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Isolation and characterization of *Chromobacterium violaceum* from a disused tin-mining lake in Malaysia

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During a survey of microorganisms from a disused tin-mining lake in Kampar, Perak, Malaysia, a distinct bacterium producing a purple pigment was isolated. The isolate was characterized by morphological observation, followed by a series of conventional biochemical tests, physiological tests, as well as antibacterial tests, and identified as *Chromobactertium violaceum.* It was a facultatively anaerobic, motile, Gram-negative bacillus. The identity of this bacterial isolate was verified by a phylogenetic analysis of its 16S rRNA sequence. The ecological, medical, pharmacological and industrial importance of this bacterium with its production of the purple pigment, violacein, was briefly discussed.

Key words: Environmental microbiology, freshwater ecology, opportunistic infection, pigmented bacteria, 16S rRNA gene.

INTRODUCTION

Tin-mining activities in Malaysia had been very active in the late 19th century. The main tin-mining areas were in the Kinta Valley of Perak State, which included districts such as Ipoh, Batu Gajah, Gopeng and Kampar (Shamshuddin et al., 1986). The tin-mining activities, however, had ceased over a hundred years ago and there are now numerous disused tin-mining lakes in the Kinta Valley, especially in the vicinity of Kampar. In these lakes, minerals and organic materials are of great abundance and encourage a rich diversity of life. The microbial communities in such aquatic system play a vital role in global ecosystem and human health (Saleem et al., 2011).

In an attempt to isolate bacteria from a tin-mining lake in Kampar, we found a distinct isolate which produced a purple pigment. This paper described the isolation and

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characterization procedures of this bacterium. Its identity to be *Chromobacterium violaceum* Bergonzini (Bergonzini, 1880) was verified by a series of morphological, physiological and biochemical tests. The phylogenetic relationship of this isolate was inferred by comparing its 16S rRNA gene sequence with that of similar taxa. Previous findings about the ecological, medical and industrial importance of *Chromobacterium* species were summarized and briefly discussed. This paper represents the first report of the occurrence of *Chromobacterium* in the disused tin-mining lakes of Kampar region.

MATERIALS AND METHODS

Source of materials

Water samples were collected into a sterile 250 ml Schott bottle from a disused tin-mining lake in Old Town, Kampar, Perak, Malaysia. The temperature of the lake water was measured using a thermometer.

Isolation of bacteria using the enrichment method

Water sample was transferred into a sterile 15 ml Falcon tube and centrifuged at 6000 rpm for 10 min. After centrifugation, the supernatant was discarded and the pellet was re-suspended in 3 ml nutrient broth. The broth was then incubated overnight at 37°C (Malghani et al., 2009).

Serial dilution, spread plate and streak plate

After incubation, the sample was serially diluted to 10^{-6} with phosphate buffer saline (PBS). Diluted samples were then spread onto nutrient agar plates and incubated at 37°C for 18 h (Lammert, 2007). The plates were checked for microbial colonies. Interesting isolates were picked and streaked on nutrient agar in order to get isolated single colonies.

Isolation of bacteria using the membrane-filtration method

Water sample was first filtered through regular filter paper (Whatman) to remove any unwanted substances such as algae and plant debris in the water. Then, 100 ml of filtered water sample was transferred into a sterile filtration unit as shown in Figure 1:1. The cellulose acetate millipore membrane of 0.2 µm pore size was used as the filter (Lammert, 2007). A vacuum pump was connected to the filtration unit to ease the filtration process. After filtration, the membrane was transferred into a sterile 50 ml Falcon tube containing 10 ml of PBS, which was then mixed well and followed by serial dilution as well as plating on nutrient agar.

Morphological examination

Bacterial isolates were examined for colony morphology, followed by Gram stain (Cappuccino and Sherman, 2013) and endospore stain (Chess, 2009). The isolates were also observed under wet-mount microscopy for motility.

Optimal growth temperature experiments

To find out the optimal temperature range for growth, bacterial isolates were streaked onto nutrient agar plates and incubated at various temperatures for 18 h. All the isolates were tested for growth at 4, 22, 37, 46 and 60°C.

Conventional biochemical tests

Presence of extracellular enzymes

Bacterial isolates were tested for starch hydrolysis, casein hydrolysis and fat hydrolysis by inoculating to specific agar media. Starch Agar, Milk Agar and Egg Yolk Agar were used to detect hydrolytic activities of amylases, proteases and lipases, respectively. Isolates were streaked on each specific medium and incubated at 37°C for 18 h. The cultures were checked for hydrolytic result, which was indicated by a clear zone surrounding the bacterial colonies (Lammert, 2007).

