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Antioxidant and cytotoxic potentials of *Streptomyces gilvigriseus* MUSC 26^T isolated from mangrove soil in Malaysia

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Abstract : Microorganisms play an important role in natural product discovery, particularly the member of genus *Streptomyces*. The Gram-positive, filamentous streptomycete, *Streptomyces gilvigriseus* MUSC 26^T was firstly isolated from mangrove forest at Tanjung Lumpur, Malaysia. After 7-day fermentation, the supernatant of MUSC 26^T was collected and subjected to chemical extraction. The resulting extract was used in antioxidant and invitro cytotoxicity assays against human colon cancer cell lines. The results showed that MUSC 26^T extract possessed significant antioxidant activity and cytotoxic effect against the tested colon cancer cell lines. Lowest cell viability was observed in HCT-116 cell lines, at 63.64 ± 3.62 % (after treated with 400 µg/mL of extract). The other cell lines exhibited cell viability ranged from 67.50 – 86.66 % when treated with 400 µg/mL of extract. Further chemical analysis on the extraction using GC-MS has revealed the production of 14 compounds, including two cyclic dipeptides which could possibly contribute to the observed bioactivities. By the same token, the availability of its genome sequence allows further investigation into the biosynthetic gene clusters responsible for the production of these compounds which could essentially accelerate its development as chemopreventive drug(s).

Keywords: *Streptomyces gilvigriseus;* cytotoxic; antioxidative; mangrove

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Introduction

Cancer remains a major threat to public health, taking second place for the leading cause of death following heart disease in the United States[1]. Despite great improvements in healthcare systems, the 5-year survival rate of colon cancer (at all stages) remains at 64.4 %, with lower survival rate at increased age[1,2]. On top of that, the emergence of chemotherapy resistance colon cancer types further complicates the current problem[3]. Thus, the hunt for effective chemotherapy drugs is still ongoing to win the war against various cancer types, including colon cancer.

Microorganisms are known to produce bioactive compounds of various structures with great potential to be developed as therapeutic drugs for humans use. In fact, many of these compounds were derived from the genus *Streptomyces*[4]. Proposed as a genus in 1943 by Waksman and Henrici, over 800 species (with validly published names) have been isolated from various environments[5]. To emphasize their contributions to mankind's health, over 10,000 bioactive compounds have been isolated from these filamentous bacteria, possessing bioactivities including an cancer properties[4,6-11]. In the current study, *Streptomyces gilvigriseus* MUSC 26^T was previously isolated as a novel strain derived from mangrove forest in the east coast of Peninsular Malaysia[12]. As an attempt to explore the bioactive potential of the strain, MUSC 26^T was subjected to fermentation process using in-house optimized media and the extract was tested for antioxidant and cytotoxic activities against several human colon cancer cell lines. Apart from that, GC-MS analysis was also carried out to identify chemical constituents present in the extract. Altogether, the well characterized strain, *S. gilvigriseus* MUSC 26^T showed its ability in producing various compounds with antioxidant and cytotoxic potential, which could account for the killing of colon cancer cells observed invitro. Further investigations into the biosynthetic gene clusters present in the strain could essentially improve the understanding of the strain, while at the same time, maximizing the production of these bioactive compounds which is crucial for the development as chemopreventive agents.

Material and methods

Sample collection and isolation of strain *Streptomyces gilvigriseus* MUSC 26^T

Originated from mangrove forest, strain MUSC 26^T was isolated from the collection site designated as MUSC-TLS1 (3°48' 3.2" N 103°20' 11.0" E, Tanjung Lumpur, Pahang, Malaysia)[9,12]. Soil samples were collected by removing the top soil and placed into sterile bags using aseptic metal trowel. These soil samples were stored at -20 °C until further processing. Wet heat (in sterilized water) was used as a selective pretreatment for the air-dried soil samples (15 min at 50 °C)[13]. Following that, the soil sample was mixed with sterilized water and mill ground before spreading onto ISP2 agar supplemented with cycloheximide (25 µg/ml) and nystatin (10 µg/ml), and incubated at 28 °C for 14 days[14]. Strain MUSC 26^T was maintained as pure cultures on ISP 2 agar slants at 28 °C and as glycerol suspensions (20 %, v/v) at -20 °C.

