ORIGINAL ARTICLE

Isolation and Characterization of *Chromobacterium* sp. from Lake Water at Manipal International University

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ABSTRACT

Introduction: The violet-pigmented *Chromobacterium* spp. are vastly located in soil and surface water of subtropical regions. Majority of the species have been identified as highly potential in bio-industries; however, the bacterial pathogenicity is largely understudied. These bacteria are resistant to multiple-drugs and infections may cause sepsis and liver abscissions. Thus, this study aimed to characterize the violet-pigmented bacteria isolated from the lake in Manipal International University and further examine its antibiotic susceptibility. **Methods:** The isolated violet bacteria (Dyh27s2016) were subjected to the morphology, physiology, biochemical and antibiotic susceptibility tests. Also, the species were scrutinized via the 16S rRNA and phylogenetic analysis. In addition, the lake water physicochemical properties were examined to understand the bacterial adaptability in this region. **Results:** Dyh27s2016 strain was found to exhibit similar morphology and physiology characteristics to *Chromobacterium* spp. and be closely related to *Chromobacterium amazonense* (98% sequence-homology). However, the biochemical analysis indicated that this strain was capable of indole production; contrarily, *Chromobacterium* spp. were found mostly indole negative. On top of that, this strain also tested resistant to most β-lactam and aminoglycoside antibiotics. The adaptability of the Dyh27s2016 strain in this region might be supported by the satisfactory physiochemical properties of the lake water and mainly by the low dissolve oxygen concentration. **Conclusion:** The morphology, physiology, biochemical and molecular characterizations of Dyh27s2016 isolate show high similarity to *Chromobacterium* spp. and the multi-drug resistance of this strain can potentially harbour a threat to public health if contacted by humans or animals via food or water.

Keywords: 16S rRNA gene amplification, Antibiotic resistance, *Chromobacterium* spp.

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INTRODUCTION

Chromobacterium spp. are saprophytic and opportunistic gram-negative bacteria commonly isolated from environmental samples of tropical and subtropical regions (1). The bacteria belong to the Neisseriacea of β-proteobacteria family and can be easily distinguished with its manifestation of a violet colony on the growth medium due to the presence of the violacein compound. The first species discovered in this genus was the *Chromobacterium violaceum* in 1881, noticed by Bergonzini when he was experimenting putrefaction (1). Fast forward to more than a century after its first discovery, this bacterium has now been extensively studied, and its potential applications in the pharmaceutical as well as in the biotechnological industries have been vastly explored. The possibly known bio-applications of *C. violaceum* are in the production of chitinase hydrolytic enzymes for the breakdown of glycosidic bonds in chitin, thus it can be used as a pest control; the violet pigment produced by the bacteria is extracted and used for textile dyeing and cosmetics, while the extracted polyhydroxyalkanoates compounds from the bacterium are used for the production of the biodegradable plastic (2–4). The violecein compound of this bacteria is also capable of inducing antimicrobial, antiparasitic, and antitumor activities, thus owning high potential for the therapeutical and pharmaceutical applications (5,6).

By 2007, *C. violaceum* was the only validated species discovered until reports started to emerge about the discovery of other 10 species within this genus (7). These isolates were recovered from multiple tropical regions and attested for distinct genetic diversities among them and warranted novel species recognition (8–17). A wide range of nutrients, diversified chemicals, physical physiology, and varied energy flow in the environment may have facilitated the bacterial metabolic adaptability; hence, the bacteria may dwell well in a heterogeneous

environment. On top of that, reports showed that these bacteria, under the nutrient-starvation and pH stresses can significantly amend their protein expression and go on to exhibit great metabolic alterations (18).

