Electronic Journal of Biotechnology 40 (2019) 40–44

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Antiproliferative activity of biomass extract from *Pseudomonas cedrina*

Leonardo Sánchez-Tafolla [a], José M. Padrón [b], Guillermo Mendoza [c], Mauricio Luna-Rodríguez [d], José J. Fernández [b], Manuel Norte [b], Ángel Trigos [c,*]

[a] Instituto de Biotecnología y Ecología Aplicada, Universidad Veracruzana, Av. de las Culturas Veracruzanas No. 101, Colonia Emiliano Zapata, 91090 Xalapa-Veracruz, Mexico
[b] Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG) Universidad de la Laguna, Avenida Astrofísico Francisco Sánchez 2, 38206 La Laguna, Spain
[c] Laboratorio de Alta Tecnología de Xalapa, Universidad Veracruzana, Calle Médicos No. 5, Col. Unidad del Bosque, 91010 Xalapa-Veracruz, Mexico
[d] Facultad de Ciencias Agrícolas, Universidad Veracruzana, Circuito Gonzalo Aguirre Beltrán s/n, Zona Universitaria, 91091 Xalapa-Veracruz, Mexico

ARTICLE INFO

Article history:
Received 1 September 2018
Accepted 29 March 2019
Available online 5 April 2019

Keywords:
Antiproliferative activity
Bioactive compounds
Cancer
Diketopiperazines
Mexico
Pinus patula
Plant-associated bacteria
Plant-associated microorganisms
Pseudomonas cedrina
Therapeutic agents

ABSTRACT

Background: The study of plant-associated microorganisms is very important in the discovery and development of bioactive compounds. *Pseudomonas* is a diverse genus of Gammaproteobacteria comprising more than 60 species capable of establishing themselves in many habitats, which include leaves and stems of many plants. There are reports of metabolites with diverse biological activity obtained from bacteria of this genus, and some of the metabolites have shown cytotoxic activity against cancer cell lines.
Because of the high incidence of cancer, research in recent years has focused on obtaining new sources of active compounds that exhibit interesting pharmacodynamic and pharmacokinetic properties that lead to the development of new therapeutic agents.
Results: A bacterial strain was isolated from tumors located in the stem of *Pinus patula*, and it was identified as *Pseudomonas cedrina*. Extracts from biomass and broth of *P. cedrina* were obtained with chloroform:methanol (1:1). Only biomass extracts exhibited antiproliferative activity against human tumor cell lines of cervix (HeLa), lung (A-549), and breast (HBL-100). In addition, a biomass extract from *P. cedrina* was fractioned by silica gel column chromatography and two diketopiperazines were isolated: cyclo-(L-Prolyl-L-Valine) and cyclo-(L-Leucyl-L-Proline).
Conclusions: This is the first report on the association of *P. cedrina* with the stems of *P. patula* in Mexico and the antiproliferative activity of extracts from this species of bacteria against human solid tumor cell lines.
How to cite: Sánchez-Tafolla L, Padrón JM, Mendoza G, et al. Antiproliferative activity of biomass extract from Pseudomonas cedrina. Electron J Biotechnol 2019;40. https://doi.org/10.1016/j.ejbt.2019.03.010.

1. Introduction

The study of plant-associated microorganisms is very important in the discovery and development of new active compounds [1,2]. These compounds are usually secondary metabolites that the microorganisms use as defense mechanisms to obtain advantage against other microorganisms and adaptation in nature [3,4].

Pseudomonas is a diverse genus of Gammaproteobacteria comprising more than 60 species capable of establishing themselves in many habitats, which include leaves and stems of many plants [5,6]. There are several reports of metabolites with diverse biological activities obtained from bacteria of this genus, and some of them such as safracin, fenazin, pyocyanin, and some rhamnolipids have shown cytotoxic activity against cancer cell lines [4,7,8,9,10]. In addition, compounds named diketopiperazines have been isolated from the genus *Pseudomonas* and other genera of bacteria, showing various biological activities (Table 1).