Other biochemical and physiological tests

Bacterial isolates were tested for a number of biochemical and physiological properties using various specific agar media following standard protocols (Cappuccino and Sherman, 2013; Chess, 2009; Lammert, 2007). The following tests were performed: Oxidation-Fermentation (OF) Tests (for glucose and sucrose), Citrate Utilization Test, SIM Agar Test (for hydrogen sulfide production, indole production and motility test), Catalase Test, Oxidase Test, and Triple-sugar Iron (TSI) Agar Test. The isolates were also streaked on Blood Agar for observation of hemolysis.

API assay

The API assay for bacterial enzymes (Humble et al., 1977) was carried out by using the API ZYM test strip from BioMeriux SA. Enzymes assayed were alkaline phosphatase, esterase, esterase lipase, lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The assay was carried out according to procedures described in the user manual of the API ZYM test kit. The API test strips were incubated for 4.5 h before colour changes were recorded and interpreted.

Antibacterial activity

Antibiotic susceptibility tests using disk-diffusion method

Isolates were subjected to antibiotic susceptibility tests according to methodology described by Bauer et al. (1966). The standard antibiotic disks used were chloramphenicol, tetracycline, penicillin, nitrofurantoin and sulfametaphazole and trimeroprim. Isolates were swabbed onto Müeller-Hinton agar plates followed by putting the antibiotic disks on the agar surface. Plates were incubated at 37°C for 18 h. After incubation, the plates were checked for antibiotic susceptibility by measuring the zones of inhibition.

Assay for antimicrobial agent using agar-well diffusion method

Isolates were also assessed for antibacterial activity against four standard indicators species using agar well-diffusion method (Liasi et al., 2009). The four standard indicator species were *Salmonella typhimurium* (ATCC 14028), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* subsp. *spizizenii* (ATCC 6633) and *Staphylococcus*