Although rapid expansion within the genus has been witnessed in recent years, there still lacks research, especially, in the pathogenicity threat toward human and animal hosts. The first human infection of *C. violaceum* case in the world was traced back from Malaysia in 1927 (19). To date, more than 150 cases of *C. violaceum* infections have been recorded worldwide, notably from Vietnam, Bangladesh, Taiwan, United States, Brazil, and Argentina (20–22). While the infections were often classified as rare, they had relatively succumbed to a high mortality rate due to the pathogenic ability to infest and set off sepsis, multiple liver abscesses and diffuse pustular dermatitis (23). Remarkably, *C. haemolyticum* was the only species besides *C. violaceum* to have been witnessed in a human case bacteremia so far (24). Although, no reports claimed clinical infections, the pathogenicity potential of the nine newly discoved *Chromobacterium* species could not be ruled out. This was especially true, when reports submitted by Santos A. B., et al (2018), mentioned the presence of pathogenecity linked genes such as pore-forming toxin alpha-hemolysin (hlyA) and extended-spectrum β-lactamases Ambler class C β-lactamase, penicillinases, Metallo-β-lactamase, and metal-dependent hydrolases of the β-lactamase superfamily I PhnP proteins, which were annotated from the genome sequence of the *C. amazonense* strain 56AF (7). Furthermore, the same strain was found to be resistant to a β-lactam group of antibiotics such as ampicillin, ampicillin/clavulanic acid, and cefotaxime (third-generation cephalosporin) (25). These reports, therefore, demanded stringent scrutinization of any *Chromobacterium* spp. and strains isolated from the environment as they presented risks to humans and animals.

In the present study, we isolated a purple-violet pigmented bacterium (Dyh27s2016 strain) from the lake water in Manipal International University (MIU), Negeri Sembilan (Malaysia) and went on to scrutinize the fundamental characteristics of this strain by studying the bacteria's physiology, morphology and biochemical properties. Besides that, we identified the strain's genus and species based on the 16S rRNA conserved gene amplification and phylogenetic analysis. Finally, the antibiotic susceptibility against β-lactam and aminoglycoside groups of antibiotics was assessed to scrutinize the antibiotic resistant profile of this strain.

MATERIALS AND METHODS

Isolation and culture of purple-violet bacteria

Water samples were carefully collected (depth: 10 – 15 cm from the water surface) from the lake located in Manipal International University (MIU) campus (2.8210° N, 101.7781° E) in sterile 300 mL Schott bottle and 100 µL of the sample was inoculated on nutrient agar (Chem Soln, India) for 24 h at 37 °C in aerobic condition. Multiple colonies of bacteria were noticed on the nutrient agar, however a distinct purple-violet bacterium was chosen for further analysis and characterization. A single colony from the original culture was then inoculated onto a fresh medium. This strain was internally assigned with ID: Dyh27s2016 and further characterized to analyse the morphology, physiology, and biochemical properties. The strain was also maintained at 4 °C and stored at - 80 °C as cell suspensions in glycerol (20 % v/v).

Morphology and physiology analysis of Dyh27s2016 strain

Gram's staining was performed and the cell's morphology, shape, and the colony colour were all observed. The bacterial physiology was analyzed by streaking them onto the Nutrient Agar containing different sodium chloride (NaCl) concentration (w/v); 0.5 %, 1.0 %, 2.0 %, 3.0 %, 3.5 % and 5.0 %. The plates were incubated at 37° C for 48 h and observed for the bacterial growth. The optimal pH and the temperature for bacterial growth were also studied. The nutrient broth was prepared with pH 2.0, 4.0. 6.0, 8.0 and 10.0 respectively. The tubes were inoculated with a loopful of bacterial strain and incubated at 37° C for 24 h. For the optimal temperature, the strain was incubated at varied temperature; 30 °C, 32 °C, 35 °C, 37 °C and 40 °C. For both experiments, bacterial growth was measured by observing the O.D value at 600 nm using a UV-spectrophotometers (Thermo Scientific, USA). Each experiment was repeated with minimal three replications.