Because of the high incidence of cancer [11] and because there are no reports of compounds obtained from *Pseudomonas cedrina* with antiproliferative activity against human solid tumor cell lines, we evaluated in our research the activity of extracts obtained from this bacterial species against five human solid tumor cell lines as well as the isolated metabolites that may be possibly responsible for this bioactivity.

2. Materials and methods

2.1. Isolation of bacterial strain from vegetal samples

Samples from pine stem of *Pinus patula* were collected from a nursery garden at Huayacocotla, Veracruz, Mexico (20° 32′N, 98° 29′W, altitude 2140 m) in February 2012. The samples of the stem segment with

* Corresponding author.
E-mail address: atrigos@uv.mx (Á. Trigos).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2019.03.010

Table 1
Bioactive diketopiperazines isolated from bacteria.

Origin	Diketopiperazine(s) isolated	Bioactivity	References
Lactobacillus plantarum LBP-K10	*Cis*-cyclo-(L-Val-L-Pro), *cis*-cyclo-(L-Phe-L-Pro) and *cis*-cyclo-(L-Leu-L-Pro)	Antifungal (*Ganoderma boninense*, *Candida albicans*)	[30]
Lactobacillus plantarum MiLAB 393	Cyclo-(L-Phe-L-Pro) and cyclo-(L-Phe-*trans*-4-OH-L-Pro)	Antifungal (*Fusarium sporotrichioides*, *Aspergillus fumigatus*, *Kluyveromyces marxianus*)	[31]
Pseudomonas rhizosphaerae	Cyclo-(L-Tyr-Pro), cyclo-(L-Tyr-Ile), cyclo-(Phe-Pro) and cyclo-(L-Val-L-Pro)	Antibacterial (*Ruegeria* sp., *Loktanella hongkongensis*, *Micrococcus luteus*, *Pseudoalteromonas piscicida*, *Bacillus cereus*)	[32,33]
Bacillus amyloliquefaciens Q-426	Cyclo-(L-Pro-Leu), cyclo-(L-Pro-Val), cyclo-(Pro-Phe) and cyclo(Ala-Val).	Inhibition of biofilm formation (*Streptococcus mutans*, *Bacillus amyloliquefaciens*)	[34]
Streptomyces fungicidicus	Cyclo-(L-Leu-L-Pro), cyclo-(L-Phe-L-Pro), cyclo-(L-Val-L-Pro), cyclo-(L-Trp-L-Pro) and cyclo-(L-Leu-L-Val)	Inhibition of fouling (*Balanus amphitrite*)	[35]
Pseudomonas rhizosphaerae	Cyclo-(L-Val-L-Pro)	Antilarval (*Balanus amphitrite*, *Bugula neritina*)	[33]
Streptomyces sp. Q24	Cyclo-(L-Phe-L-4-OH-Pro), cyclo-(L-Phe-D-4-OH-Pro) and cyclo-(L-Leu-L-Pro)	Antiproliferative properties against glioblastoma cells (U87-MG and U251)	[36]
Pseudomonas fluorescens H40	Cyclo-(L-Leu-L-Pro)	Cytotoxic against cancer cell lines (Hep-2)	[10]

the tumor were washed with sterile water and disinfected with NaClO (2% v/v), cut as small pieces with sterilized scalpel, and then macerated with sterile deionized water. Serial dilutions were prepared (1:10), and then, the suspensions were plated on King's B agar (KB, Mast Group Ltd., UK) and yeast dextrose carbonate medium (YDC, Duchefa Biochemie, The Netherlands). The plates were incubated at 27°C ± 1 for 48 h (Arsa mod. AR-130D, Mexico) [12].