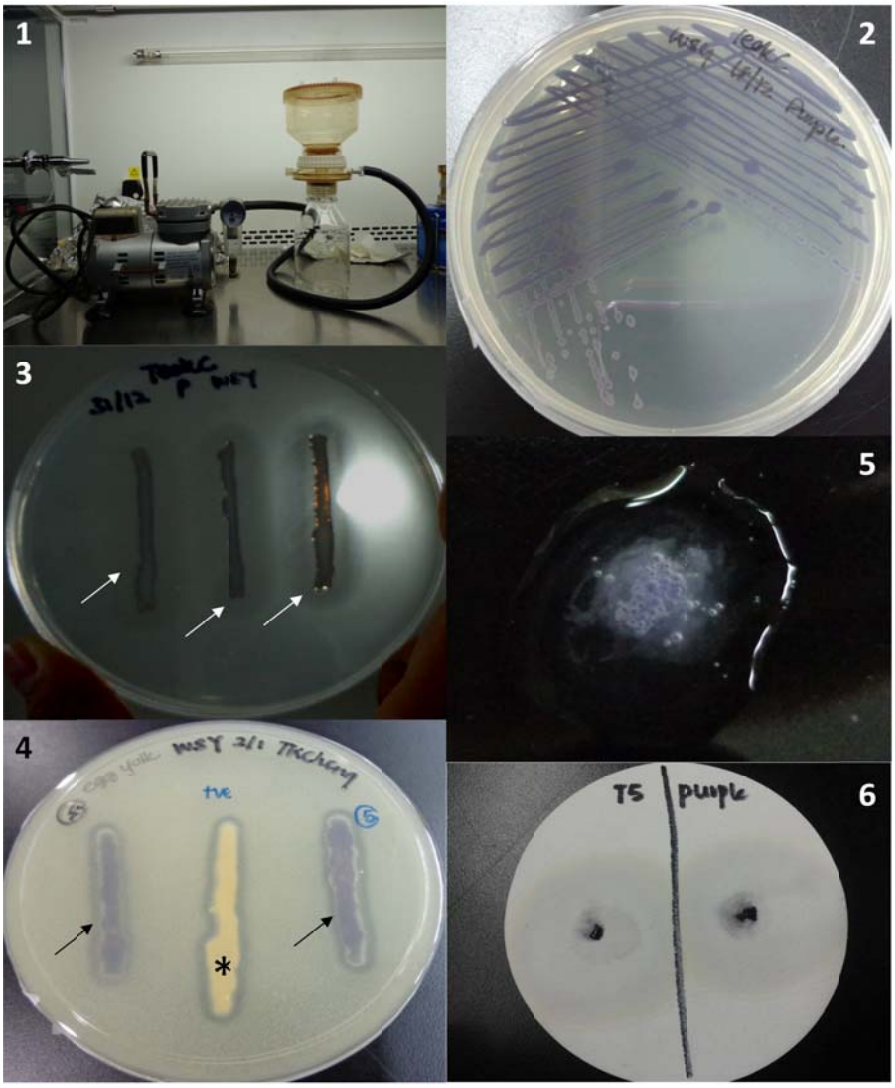

Figure 1. *Chromobacterium violaceum.* 1. Set-up of the membrane-filtration method for the isolation of bacteria from water samples. 2. Colonies on nutrient agar plate. 3. Casein Hydrolysis Test result (positive) in triplicates. Arrows point to clear zones due to hydrolysis. 4. Fat Hydrolysis Test result (positive) in duplicates. Arrows point to clear zones due to hydrolysis. Asterisk indicates position of positive-control isolate. 5. Catalase Test. Gas evolution indicates a positive result. 6– Oxidase Test. Test sample turns blue-black indicating a positive result.

aureus (ATCC 6538). Isolates were prepared in 0.85% saline. The bacterial inoculum's turbidity was referred to McFarland standard, for which the OD was within 0.1 and 0.2. Müeller-Hinton agar plates were punched with wells and the indicator species were swabbed onto the agar surface. 40 µL of the bacterial inoculum were transferred into the well, and the inoculated plates were incubated at 37°C for 18 h. After incubation, the plates were checked for the presence of zones of inhibition.

Phylogenetic analysis

Genomic DNA extraction

The genomic DNA of the present bacterial isolate (PB_Malaysia) was extracted using fast boil method (Holmes and Quigley, 1981). The bacterial samples were inoculated into LB broth for 24 h at 37°C with agitation of 200 rpm. After 24 h incubation, 1 mL of bacterial culture was centrifuged at 13,000 rpm for 5 min. The cell pellet was resuspended with 50 µL of sterile distilled water and incubated in water bath at 70°C for 20 min. After incubation, the sample was centrifuged at 13,000 rpm for another 5 min and the supernatant was transferred into a new sterile Eppendorf tube. The final concentration of the DNA was measured using Nanodrop 1000 (Thermo Scientific) and stored at -20°C for future use.

Polymerase chain reaction (PCR)

Partial 16S rRNA amplification was done by PCR with the genomic

DNA of isolate as template. The amplification reaction was made using the Taq DNA polymerase by 1st base and oligo 14-F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492-R (5'-GGTTACCTTGTTAGGACTT-3') as universal bacterial primers. PCR program was carried out in Thermal Cycler (Bio-rad) which comprised three steps: 1) denaturation at 94°C for 30 s; 2) annealing at 52.7°C for 30 s; and 3) extension at 72°C for 1 min. The amplicon was purified using a PCR purification kit (Philekorea Technology) and source out for sequencing analysis.

Phylogenetic analysis

The isolated 16S rRNA sequences were aligned and compared with available online database of other bacterial isolates using Basic Local Alignment Search Tool (BLAST) from National Center of Biotechnology Information (NCBI). The phylogenetic analysis was carried out based on the 16S rRNA sequences of the isolate being studied (PB_Malaysia). Neighbor-joining phylogenetic tree was constructed using MEGA 5.2 software and the bootstrap test was performed with 1000 replicates.

RESULTS

Growth and basic morphology

Water samples were collected from a disused tin-mining lake at Kampar, with water temperature ranging from 32 to 33°C. A number of different bacterial isolates were obtained from the collected water samples, among them, *Chromobacterium violaceum* was spotted easily due to its distinct purple colonies (Figure 1: 2). It was isolated from both the enrichment method and the filtration method. It grew well at 22 and 37°C, but not at 4, 46 and 60°C. The colonies on nutrient agar plates incubated at optimal temperature were purple in colour, circular, slight raised, with an entire margin and smooth surface. *C. violaceum* is motile, Gram-negative, non-sporing coccobacillus.