Biochemical analysis

The isolated bacteria were subjected to Indole, citrate utilization, urease, catalase production, haemolysis and starch hydrolysis tests. These tests were conducted by the conventional biochemical analysis (6,26). The Tween 80 and Tween 40 hydrolysis tests were performed as defined by Crombach W. H. J., (1978) with incubation time for Tween 80 hydrolysis test was 14 days at 37 °C while the Tween 40 hydrolysis test was incubated for 48 h at 37 °C (27). Tubes without bacterial culture were used as a negative control for all conventional biochemical tests and each experiment was repeated with minimal three replications.

Antibiotic susceptibility test

Antibiotic susceptibility test against non-Enterobacteriaceae with the protocol defined by Kirby-Bauer was adopted. Briefly, the bacterial strain was swabbed evenly on Mueller-Hinton agar (MHA) plate and after 5 min, the antibiotic disks were placed gently onto the agar surface. Agar plates were then incubated at 37 °C for 24 h and thereafter, the diameter of the inhibition zone was measured (28). Clinical

and Laboratory Standards Institute (CLSI) guidelines were used to determine the antibiotic susceptibility profiles of this strain using β-lactam (imipenem- 10 µg, ampicillin- 10 µg and ticarcillin/clavulanate- 75/10 µg), aminoglycoside (gentamicin - 10 µg, kanamycin- 10 µg and streptomycin- 10 µg) and cephems (cefotaxime- 30 µg) antibiotics in this study. *Escherichia coli* DH5α was used as control and each experiment was repeated with minimal three replications.

Amplification of 16S rRNA and purification of the amplicon

The strain Dyh27s2016 maintained in nutrient agar was inoculated into the nutrient broth for 24 h before genomic DNA was isolated using GF-1 Bacteria DNA Extraction Kit (Vivantis Technologies, Malaysia). The gene 16S rRNA of the bacteria was amplified using a set of bacterial conserved primers sequence (Forward 5′-GTTTGATCCTGGCTCAG-3′ and Reverse 5′-TACCTTGTTACGACTTCA-3′). Master mix for the polymerase chain reaction (PCR) was prepared using a 2X Taq Master Mix reagent (Vivantis Technologies, Malaysia). The PCR cycle and the temperature were set according to the information given by the manufacturer. The product of PCR was purified thereafter using the QIAquick PCR purification kits (Qiagen, Germany) and was sequenced using the Next-Generation Sequencing service from 1st Base service (Malaysia). Trimmed 16S rRNA region of the Dyh27s2016 isolate nucleotide sequence was deposited into GeneBank nucleotide database and the partial gene sequence was assigned with accession number MK673984.

16S rRNA Sequence and phylogenetic tree analysis

Bacterial genus and species were scrutinized using a multiple sequence alignment (MSA) tool for the consensus sequences of the known 16S rRNA gene retrieved from GenBank database library (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A query of 12 sequences from the GenBank database showing > 97% identity score as well as the next four query sequences showing 94% - 95% identity score were used for MSA. A maximum-likelihood phylogenetic tree was constructed using MEGA version 7 software and Tamura-Nei model for this purpose. A Bootstrap consensus tree was constructed with 1000 replicates to represent the evolutionary history with the initial tree was inferred to Neighbor-Join and BioNJ algorithm to a matrix of pairwise distances estimated by using the Maximum Composite Likelihood (MCL) approach (29).

Physicochemical Analysis of Lake Water

Water was sampled carefully (depth: 10 – 15 cm from the surface) from the lake. Physicochemical properties of the water were analyzed by assessing the dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) according to the American Public Health Association (APHA) (2005) standards. Briefly, the DO of the water sample was examined using the Azide Modification method while BOD analysis was made using the 5-Day BOD (BOD5) method. The COD analysis, on the other hand, was measured by refluxing the acidified water sample with a known concentration of Potassium dichromate ($K_2Cr_2O_7$) in a Liebig condenser. Besides DO, BOD and COD, the temperature (thermometer) and pH (pH meter) of lake water at the point of sampling were also recorded. The samples were consistently collected every forth night, approximately from 0900 to 1300 and the sampling was carried out from May to August 2017.