Genomic and phylogenetic characterization of MUSC 26^T

Using polyphasic approach, strain MUSC 26^T was characterized in-depth, combining observations based on genomic, phylogenetic, biochemical and phenotypic characteristics. Extraction of genomic DNA[6], 16S rRNA gene amplification and sequencing were carried out as described previously[8]. Upon retrieving the 16S rRNA gene sequence of related type strains of the genus Streptomyces from the GenBank/EMBL/DDBJ databases, these sequences was aligned with the gene sequence of MUSC 26^T using CLUSTAL-X software[15], while the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) was used for calculations of sequence similarity[16]. The alignment was verified manually and adjusted prior to the reconstruction of phylogenetic trees using maximum-likelihood algorithm[17] (Figure 1) with MEGA ver sion 6.0[18]. The stability of the resultant trees topologies were evaluated by bootstrap analysis with 1000 replications[19]. Additional characterization of strain MUSC 26^T and the closely related strains was performed using BOX-PCR fingerprint analysis using BOX-A1R primer (5'-CTACGGCAAGGCGACGCTGACG-3')with PCR cycling conditions described by Lee *et al.*[20,21]. The PCR products were observed by 2% agarose gel electrophoresis.

DNA-DNA relatedness of MUSC 26^T with the closely related strains were evaluated using hybridization technique. Genomic DNA of MUSC 26^T and the selected type strains was extracted[22] before commencing DNA-DNA hybridization (performed by the Identification Service of the DSMZ, Braunschweig, Germany according to the protocol of De Ley *et al.*[23] under consideration of the modifications described by Huss *et al.*[24].

Phenotypic characterization

Cultural properties of strain MUSC 26Twere recorded following growth on ISP2 and ISP7 agar[14], starch casein agar (SCA)[25], Streptomyces agar (SA)[26], Actinomycetes isolation agar (AIA)[26] and nutrient agar[27] (7 – 14 days at 28 °C).The designation of colony color was determined by using the ISCC-NBS color charts[28]. Apart from that, cell morphology of MUSC 26T (7-14 day-old colonies) was visualized using light microscopy (80i, Nikon) and scanning electron microscopy (Tabletop Microscope TM3000; Hitachi). The intact arrangement of the aerial hyphae was observed on ISP 2 agar after 10 days at 28 °C using the coverslip technique. Gram staining was performed by standard

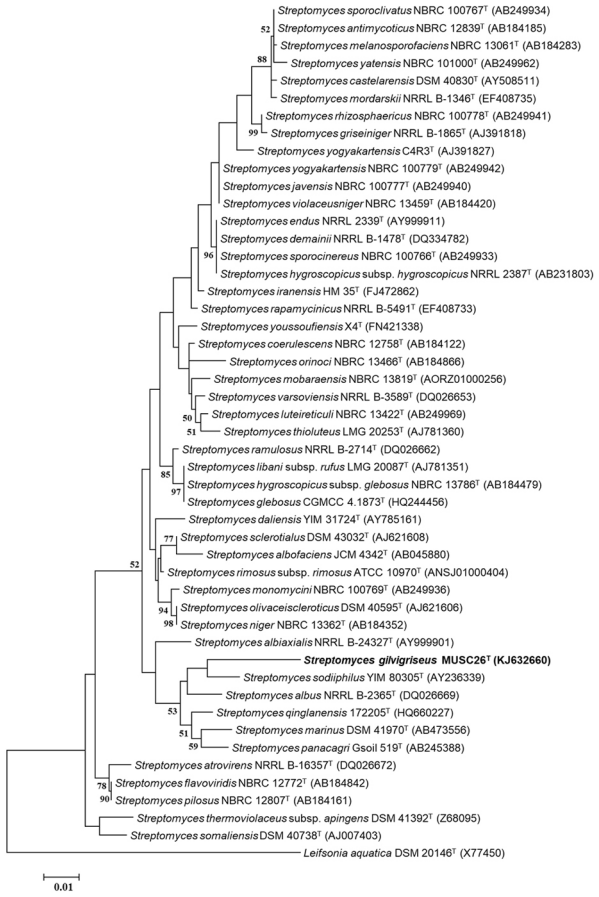

Figure 1. Maximum-likelihood phylogenetic tree based on almost complete 16S rRNA sequences (1447 nucleotides) showing the relationship between strain MUSC 26^T and representatives of some other related taxa. Numbers at nodes indicate percentages of 1000 bootstrap re-samplings, only values above 50% are shown. Bar, 0.002 substitutions per site. *Leifsonia aquatica* DSM 20146^T was used as an outgroup.

gram reaction and confirmed by using KOH lysi[s[29]]. Growth rates were evaluated in tryptic soy broth (TSB): (a) NaCl tolerance (0–10 % w/v) and (b) pH (4.0–10.0), and ISP2 agar for temperatures (4–40 °C) for 14 days.