Biochemical and physiological properties

Our isolate of *C. violaceum* was able to hydrolyze casein (Figure 1: 3) and fat (Figure 1: 4), but not starch. It was catalase-positive (Figure 1: 5) and oxidase-positive (Figure 1: 6). It oxidized and fermented glucose (Figure 2: 7), but not sucrose. It was unable to utilize citrate as the sole carbon source. On SIM agar, it neither produced hydrogen sulfide nor indole, but it showed motility (Figure 2: 8).

On Blood Agar (Figure 2: 9), our isolate was distinctly β-hemolytic, showing clear zones surrounding the bacterial colonies (Figure 2: 10). On TSI medium, our isolate showed an alkaline slant and an acidic butt without H_2S or gas production (Figure 2: 11). Proteolysis and fermentation of glucose occurred, but lactose and sucrose were not utilized.

In API Test (Table 1 and Figure 2: 12), our isolate was positive in alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase and Naphthol-AS-BI-phosphohydrolase. Table 2 summarizes the results of various biochemical tests of our *Chromobacterium* isolate in this study. The results of selected biochemical tests of our *Chromobacterium* isolate were compared with those of other isolates from other countries, based on the literature (Table 3).

Antibacterial activities

In the results of antibiotic disk-diffusion tests (Figures 3: 13 to 16), our isolate of *C. violaceum* showed susceptibility to chloramphenicol, tetracycline, nitrofurantoin and sulfametaphazole and trimeroprim. It was resistant to penicillin.

Our isolate of *C. violaceum* showed antibacterial ability to *Bacillus subtilis* subsp. *spizizenii* (Figure 3: 17) as well as *Staphylococcus aureus* (Figure 3: 18), but not *Salmonella typhimurium* and *Escherichia coli*

Molecular phylogenetic analysis

The 16S rRNA sequence analysis of our bacterial isolate showed a high identity (99%) with *C. violaceum*. The phylogenic tree (Figure 4) showed that our isolate clustered together with other *C. violaceum* isolates in a distinct clade supported by 99% bootstrap values. It was closer to, but distinct from *Vogesella indigofera*, another bacterium that produces a purple pigment.

DISCUSSION

The bacterium that was obtained from the disused tin-mining lake at Kampar, Malaysia, was identified as *C. violaceum*. Characterization of the isolate by various biochemical and physiological tests supported the identification of this bacterial species. Its identity was confirmed by our molecular phylogenetic analysis as well as the comparison of various biochemical test results with those reported from other countries (Antunes et al., 2006; Chang et al., 2007; Jitmuang, 2008; Kaufman et al., 1986; Kumar, 2012; Lee et al., 1999).

In phylogenetic studies, we compared our Malaysian isolate (PB_Malaysia) of *C. violaceum* with other pigment-producing bacterial taxa, and the identity of our isolate in *Chromobacterium* was unambiguous. The closest taxon to *Chromobacterium* is *V. indigofera* which produces a blue pigment (indigoidine). Although it is also commonly found in freshwater, it is not known to be pathogenic (Grimes et al., 1997). Other purple or blue pigment-producing bacterial taxa in our comparison were *Arthrobacter*, *Corynebacterium*, *Erwinia*, *Iodobacter* and *Janthinobacter* (Cardona-Cardona et al., 2010), but they were phylogenetically distant from *Chromobacterium*.