Statistical Analysis

The data were expressed as mean (±SD). Student T-Test was also performed to evaluate the significance of data groups.

RESULTS

Physiology, Morphology and Biochemical analysis of Dyh27s2016 strain

A distinguished pigmented bacterium was isolated from the lake located near Manipal International University and later it was denoted as the Dyh27s2016 strain. Physiology analysis showed that Dyh27s2016 strain had an optimal growth rate at a temperature of 37 °C (Fig. 1a). This strain was also able to grow optimal at pH range from 6.0 to 8.0 (Fig. 1b) and the salinity test, on the other hand, showed that Dyh27s2016 strain was not capable of growing at NaCl concentration > 1% in nutrient agar. For the morphology analysis, the strain was observed forming colonies with circular morphology, purple-violet pigmentation and having a smooth texture with the

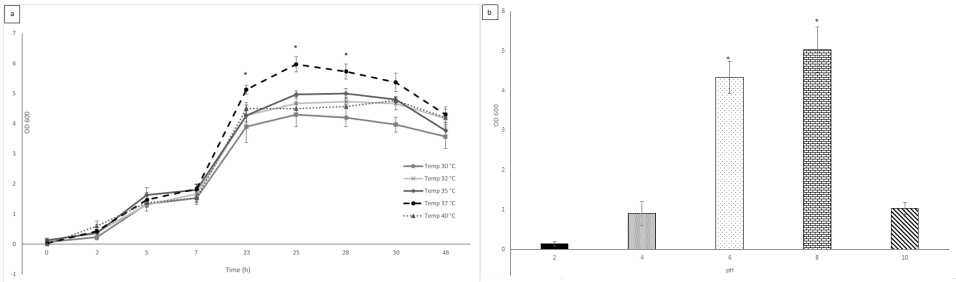

Figure 1: Optimal temperature and pH for Dyh27s2016 bacterial strain growth. (a) The strain grows optimum at temperature 37 °C. At this temperature, the growth is significant (*p-value < 0.05) compared to other temperature ranges at least for the time interval at 23 h, 25 h and 28 h. (b) At pH 6 and 8, Dyh27s2016 strain growth was found significant (*p-value < 0.05) compared to pH 2, pH 4 and pH 10.

cells being slightly elevated (Fig. 2a). The bacterium was also confirmed as Gram-negative and rod-shaped (Fig. 2b). The biochemical tests, in contrast, showed positive for most of the tests except for the citrate utilization, urease and starch hydrolysis. The bacteria were notably positive for indole production (Fig. 2c) and hydrogen peroxide (Fig. 2d). A full profile of the biochemical test is shown in Table I and comparisons were made against other *Chromobacterium* spp. isolates (8–17).