2.2. Identification of the bacterial strain

The strain isolated was identified by the morphology of colonies, biochemical test (LOPAT) [12], and hypersensitive response (HR) in *Nicotiana tabacum* leaves [13]. Microscopic observations were made with an optical microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Additionally, the strain was identified by analyzing the sequences of 16S rRNA. Isolation of the genomic DNA was performed using the technique for Gram-negative bacteria described by Cheng and Jian [14]. Amplification was performed in a 25 μL reaction mix containing DNA, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 200 μM dNTPs, 30 pmol of each primer, 1.5 units of Taq DNA polymerase (Promega, USA), and 1 μL of isolated DNA. PCR amplifications were performed in a Mastercycler (Eppendorf AG, Germany). The primers used were 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′, TM = 58°C) and 1512R (5′-GTG AAG CTT ACG GYT AGC TTG TTA CGA CTT-3′, TM = 58°C) to amplify the 16S rRNA region [15]. Purification of PCR products was carried out with a Wizard SV Gel and PCR Clean-Up System (Promega, USA), after which sequencing was performed (Applied Biosystems, mod. 3130xl, USA). The sequence of nucleotides was compared with those present in the GenBank, NCBI, by employing the BLASTN tool to confirm identity between species. For phylogenetic analysis, the sequences were aligned using the algorithm ClustalW of MegaAlign from MEGA7 software [16]. The phylogenetic tree was constructed using the maximum likelihood estimation (Kimura 2-parameter model) of MEGA7 software. Bootstrap analysis was performed with 1000 replicates. The nucleotide sequence obtained was submitted to GenBank, and the accession number provided was **MF962580**.

2.3. Bacterial culture and preparation of extracts

The *P. cedrina* strain was cultivated in Petri dishes containing a solid Luria–Bertani medium (LB, Dibico, Mexico) incubated for 48 h at 27°C ± 1. After that, one bacterial colony was used to inoculate five Erlenmeyer flasks (500 mL) with 100 mL of LB broth; the flasks were placed in an orbital shaker (SEV mod. 6090, Mexico) for 48 h at 27°C ± 1 [12]. Then, 1 mL of bacterial suspension was used to scale up the culture in 40 L of LB broth distributed in Erlenmeyer flasks (500 mL) with 100 mL of medium. Subsequently, the broth culture was sonicated (Bransonic mod. 3510R-MT, USA) for 1 h, and then, the biomass and broth were separated by centrifugation (Eppendorf mod. 5416, Germany) at 7500 rpm for 10 min. Both biomass and broth were first frozen and then lyophilized (−40°C, 0.015 mbar, LABCONCO FreeZone Plus 6, Missouri). Once dry, the broth and biomass were extracted separately with a mixture of chloroform: methanol (1:1) for five days at room temperature. The extraction was repeated five times; the extracts obtained were filtered with a filtration system (Sterifil, Millipore, Germany) at room temperature and concentrated in a rotatory evaporator (40°C, 330 mbar, Büchi Olibath B-485, Flawil), and finally, the lyophilized extracts were used in an antiproliferative assay.

2.4. Cell lines and culture

The human solid tumor cell lines A-549, HBL-100, HeLa, T-47D, and WiDr, donated by Prof. G. J. Peters (VU Medical Center, Amsterdam, The Netherlands), were used in this study. The cells were maintained in 25 cm^2 culture flasks in RPMI 1640 (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 5% heat-inactivated fetal calf serum and 2 mM L-glutamine (Lonza BioWhittaker, Verviers, Belgium) in an incubator at 37°C, 5% CO_2, and 95% air humidity (Steri-Cycle CO_2 Incubator, Thermo Electron Corporation, Waltham, MA, USA). Cells growing in the exponential phase were trypsinized (Lonza BioWhittaker, Verviers, Belgium) and resuspended in an antibiotic-containing medium (100 units of penicillin G and 0.1 mg of streptomycin per mL) (Lonza BioWhittaker, Verviers, Belgium). Single-cell suspensions were counted using Orflo's Moxi Z automated cell counter (Orflow, Ketchum, ID, USA), and dilutions were made to give the appropriate cell densities for the inoculation onto 96-well microtiter plates. Based on their doubling times, the cells were inoculated in 100 μL per well at 10,000 (A-549, HBL-100, and HeLa), 15,000 (T-47D), and 20,000 (WiDr) cells per well.