C. violaceum is a facultatively anaerobic, motile, Gram-negative bacillus. Currently there are a great number of

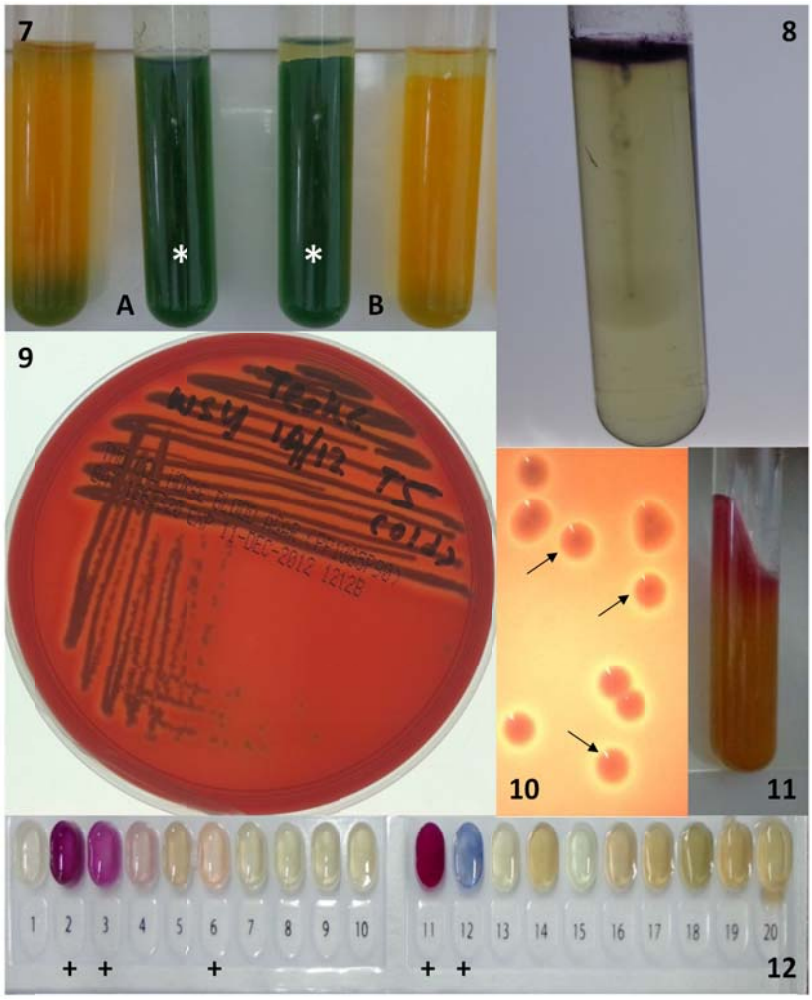

Figure 2. *Chromobacterium violaceum*. 7. Glucose Utilization Test result. A = aerobic (oxidative), positive; anaerobic (fermentative), positive. Asterisks indicate uninoculated control tubes. 8. SIM Agar Test result showing negative in hydrogen sulfide and indole production and positive in motility. 9-10. Colonies on blood agar showing b-hemolysis. Arrows point to clear zone due to b-hemolysis. 11. TSI Agar Test result showing alkaline slant (red), acidic butt (yellow) and no gas production. 12. Biochemical assay using the API test strip. Result show positive in alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase and Naphthol-AS-BI-phosphohydrolase.

publications worldwide about this bacterium. It is a common inhabitant of soil and water confined to tropical and subtropical regions. Generally, it behaves as a saprophyte, but sporadically it becomes an aggressive opportunistic animal (including human) pathogen, causing serious infections with a high mortality in immune-deficient individuals. The production of violacean, the purple pigment, has attracted many researchers worldwide to study on this bacterium. With these many achievements in research on this bacterium throughout the decades, we therefore did not carry out any further research with our Malaysian isolate, but briefly presented in the following sections the various scientific findings, highlighting its medical, ecological and industrial importance.

Medical importance

In recent decades, *C. violaceum* has been noticed to cause severe systemic infection of humans, usually via an open wound (Jitmuang, 2008; Kumar, 2012). It is noteworthy for its difficult-to-treat entity characterized by a high frequency of sepsis and high mortality rate (Ang, 2004; Kaufman et al., 1986). The pathogenic potential of *C. violaceum* was first described by Wooley (1905) from a fatal infection in buffalo, while human infection was first reported in 1927 from Malaysia by Lessler (Sneath et al., 1953).

The most recent comprehensive review of human infections by *C. violaceum* worldwide was given by Yang and Li (2011). They studied 106 cases of *C. violaceum*

Table 1. Biochemical test results of *C. violaceum* using the API ZYM test strip.

Types of enzyme	Reaction
Control	–
Alkaline phosphatase	+
Esterase (C 4)	+
Esterase lipase (C 8)	–
Lipase (C 14)	–
Leucine arylamidase	+
Valine arylamidase	–
Cystine arylamidase	–
Trypsin	–
α-chymotrypsin	–
Acid phosphatase	+
Naphthol-AS-BI-phosphohydrolase	+
α-galactosidase	–
β-galactosidase	–
β-glucuronidase	–
α-glucosidase	–
β-glucosidase	–
N-acetyl-β-glucosaminidase	–
α-mannosidase	–
α-fucosidase	–

Table 2. Results of various biochemical tests of *C. violaceum*.