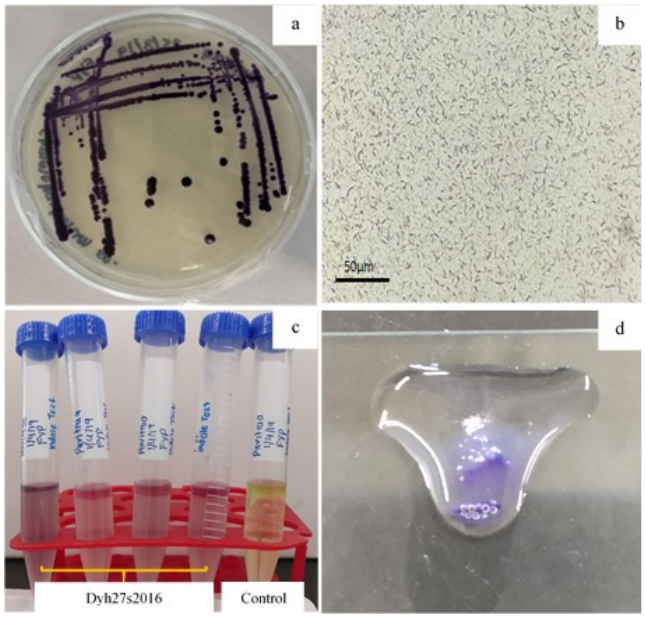

Figure 2: Morphology, Gram's staining and biochemical test of Dyh27s2016 strain. (a) Purple-violet pigmented bacterial colony growth was observed in the Nutrient agar incubated at 37 °C in aerobic condition. (b) Gram's staining showing pink and rod-shaped bacterial colony indicating lack of peptidoglycan and the isolate is a Gram-negative. (c) The presence of a cherry-red ring during Indole test shows the ability of the isolate to metabolize tryptophan in indole production. (d) Bubble formation occurs when hydrogen peroxide (H_2O_2) was added to this isolate suggesting the bacterial ability to synthesis catalase enzyme.

Antibiotic susceptibility

The antibiotic susceptibility profile of Dyh27s2016 isolate is shown in Table II. While susceptible to streptomycin, the isolate was found resistant to gentamicin and kanamycin. For β-lactam antibiotics susceptibility, the Dyh27s2016 strain was found resistant to imipenem and ticarcillin/clavulanate but susceptible to ampicillin. This strain was also found resistant to cefotaxime, a cephalosporin class IIIc antibiotics.

Table II: Antibiotic susceptibility profiles of Dyh27s2016 isolate

Antimicrobial Class	Antibiotics/ Generic Names	Zone of Inhibition (mm)	Susceptibility
	Ampicillin (10 µg)	22.2 ± 0.6	Susceptible
β-Lactam/ β-lactamase inhibitor combinations	Ticarcillin/clavulanate (75/10 µg)	< 6.0	Resistant
	Imipenem (10 µg)	< 6.0	Resistant
Cephems (Subclass: Cephalosporin IIIc)	Cefotaxime (30 µg)	< 6.0	Resistant
	Gentamicin (10 µg)	< 6.0	Resistant
Aminoglycosides	Kanamycin (10 µg)	< 6.0	Resistant
	Streptomycin (10 µg)	23.3 ± 1.5	Susceptible

16S rRNA Sequence and phylogenetic tree analysis

The sequencing of the conserved region for 16S rRNA and the subsequent primary phylogenetic tree construction disclosed that Dyh27s2016 isolate was highly related to the *Chromobacterium amazonense* strain CBMAI 310 (98% of identity) with the pairwise alignment showing 99% homology (Fig. 3).

Table I: Summary of morphology, physiology and biochemical analysis performed on the Dyh27s2016 strain isolated from the lake water in MIU compared to other *Chromobacterium* sp. strains