Using blood agar medium containing 5 % (w/v) peptone, 3 % (w/v) yeast extract, 5 % (w/v) NaCl and 5 % (v/v) horse blood, hemolytic activity was assessed[30], while catalase activity and production of melanoid pigments were investigated following protocols described by Lee et al.[31]. Amylolytic, lipase, protease cellulase, chitinase, and xylanase activities were examined with ISP2 agar as described by Meena et al.[32]. Using disc diffusion method, antibiotic susceptibility tests were performed[33]. Carbon-source utilization and chemical sensitivity assays were tested on Biolog Onmilog systems with GenIII MicroPlates (Biolog, USA).

Genome sequencing of MUSC 26^T

Genomic DNA extraction of MUSC 26^T was carried out with MasterpureTM DNA purification kit (Epicentre, Illumina Inc., Madison, WI, USA) before RNase (Qiagen, USA) treatment[34,35]. DNA quality was accessed using NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA) and a Qubit version 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA). DNA library was constructed using Nextera ™ DNA Sample Preparation kit (Nextera, USA) and the library quality was checked by Bioanalyzer 2100 high sensitivity DNA kit (Agilent Technologies,

Palo Alto, CA) prior to genome sequencing on MiSeq platform with MiSeq Reagent Kit 2 (2 × 250 bp; Illumina Inc., Madison, WI, USA).After trimming the paired-end reads were trimmed and de novo assembled with CLC Genomics Workbench version 7 (CLC bio, Denmark), gene prediction was carried out with Prodigal version 2.6[36]. Prediction of rRNA and tRNA were performed using RNAmmer[37] and tRNAscan SE version 1.21[38]. The assembly was annotated using Rapid Annotation using Subsystem Technology (RAST)[39] and NCBI annotation pipeline. Further bioinformatics analyses were carried out on antibiotics & Secondary Metabolite analysis shell (antiSMASH) server to investigate the presence of biosynthetic gene clusters[40].

Fermentation process and preparation of MUSC 26^T

Seed medium (TSB) of MUSC 26T was prepared by growing the cells for 7 days prior to inoculation of fermentation media (FM3) [41,42]. Fermentation process was carried out for 7 days with 200 mL of sterile FM3 (in 500 mL Erlenmeyer flask, 28 °C/200 rpm). Following fermentation, FM3 medium containing metabolites of MUSC 26^T was recovered by centrifugation at 12000 ×g for 15 min. The filtered supernatant was freeze-dried and repeatedly extracted with methanol. After removing the solvent using rotary vacuum evaporator at 40 °C, the final extract was obtained and suspended in dimethyl sulphoxide (DMSO) as a vehicle reagent prior to assay.

Investigation of antioxidant activity of MUSC 26^T extract

Antioxidant activity of MUSC 26^T extract was examined using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) [42,43]. ABTS radical cation (ABTS·) was created through reaction between ABTS stock solution (7 mM) and potassium persulphate (2.45 mM). The change in radical amount was indicated by decrease in absorbance at 743 nm and ABTS scavenging activity was calculated in percentage based on the differences in absorbance of control and sample.

Metal-chelating activity was measured as previously described[42,43]. 2 mM of FeSO4 was mixed with the extract and the reaction was initiated by adding 5 mM of ferrozine. The measurement of metal-chelating activity was done using spectrophotometer (562 nm) and was computed as follows:

$$\text{Metal-chelating activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Cell culture and maintenance

Four human derived colon cancer cell lines were included for this study: HCT-116, HT-29, Caco-2 an d SW480[42-44]. All the cell lines were cultivated in RPMI 1640 supplemented with 10% FBS in humidified incubator (5 % CO2 in air at 37 °C).

Cytotoxicity determination using 3-(4,5-dimethylthazol-2yl)-2,5-diphenyl tetrazolium-bromide (MTT) assay

Cells were seeded into a sterile flat bottom 96-well plate at a density of 5 × 103 cells/well and allowed to adhere overnight. Cells were treated with MUSC 26^T extract for 72 hour before performing MTT assay. Upon completion of treatment, MTT solution was loaded into each well and further incubated for 4 hours (37 °C, 5 % CO2, 95 % air). The medium was then gently removed before adding dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. The amount of formazan product was measured spectrophotometrically at 570 nm (with 650 nm as reference wavelength) and cell viability was calculated (in percentage) with the following formula:

$$\text{Percentage of cell viability} = \frac{\text{Absorbance of treated cell}}{\text{Absorbance of untreated cell}} \times 100\%$$

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Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was performed on Agilent Technologies 6980N (GC) equipped with 5979 Mass Selective Detector (MS), HP-5MS (5% phenyl methyl siloxane) capillary column of dimensions 30.0 m × 250 µm × 0.25 µm, with helium as carrier gas at 1 mL/min[45]. The column temperature was set to 40 °C for 10 min, followed by temperature hike of 3 °C/min to 250 °C before it was kept isothermally for 5 min; while the MS unit was set to be functioning at 70 eV. The constituents were identified by comparing their mass spectral data to those from NIST 05 Spectral Library.