Biochemical tests	*Chromobacterium* Isolate
Starch Hydrolysis Test	–
Casein Hydrolysis Test	+
Fat Hydrolysis Test	+
Oxidase Test	+
Catalase Test	+
Citrate Utilization Test	–
SIM Agar Test	
- hydrogen sulfide production	–
- indole production	–
- motility	+
Triple Sugar Iron Agar Test	Alkaline slant, acidic butt.
Oxidation-fermentation test	
- Glucose – aerobic	Glucose oxidized
- Glucose – anaerobic	Glucose fermented
- Sucrose – aerobic	No Sucrose oxidation
- Sucrose – anaerobic	No Sucrose fermentation

human infections from the literature, and provided a demographic data of the patients. Forty two percent of the cases were reported in the region of Americas (including Argentina, Brazil, Colombia, Guvana,and USA), 41% in the East Western Pacific (including Australia, Cambodia, Hong Kong, Japan, Korea, Laos,

Table 3. Comparison of selected biochemical test results of *C. violaceum* from different localities.

Biochemical tests	Test results of Malaysian isolate (this paper)	Test results of foreign isolates (from literature)	Localities and year of publications in previous studies*
Indole from SIM test	Negative	Negative	Brazil (2006), Taiwan (2007), Argentina (1986),Korea (1999), India (2012)
Motility from SIM test	Positive	Positive	Brazil (2006), Taiwan (2007), Argentina (1986)
Catalase	Positive	Positive	Brazil (2006), Thailand (2008), Korea (1999), India (2012)
Oxidase	Positive	Positive	Brazil (2006), Thailand (2008), Taiwan (2007), India (2012), Korea (1999)
TSI slant	Alkaline	Alkaline	Taiwan (2007), Argentina (1986), India (2012)
TSI butt	Acidic	Acidic	Taiwan (2007), Argentina (1986), India (2012)
Gas production from TSI test	Negative	Negative	Taiwan (2007), Argentina (1986), India (2012)
Hydrogen sulfide production from TSI test	Negative	Negative	Taiwan (2006), Argentina (1986), India (2012)

*Information regarding localities and year of publications are based on the following references: Argentina (Kaufman et al., 1986), Brazil (Antunes et al., 2006), India (Kumar, 2012), Korea (Lee et al., 1999), Taiwan (Chang et al., 2007), and Thailand (Jitmuang, 2008).

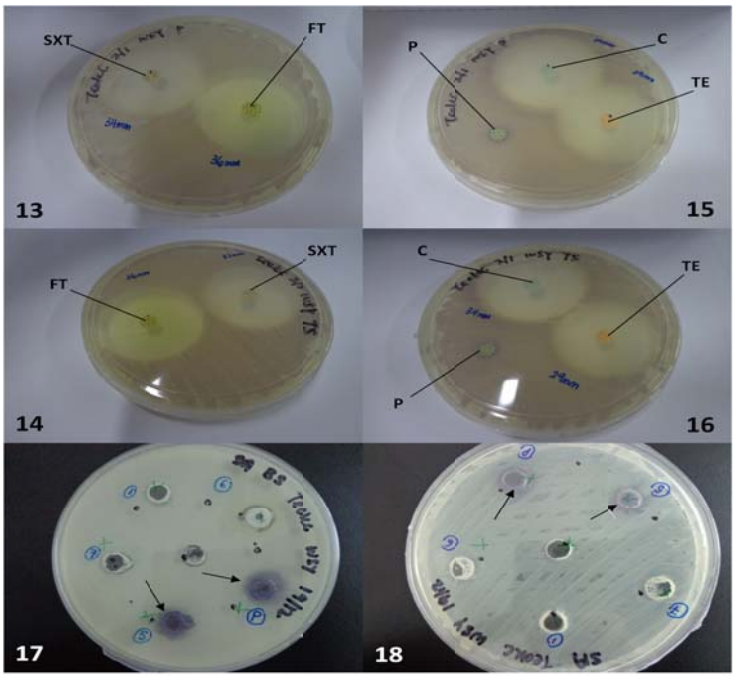

Figure 3. *Chromobacterium violaceum*. 13,14-Antibiotic susceptibility test by disk-diffusion method showing susceptibility to nitrofurantoin (FT) and sulfametaphazole and trimeroprim (SXT). 15, 16- Antibiotic susceptibility test by disk-diffusion method showing susceptibility to chloramphenicol (C) and tetracycline (TE), and resistance to penicillin (P). 17- Antibacterial activity test with *Bacillus subtilis* subsp. spizizenii (ATCC 6633) as the indicator species. Arrows point to inhibition zones against the indicator species. 18- Antibacterial activity test with *Staphylococcus aureus* (ATCC 6538) as the indicator species. Arrows point to inhibition zones against the indicator species.