Analysis	Test	1	2	3	4	5	6	7	8	9	10	11	12
Morphology	Gram's staining	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve	- ve
	Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
	Colony colour	Purple violet	Violet	Non-pigmented	Deep-violet	Tan-coloured	Violet	Violet	Tan-coloured	Deep purple	Non pigmented	Violet	Violet
	Colony morphology	Round	Na	Round	Round	Round	Round	Round	Na	Round	Round	Round	Round
	Elevation	Raised	Na	Raised	Raised	Raised	Raised	Raised	Na	Raised	Raised	Raised	Raised
Physiology	Optimal pH (growth)	6.0 - 8.0	6.0 - 8.0	Na	6.0 - 8.0	6.0	6.0	6.0	7.0	Na	8.0	4.5 - 9.0	5.0 - 9.0
	Optimal temperature (growth)	37 °C	28-30 °C	Na	25-28 °C	32 °C	32 °C	32 °C	30 °C.	25–26 °C	30 °C.	30 °C.	35-37 °C
	Salinity tolerance	< 1.0 %	≤ 3.5 %	Na	≤ 2.0 %	≤ 3.5 %	≤ 3.5 %	≤ 3.5 %	≤ 3.5 %	≤ 3.0 %	≤ 2.0 %	≤ 2.0 %	≤ 2.0 %
Biochemical	Indole production	+ ve	- ve	- ve	Na	- ve	Na	Na	Na	+ ve	- ve	- ve	- ve
	Citrate utilization	- ve	Na	+ ve	Na	+ ve	Na	Na	Na	Na	+ ve	Na	- ve
	Catalase production	+ ve	+ ve	+ ve (week)	Na	- ve	- ve	- ve	+ ve	+ ve	+ ve	Na	+ ve
	Gelatin hydrolysis	+ ve	+ ve	+ ve	Na	+ ve	Na	Na	Na	+ ve	+ ve	+ ve	+ ve
	Carbohydrate fermentation (Glucose)	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	- ve	+ ve	- ve	+ ve
	Haemolysis- Horse blood agar (Beta haemolysis)	+ ve	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na	Na
	Haemolysis-Sheep blood agar (Alpha haemolysis)	+ ve	Na	+ ve	+ ve	+ ve	Na	Na	+ ve	Na	Na	Week	+ ve
	Tween 40 & 80 hydrolysis	+ ve	+ ve	Na	+ ve	+ ve	Na	Na	+ ve	Na	+ ve	Na	+ ve
	Urease	- ve	- ve	Na	Na	- ve	Na	Na	Na	-ve	+ ve	-ve	-ve
	Starch hydrolysis	- ve	Na	Na	Na	Na	Na	Na	Na	Na	- ve	Na	- ve

Legend
1. Dyh27s2016 isolate,
2. *C. amazonense* (8),
3. *C. haemolyticum* (9),
4. *C. subtsugae* (10),
5. *C. aquaticum* (11),
6. *C. piscinae* (12),
7. *C. pseudoviolaceum* (12),
8. *C. rhizoryzae* (13),
9. *C. vaccinii* (14),
10. *C. alkanivorans* (15),
11. *C. sphagni* (16),
12. *C. violaceum* (17),
13. Na- Not applicable/ not stated

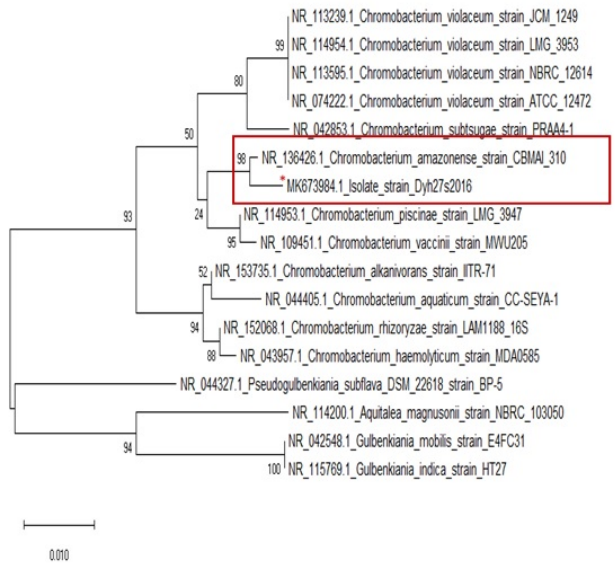

Figure 3: The Maximum Likelihood phylogenetic tree. The tree depicts isolate strain Dyh27s2016 (*MK673984: NCBI accession number) is closely related to *Chromobacterium amazonense* strain CBMAI 310 (NR136426: NCBI accession number). The evolutionary analysis was inferred to the Tamura-Nei model with 1000 bootstrap replication.

Physicochemical analysis of Lake Water

Water sampled from the lake in MIU showed an average pH value of 6.3 ± 0.5 and temperature approximately 28.3 ± 0.7 °C. The DO, BOD and COD indexes are shown in Table III. The lake water is classified as 'natural environment' based on standards set by both the National Water Quality Standards for Malaysia (NWQS) and the Department of Environment Water Quality Index Classification.