Statistical analysis

Assays used to test for antioxidant and cytotoxic activities were carried out in quadruplicate. Results were analyzed with SPSS statistical analysis soft ware and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) wasused for comparison of more than two means. A difference was considered statistically significant when $p \leq 0.05$.

Results

Phenotypic characteristics of strain *S. gilvigriseus* MUSC 26^T

Strain MUSC 26^T was found to grow well on ISP 2 medium, ISP medium, Streptomyces agar, starch casein agar and tryptic soy agar and to grow moderately on actinomycetes isolation agar, Luria Bertani agar and nutrient agar. The colors of the aerial and substrate mycelium were media-dependent. Scanning electron microscope images and morphological observation of a 15-day-old culture grown on ISP 2 medium displayed a smooth spore surface with abundant growth of both aerial and vegetative hyphae, which were well developed and not fragmented (Figure 2).

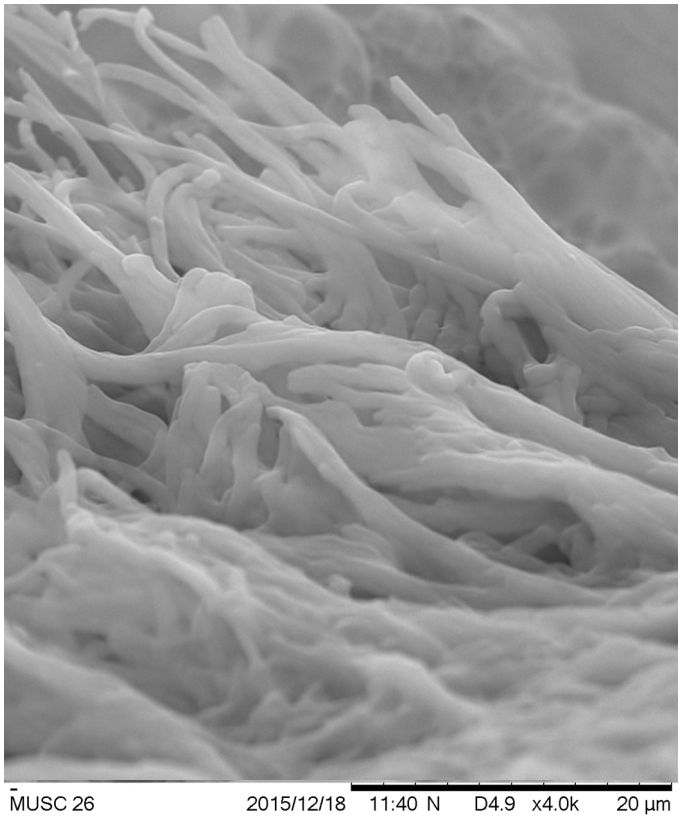

Figure 2. Scanning electron microscope of *Streptomyces gilvigriseus* MUSC 26^T.

These morphological features are consistent with assignment of the strain to the genus Streptomyces[46]. Growth was found to occur at pH ranged from 5.0 to 8.0 (with optimum growth observed at pH 6.0 -7.0). The strain could tolerate salinity up to 6 % NaCl (optimum 2-4 %) and growth was observed at temperature of 20 - 36 °C (optimum 28 - 32 °C). Cells were found to be positive for catalase but negative for melanoid pigment production and hemolytic activity. Hydrolysis of carboxymethylcellulose and soluble starch were found to be positive, but negative for hydrolysis of casein, chitin, tributyrin (lipase) and xylan. Strain MUSC 26^T was found to be able to utilize various compounds as carbon sources (Supplementary Table 1). Cells are sensitive to resistant to aztreonam, D-serine, fusidic acid, guanine HCl, lincomycin, lithium chloride, minocycline, 8 % NaCl, nalidixic acid, niaproof 4, potassium tellurite, rifamycin RV, sodium bromate, sodium butyrate, 1 % sodium lactate, tetrazolium blue, tetrazolium violet and troleandomycin.