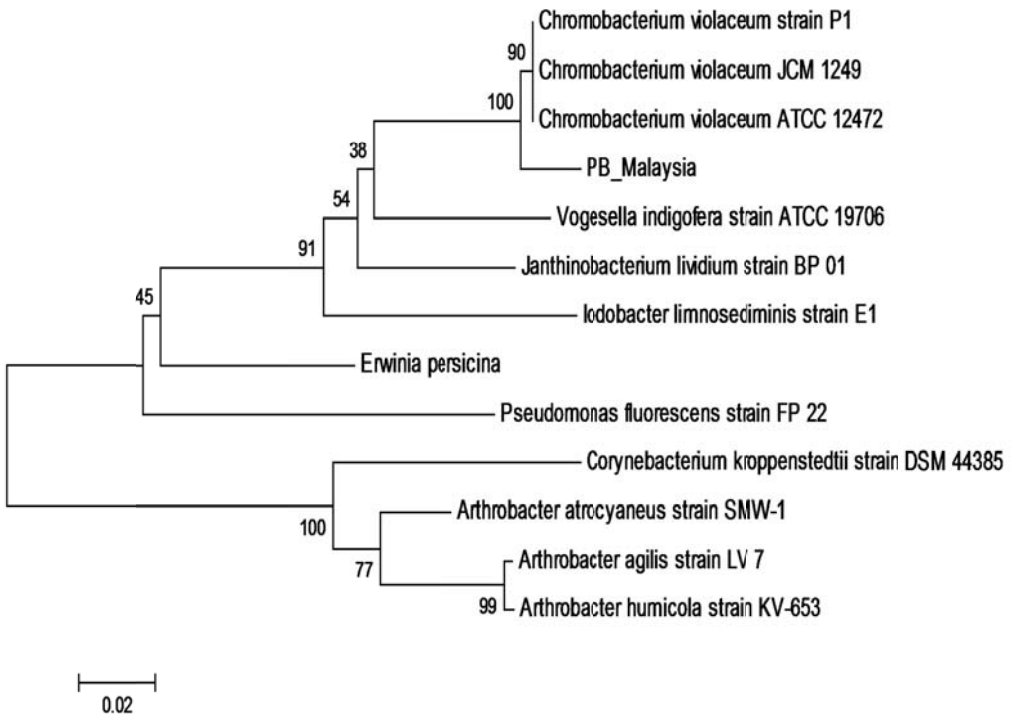

Figure 4. Neighbor-joining phylogenetic tree of *Chromobacterium violaceum* and similar bacterial taxa, based on the16S rRNA gene sequences. Bootstrap percentage values are given at the nodes of the tree. Bar represents 0.02 substitutions per nucleotide position. Result shows that the present Malaysian isolate of purple bacterium (PB_Malaysia) clearly clustered within the clade of *C. violaceum* with a bootstrap value of 100%.

Mainland China, Malaysia, Papua New Guinea, Singapore, Taiwan,and Vietnam). There were 16 reported cases in the South East Asia (including India, Sri Lanka, and Thailand), and 3 cases in Africa (including Nigeria and South Africa). Two other cases were recently added to the history: one from Italy (Arosio et al., 2011), and the other from Vietnam (Campbell et al., 2013). *C. violaceum* was known to be sensitive to temperature and thus it has a predilection to the tropical and subtropical areas (Saleem et al., 2011). Yang and Li (2011) commented that the effects of global warming may affect geographical distribution of this bacterium, and cases of infections may significantly increase in future.

Since the first report of *C. violaceum* occurrence in Malaysia in 1927 (Sneath et al., 1953), only during the past 20 years were additional cases of human infections by this bacterium recorded for this country (Saigin et al., 1994). Several local cases of *Chromobacaterium* infections were recorded in recent years, patients of which were children and adults, including a 4-year-old boy and an 11-year-old girl (Ang, 2004), 12-year-old boy (Sureisen et al., 2008), and a 45-year old man (Cheong, 2010). In Malaysia, fatal infections with *C. violaceum* were also observed in animals. Groves et al. (1969) reported infections in 9 gibbons and one Malayan sun bear in National Zoo, Kuala Lumpur. The disease symptoms in animals were similar to those in humans. Typical septicemic process with hepatic abscesses in all animals and pulmonary abscesses in most cases were reported. Our present paper represents the first record of occurrence of this bacterium in disused tin-mining lakes, but we did not encounter any infection in our study.

