in mangrove plants, as endophytes have excellence in producing plant hormones (Khalifa and Almalki, 2015) and bioactive compounds that can be developed as medicine (Prihatiningtias and Sri, 2011). Furthermore, endophytes are potential to increase crops yields if the information about their ecology is discovered (Rosenblueth and Martínez-Romero, 2006).

KESIMPULAN

The contaminants inside the surface sterilized leaf discs of *Avicennia marina* Forsk. are endophytic bacteria. After the isolation and characterization procedures, it is known that the endophytes are *Bacillus megaterium*, *Corynebacterium kutscheri* and *Shigella sonnei* or *Yersinia pestis*.

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