Phylogenetic and genomic analyses

The almost-complete 16S rRNA gene sequence of MUSC 26T was aligned manually with the corresponding partial 16S rRNA gene sequences of type strains of representative members of the genus Streptomyces retrieved from GenBank/EMBL/DDBJ databases. Phylogenetic tree constructed using 16S rRNA sequence revealed that MUSC 26T formed a distinct clade with type strain Streptomyces sodiiphilus YIM 80305T (Figure 1).Pairwise comparison of the 16S rRNA gene sequence showed that strain MUSC 26^T exhibited highest gene similarity with *S. sodiiphilus* YIM 80305^T (96.48 %), followed by *S. qinglanensis*172205^T (96.41 %), which corresponds to 52 nucleotide differences at 1447 locations with gaps, and lower similarity values to *S. rimosus subsp. rimosus*ATCC 10970^T (96.27 %, 54 nucleotide differences at 1446 locations with gaps).

The DNA–DNA relatedness values between strain MUSC 26^T and the selected type strains were previously reported: (a) *S. qinglanensis* DSM 42035^T (33.2 ± 5.3 %), (b) *S. sodiiphilus* DSM 41839T (17.0 ± 2.2 %) and (c) *S. rimosus subsp. rimosus* NBRC 12907T (29.4 ± 3.1 %); these values were significantly below 70 %, the threshold value for the delineation of bacterial species[47]. Furthermore, BOX-PCR results demonstrated that strain MUSC 26^T yielded a unique BOX-PCR fingerprint compared with the closely related type strains (Figure 3).

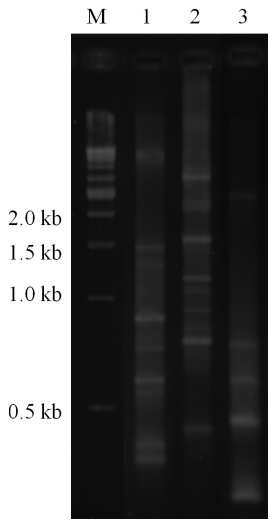

Figure 3. BOX-PCR comparison of strain MUSC 26^T and the closest related type strains. Lanes: 1, Streptomyces gilvigriseus MUSC 26^T; 2, Streptomyces qinglanensis DSM 42035^T; 3, Streptomyces rimosus subsp. rimosus NBRC 12907^T. M, GeneRuler 1kb DNA ladder marker.

Altogether, these results were in agreement with results of DNA-DNA hybridizations, which indicate that strain MUSC 26^T represents a novel species.

Using the MiSeq platform, the genome size of MUSC 26^T was found to be made up of 5,213,277 bp, with an average coverage of 40.0-fold and G + C content of 73.0%. The whole genome project of MUSC 26^T was deposited at DDBJ/EMBL/GenBank under accession number MLCF00000000 and the version described in this paper is the first version (MLCF01000000). A to-

tal of 4,337 protein-and the version described in this paper is the first version(MLCF01000000). A total of 4,337 protein-encoding genes was predicted and assigned to 363 subsystems with highest number of genes involved in carbohydrates metabolism (7.39 %), followed by amino acids and derivatives metabolism (5.90 %) and protein metabolism subsystems (5.81 %).Further bioinformatics analyses using antiSMASH server reflected a total of 40 biosynthetic gene clusters; two of which were responsible for siderophores production. One of the biosynthetic gene clusters related to siderophores production was revealed to reflect 40 % gene similarities to desferrioxamine B biosynthetic gene cluster.

Antioxidant and cytotoxic activities of MUSC 26^T extract

Antioxidant activity of MUSC 26^T was evaluated using two assays – ABTS and metal chelating studies (Table 1).

Table 1. Antioxidant activity of MUSC 26^T extract.

Concentration of MUSC 26^T extract (mg/mL)	Mean ± standard deviation (%)	
	ABTS	Metal-chelating
0.125	8.98 ± 2.74	N.A.
0.25	9.04 ± 0.55	16.07 ± 1.27
0.5	20.14 ± 0.67	32.79 ± 2.29
1	36.93 ± 2.62	49.71 ± 2.66
2	69.15 ± 1.81	78.92 ± 1.21
4	96.42 ± 0.84	92.99 ± 0.83

ABTS and metal chelating assays revealed significant antioxidant activity of MUSC 26^T extract with activities as high as 96.42 ± 0.84 % and 92.99 ± 0.83 % observed at 4 mg/mL. For the determination of cytotoxic activity, four human colon cancer cell lines were included for the study: Caco-2, SW480, HCT-116 and HT-29 cell lines. Based on MTT results, there is an increase in cyto toxic activity with increasing concentration of MUSC 26^T extract against some of the tested cell lines (Figure 4). When treated with 400 µg/mL of MUSC 26^T extract, lowest cell viability was observed in HCT-116 cell line at 63.64 ± 3.62 %. Compared to HCT-116, at the same tested extract concentration, other colon cell lines SW-480 and HT-29 exhibited higher cell viability at 67.50 ± 1.52 % and 69.05 ± 6.38 %, respectively. Highest cell viability was observed in Caco-2 cell lines – 86.88 ± 4.41 % when treated with 400 µg/mL.