Biological activities of violacean and its pharmacological importance

The most notable characteristic of *C. violaceum* is the production of violacein. This purple pigment was first isolated in 1944 (Strong, 1944), and chemically characterized a few years later (Ballantine et al., 1958). This pigment is regarded as a natural antibiotic and hitherto there are a tremendous amount of literatures about this pigment. Work has been done to extract, purify, and produce the pigment in a larger scale (Rettori and Durán, 1998). Violacein is not diffusible, but soluble in ethanol and insoluble in water and chloroform (Moore et al., 2001). It has been used as an antimicrobial agent (Lichstein and Van de Sand, 1945; Durán et al., 1983; Durán and Menck, 2001), an insecticide and an anti-cancer agent (de Carvalho et al., 2006; Durán et al., 2007). It has been shown to have antifungal effect which

can protect amphibians from fungal infection (Becker et al., 2009). There are also research evidences of violacean that showed its successful inhibitory actions against certain pathogenic protozoa: against *Plasmodium* that causes malaria (Lopes et al., 2009), against *Trypanosoma cruzi* and *Leishmania amazonensis* (Leon et al., 2001). Violacean also has inhibitory effects against the nano-flagellates and some bacteriovorous protozoa (Matz et al., 2004). The versatility of violacean in terms of its bactericidal, tumoricidal, trypanocidal and antileishmanial activities was discussed by Leon et al. (2001). A potential application of its antiviral activity is discussed by Andrighetti-Fröhner et al. (2003).

Genetic analysis and violacein biosynthesis

C. violaceum has been reviewed as possessing many pharmacological and industrial perspectives (Durán and Menck, 2001). Many researchers were interested in cracking the genome of this bacterium (Brito et al., 2004). The complete genome sequence of this bacterium has revealed remarkable and exploitable biotechnological potentials (Brazilian National Genome Project Consortium, 2003). Molecular studies of the violacein-producing gene and the biosynthetic pathway of the pigment had been accomplished during the past decade (Antônio and Creczynski-Pasa, 2004; August et al., 2000)

Quorum sensing is a system of stimulus and response correlated to population density of organisms (Miller and Bassler, 2001). Bacteria use quorum sensing to coordinate certain behavior such as biofilm formation, virulence and antibiotic resistance. Production of violacean by *C. violaceum* has been used in studies of bacterial quorum sensing inhibition (Choo et al., 2006; McClean et al., 1997). Researchers are interested in the mechanism of molecular signaling in bacterial population. Recent studies by Anthony et al. (2013) explored the difference in quorum sensing regulation and violacein synthesis between the wild and mutant *C. violaceum*. They also have evaluated the reason behind the inhibition of violacein synthesis in *C. violaceum* mutant.

Biochemical, ecological and industrial importance

In the 1960's, *C. violaceum* was already known to produce polysaccharides that contribute to more stable soil structure in terms of enhancing humus production and affecting decomposition rate of soil (Martin and Richard, 1963; Corpe, 1964). This bacterium also exhibits certain chitinolytic activity which is involved in the regulation of quorum sensing (Chernin et al., 1998). A comprehensive account of the ecological versatility of *C. violaceum* was given by Hungria et al. (2004).

Cyanide-producing microorganisms are able to assimilate or detoxify cyanide by a variety of pathways (Knowles, 1976). Besides a few soil borne pseudomonads, *C. violaceum* is the only bacterium known to produce cyanide (Rodgers and Knowles, 1978). Moreover, *C. violaceum* also forms rhodanese as a possible detoxifying agent (Knowles, 1976). It is thus a potential agent for environmental bioremediation (Faramarzi et al., 2004).

Researchers in this century are keen to exploit intriguing microbial polymers. In a recent study by Bhubalan et al. (2010), a Malaysian isolate of *C. violaceum* was found to produce polyesters which can be used to make polyhdroxyalkanoates (PHA's). PHA's are an alternative to plastics made by petrochemicals. Cloning of gene in *C. violaceum* for the production of PHA has been accomplished by Bhubalan and his co-workers (Bhubalan et al., 2010).

Conflict of Interests

The authors have not declared any conflict of interests.

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