Table III: Physicochemical Analysis of Water Sampled from MIU Lake

Physicochemical Analysis	Result
Dissolved Oxygen (DO)	7.19 ± 0.22 mg/L
Biological Oxygen Demand (BOD)	0.78 ± 0.12 mg/L
Chemical Oxygen Demand (COD)	10.74 ± 0.43 mg/L
Temperature	28.3 ± 0.7 °C
pH	6.3 ± 0.5

DISCUSSION

When random bacterial isolation was performed, a unique purple-violet pigmented colony was noticed, and this bacterial strain was then subjected to physiology, morphology and biochemical analysis. The bacterial strain had optimal growth rate at a temperature of 37 °C and significant growth at pH 6 and pH 8. Results from the previous study indicate that the *Chromobacterium* spp. were able to propagate at a temperature beyond 40 °C as well as be capable of survival in a highly acidic environment (pH 4.0) (30). The salinity test showed the bacterial strain was not capable of growing at NaCl concentration beyond 1% in nutrient agar. The Dyh27s2016 strain was isolated from a freshwater lake. The failure of this strain to grow at salinity beyond 1% in nutrient agar might be linked to their inhabiting geographic and metabolic adaptability. This is supported by the physicochemical analysis that showed the lake water is clean like the 'natural environment'. Furthermore, most *Chromobacterium* spp. were also found not capable of growing at salinity beyond 3% (1,8). On the other hand, morphology analysis of the Dyh27s2016 strain such as the Gram's staining, bacterial shape, bacterial colony morphology and the bacterial colony elevation on the agar were highly similar to other species within this genus (Table I). Besides, the purple-violet shade on this strain was also found similar to most *Chromobacterium* spp. Purple colour pigmentation usually expressed within this genus is due to the presence of the violacein compound (31).

Biochemical analysis of *Chromobacterium* spp. had generally demonstrated variations when compared to one species or another. However, one notable feature that was unanimously present in almost all species across the genus was the bacteria's inability for indole production. This at least was confirmed in the isolates *C. amazonense* CBMAI 310T, *C. vaccini* DSM 25150T, *C. violaceum* ATCC 12472T, *C. subtsugae* DSM 17043T, *C. haemolyticum* CCUG 53230T and *C. aquaticum* SEYA-1T (8,9,11). Indole synthesis in bacteria (other than *Chromobacterium*) occurs due to their ability to metabolize tryptophan and convert them to indole derivatives (32). On the other hand, *Chromobacterium* spp. utilizes tryptophan only for violacein synthesis but not for growth; this has been previously proven (33). Surprisingly the Dyh27s2016 strain showed positive for the indole test. The reason for such an outcome is not clear and not possible to be elucidated at this point; however, several studies have indicated that some *Chromobacterium* spp. are also capable of indole positive (14,34).

The Dyh27s2016 strain was also found susceptible to ampicillin and streptomycin while in the rest of the antibiotics, this strain was resistant. Generally, *Chromobacterium* spp. are resistant towards β-lactam antibiotic but sensitive towards aminoglycoside (35,36). An analysis on *C. violaceum* ATCC 12472T genome found a large number of genes related to the antibiotic resistance, which includes β-lactam and many multi-drug resistant genes (37). On top of that, *Chromobacterium* spp. isolated from surface water were also proven to possess many extended-spectrum β-lactamase (ESBL) (38). This may support the nature of the Dyh27s2016 strain being resistant to most β-lactam

class, aminoglycoside, and cephems antibiotics. On top of that, the quorum sensing mechanism presented by *Chromobacterium* spp. in general is capable of inducing pathogenicity and mediating resistance against antibiotics. Quorum sensing is a tool generally used by Gram-negative bacteria during cell-to-cell communication when extracellular signaling molecules (autoinducers) are presented to them. The bacteria, in response, may stir up high cell density and their gene expression pattern modifications enable them to control their physiological functions, and thereby, facilitate cell pathogenicity and survival (39). A similar mechanism could have been adopted by the Dyh27s2016 strain to induce resistance towards the β-lactam and aminoglycoside antibiotics in this study, nevertheless, this remains to be elucidated.