A total of 14 compounds (Table 1) and their chemical structures (Figure 5) were identified using GC-MS as Butanoic acid, 3-methyl- (1), Trisulfide, dimethyl- (2), 1-Butanamine (3), 1H-Indole (4), 3-Aminopiperidin-2-one (5), Phenol, 2,4-bis(1,1-dimethylethyl)-(6),(3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione (7), Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro- (8), Methyl 13-methyltetradecanoate (9), Tetradecanoic acid, 12-methyl-, methyl ester (10), Hexadecanoic acid, methyl ester (11), 3,9-Diazatricyclo[7.3.0.0(3.7)]dodecan-2,8-dione (12), 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonan (13), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)- (14).

Chemical profiling of MUSC 26T extract

A total of 14 compounds (Table 2) and their chemical structures (Figure 5) were identified using GC-MS as Butanoic acid, 3-methyl- (1), Trisulfide, dimethyl- (2), 1-Butanamine (3), 1H-Indole (4), 3-Aminopiperidin-2-one (5), Phenol, 2,4-bis(1,1-dimethylethyl)-(6),(3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione(7),Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro- (8), Methyl 13-methyltetradecanoate (9), Tetradecanoic acid, 12-methyl-, methyl ester (10), Hexadecanoic acid, methyl ester (11),3,9-Diazatricyclo[7.3.0.0(3.7)]dodecan-2,8-dione (12), 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonan (13), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)- (14).

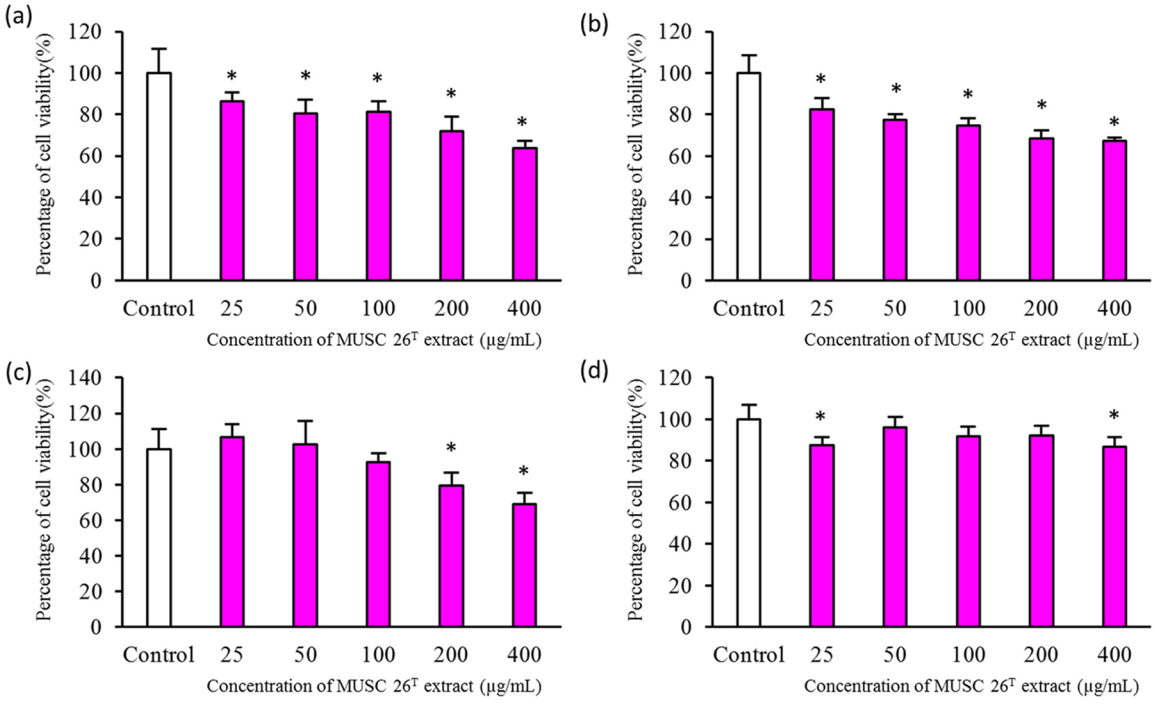

Figure 4. Cytotoxic activity of MUSC 26Textract against human colon cancer cell lines. Cell viability was measured using MTT assay. The graphs show cytotoxicity effects of MUSC 26^T extract against (a) HCT-116, (b) SW480, (c) HT-29, (d) Caco-2.

Table 2. Compounds identified from MUSC 26^T extract through

No	Retention time (min)	Compound	Formula	Molecular weight (MW)	Quality (%)
1	9.221	Butanoic acid, 3-methyl-	$C_5H_{10}O_2$	102	74
2	17.140	Trisulfide, dimethyl-	$C_2H_6S_3$	126	90
7	51.620	(3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_8H_{12}N_2O_2$	168	90
8	53.114	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154	94
9	54.687	Methyl 13-methyltetradecanoate	$C_{16}H_{32}O_2$	256	97
10	54.956	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256	94
11	58.075	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	96
12	59.248	3,9-Diazatricyclo[7.3.0.0(3.7)]dodecan-2,8-dione	$C_{10}H_{14}N_2O_2$	194	90
13	59.379	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonan	$C_{11}H_{18}N_2O_2$	210	83
14	72.071	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)-	$C_{14}H_{16}N_2O_2$	244	93

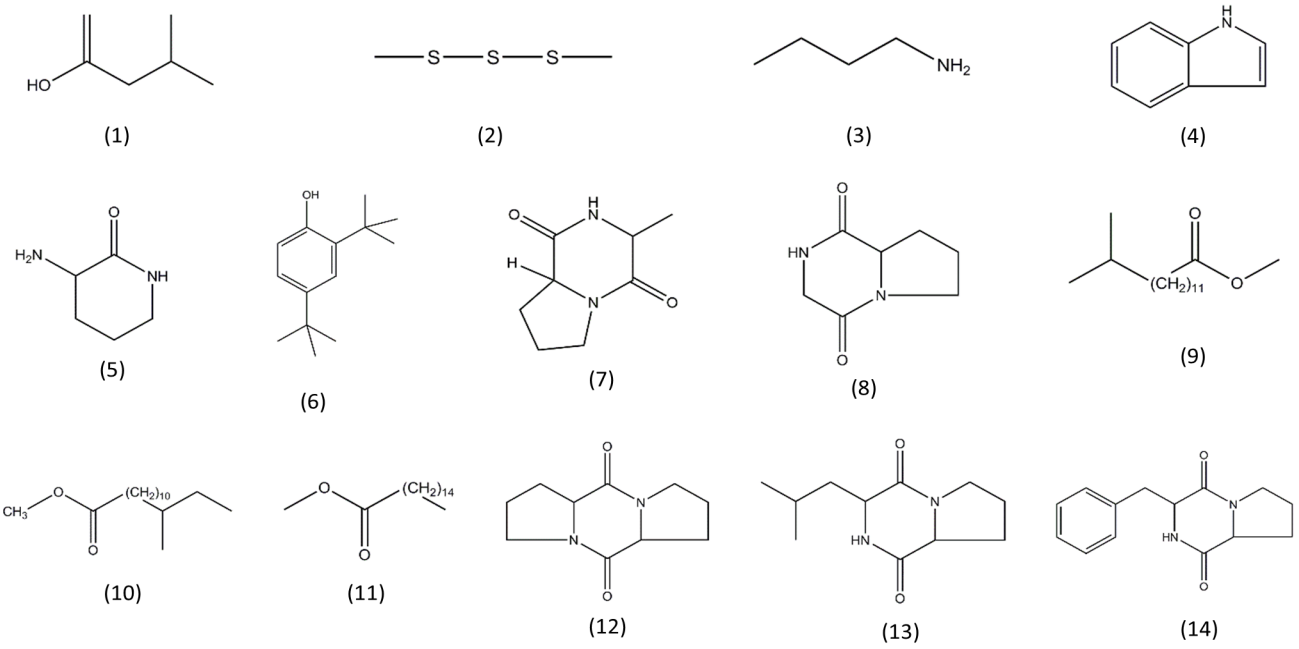

Figure 5. Chemical structures of the identified compounds from MUSC 26^T.(1), Butanoic acid, 3-methyl-; (2), Trisulfide, dimethyl-; (3), 1-Butanamine; (4), 1H-Indole; (5), 3-Aminopiperidin-2-one; (6), Phenol, 2,4-bis(1,1-dimethylethyl)-; (7), (3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione; (8), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-; (9), Methyl 13-methyltetradecanoate; (10), Tetradecanoic acid, 12-methyl-, methyl ester; (11), Hexadecanoic acid, methyl ester; (12), 3,9-Diazatricyclo[7.3.0.0(3.7)]dodecan-2,8-dione; (13), 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonan; (14), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)-.