Molecular characterization of the bacterium showed that this strain is closely related to the *Chromobacterium amazonense* strain CBMAI 310. In the NCBI blast analysis, there were only 12 queries with sequence homology of > 97% found. Besides, the next four queries (non-*Chromobacterium*) showing sequence homology of < 97% (between 94 – 95%) were also included in the phylogeny analysis to approximate Dyh27s2016 isolate's clade. The 16S rRNA sequence approximation is a proven method for the genus identification in most cases (> 90%). However, for the species level, a secondary analysis, especially the DNA-DNA hybridization method is required. This is mainly, bacterial identification using 16S rRNA which may only estimate up to 83% for its species (40). Hence, this strain may require further species classification and phylogenetic analysis in future.

Chromobacterium spp. usually exhibits a vast range of metabolic flexibility, as a result, the isolation of this bacteria from various regions has been studied extensively in the past (1,41,42). Although, the physicochemical properties of its natural habitation are often failed to be reported, the analysis of such properties is vital as it provides a dynamic understanding of the bacteria's environmental adaptation and its metabolic modifications for survival. The lake in MIU was once used as a source of water for construction site when the University and nearby apartment residence were being built. Today, the lake serves as a recreational site for the campus, and not much development has taken place in recent years. Consequently, the natural coherence of the lake water is not known, so the physicochemical analysis is necessary to understand the possible survival and metabolic adaptability of the Dyh27s2016 strain in this lake. When the lake water was sampled, the pH value found (6.3 ± 0.5) suitable for the sustenance of most aquatic organisms including microorganisms and to support its physiological functions. Although freshwater aquatic biotas are capable of tolerating a wide range of pH, the lethal free pH zone between 5.0 - 9.0 generally provides adequate protection for its bottom-dwelling organisms (43). Besides pH, dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) indexes of the water were also scrutinized in this study. According to the National Water Quality Standards for Malaysia (NWQS) and the Department of Environment Water Quality Index Classification, lake water can be classified into class I and/or IIa based on the BOD, COD and DO results. Therefore, the water is generally clean though it may still require conventional water treatment. The lake serving as 'natural environment' may therefore, support the breeding of many sensitive aquatic organisms including the exceptional microorganism species (43). Moreover, low concentration of both BOD and COD suggest a minimal biological and chemical contamination of the water. On the other hand, the DO concentration (7.19 ± 0.22 mg/L) of the lake water in MIU was found to be near the microbial threshold. Spietz R. L., et al. (2015) have shown the DO concentration between 5.18 and 7.12 mg/L as the microbial threshold, whereby a significant shift in the microbial taxa richness has been evident at this range. Lower DO concentration in the MIU lake water might has contributed to the bacterial community composition shift and stem into microbial taxa richness (44). Dissolved oxygen of a water body is the most vital component of all abiotic factors as it determines the bacterial community composition in an ecosystem. When the DO concentration drops, energy will be transferred from macrofauna to microbes, accommodating multiple taxa coexistence in an ecosystem (44). This may substantially add greater biodiversity in an ecosystem and progressively support the growth of many unique micro-bacterium, such in the case of *Chromobacterium* spp. survival in the lake water located at MIU.

CONCLUSION

In this study, we isolated a bacterial strain Dyh27s2016 expressing purple-violet pigmentation from the lake water at MIU and subsequent phylogenetic analysis showed that this isolate is closely related to *Chromobacterium amazonense* strain CBMAI 310 (98% sequence analogy). The isolate found expressed unique capability for indole production which was not evident in most other species within the genus. The Physicochemical analysis of the lake revealed that the water is generally clean and has low dissolve oxygen concentration. These physiochemical properties may have substantially supported the adaptability of this strain in this region. Though the strain is resistance towards β-lactam and aminoglycoside antibiotics highlights the potentially threat it could harbour to humans and animals, further analysis is possibly required to confirm the pathogenicity of this strain in humans and animals.

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