Discussion

Members of *Streptomyces* genus have always been in the limelight for the search of valuable drugs. Given that these microbes are capable of producing over 10,000 bioactive compounds with various structures, many researchers have attempted to isolate them from various habitats including both terrestrial and marine environments. In fact, recent studies have indicated the importance of special environments like the underexplored mangrove forest as numerous interesting bioactive compounds have been recovered from streptomycetes derived from this region[6,8,42-45]. The mangrove forest is known to be a unique woody plant area of intertidal coasts, commonly found in tropical and subtropical coastal regions. It is suggested that the constant changes in salinity and tidal gradient in the mangrove ecosystem turned out to be the driving forces for metabolic pathway adaptations which could result in the production of valuable metabolites[6,8]. In this current study, MUSC 26^T was previously isolated as novel streptomycete derived from mangrove forest in the east coast of Peninsular Malaysia. Overall, results from phylogenetic and genomic analyses based on 16S rRNA gene sequence and DNA-DNA hybridization showed that strain MUSC 26^T was indeed a novel strain belong to the genus *Streptomyces*.

As an effort to study the bioactive potential of the strain, antioxidant assays were conducted on the extract of MUSC 26^T. Results from these assays reflected that the strain was capable of reducing amount of free radicals and also chelate metal ions. In fact, when we took a closer look at its genome, antiSMASH server detected two gene clusters associated with siderophores production; one of which showed 40% similarities to desferrioxamine B biosynthetic gene cluster. Desferrioxamine (Prescription name: Desferal) is currently listed on World Health Organization's List of Essential Medicines, owing to its medical importance as iron chelator and to defend against iron-induced oxidative stress[48]. Besides preventing accumulation of free radicals, this trihydroxamate molecule is capable of inhibiting growth of various cancer cell lines and tumor by altering gene expression of proto-oncogenes and signaling pathways which are critical for cell proliferation and apoptosis[49-54]. The presence of sidero

phores gene clusters does not only suggesting its antioxidant potential of MUSC 26^T, but also indicating possiblecytotoxic activities against cancer cells. In current study, MUSC 26^T extract was tested against several human colon cancer cell lines. Through MTT assay, lowest cell viability was observed in HCT-116 cell line at 63.64 ± 3.62 % when treated with 400 µg/mL of the extract. Higheramount of cells was observed in SW-480 and HT-29 cell lines with cell viability recorded at 67.50 ± 1.52 % and 69.05 ± 6.38 %, respectively after treated with 400 µg/mL of extract. Among the four colon cancer cell lines, Caco-2 was found to be least sensitive to the extract treatment as 86.88 ± 4.41 % of cell viability was observed (even after treated with 400 µg/mL). The difference in susceptibility to the extract treatment may possibly be due to the nature of genetic makeup of these cell lines. In fact, previous reports have discussed the involvement of tumor suppressor gene, p53 in determining susceptibility of these cancer cell lines. Except HCT-116, HT-29, Caco-2 and SW480 cell lines were known to possess dysfunction p53 tumor suppressor protein[55,56]. However, further investigations are still required to pinpoint the target of mechanism(s) of action for MUSC 26^T extract.

Apart from conducting bioactivities screening, GC-MS analysis was also carried out to examine the chemical constituent present in MUSC 26^T extract. This technique has long been in used in natural product discovery, including detecting those derived from *Streptomyces* species[57-60]. Based on their fragmentation patterns, a total of 14 compounds were identified in the extract of MUSC 26^T. Most of them were present as heterocyclic compounds, belonging to families such as phenols (e.g. phenol, 2,4-bis(1,1-dimethylethyl)-) and cyclic dipeptides (e.g. 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonan and pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)-). As a matter of fact, cyclic dipeptides are the smallest peptide derivatives in nature, which some of them possessing interesting bioactivities[61,62]. For instance, 1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonan has been previously isolated from marine-associated microbe and it was shown to possess cytotoxic potential against human cancer cell lines[6,62,63]. The compound demonstrated in vitro anticancer potential against lung (A549) and cervical (HeLa) cancer cells in a dose-dependent manner with the IC50 concentration of 19.94 ± 1.23 and 16.73 ± 1.78 µg/mL respectively[62].Similarly,pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-phenylmethyl)- was discovered to possess antibacterial, anticandidal activity as well as anticancer activity against colon cancer cells[64-66]. These findings from GC-MS have further highlighted the potential exploitation of the mangrove-derived streptomycete strain MUSC 26^T, given that the strain is capable of producing bioactive compounds with antioxidant and cytotoxic potentials. Nonetheless, by combining information obtained from its whole genome sequence, further in-depth investigation into the biosynthetic gene clusters responsible for the production of these compounds could essentially hasten the process for development of clinically useful drugs against cancer.

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