2.5. Antiproliferative activity

Dry extracts were initially dissolved in DMSO (Sigma-Aldrich, St. Louis, MO, USA) at 400 times the desired final maximum test concentration, i.e., 10 mg/mL and diluted in the culture media until they reached an assay concentration of 250 μg mL^{-1} [17]. Control cells were exposed to an equivalent concentration of DMSO but with no extract (0.25% v/v, negative control). The extract (i.e., drug) treatment began on the first day after plating. The extracts were incubated for 48 h, and after that, the cells were precipitated with 25 μL ice-cold TCA (50% w/v) and fixed for 60 min at 4°C. Then, the SRB assay was

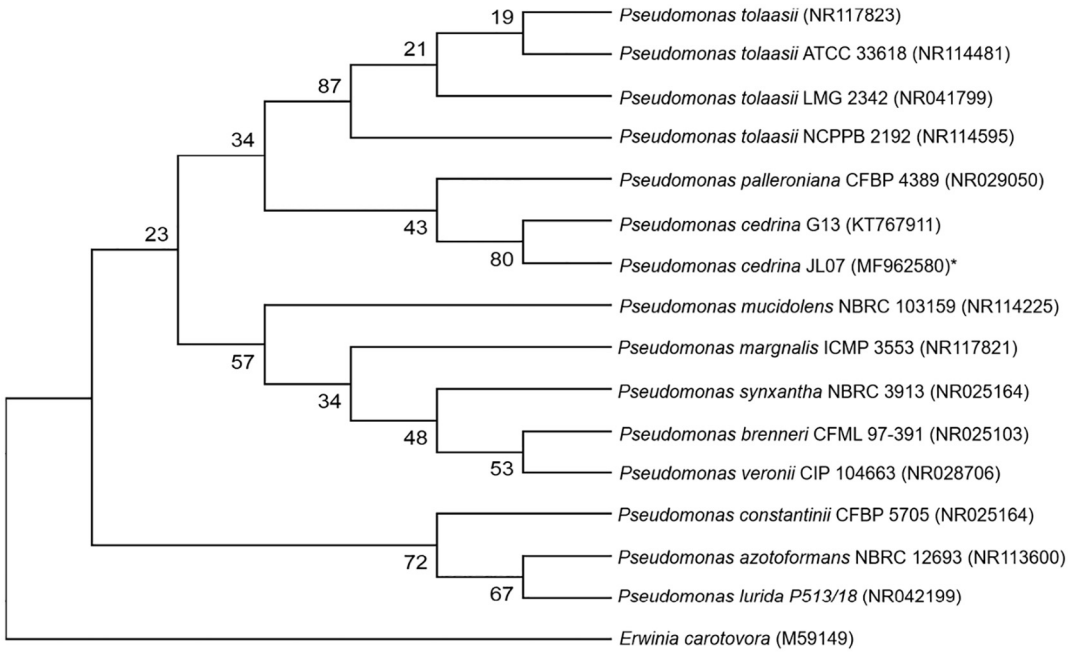

Fig. 1. Molecular phylogenetic analysis of *P. cedrina* strain used in the research (*). The tree was constructed using a Maximum Likelihood algorithm based on the Kimura 2-parameter model with bootstrap analysis (1000 replicates). *Erwinia carotovora* was used as an external group. Figure created with MEGA7 software.

performed using the technique described by Skehan et al. [18]. The optical density (OD) of each well was measured at 492 nm using BioTek's PowerWave XS Absorbance Microplate Reader (BioTek, Winooski, VT, USA). The percentage growth was calculated as the OD difference between the start and end of each treatment level corrected for background OD of the control and compared with untreated cells. The results were expressed as the concentration of extract causing 50% reduction in the proliferation of cancer cells (GI_{50}) [17].

2.6. Purification and structural elucidation of compounds

The biomass extract was purified by silica gel column chromatography (Merck 0.040–0.063 mm) as the static phase and n-hexane-ethyl acetate gradient as the eluent. The chromatography process was monitored by TLC (Merck 60 GF_{254}, 0.2 mm of thickness) staining under UV light (254 and 365 nm) and with iodine vapors. The compounds were identified by analysis of ^{1}H and ^{13}C NMR and a comparison of their spectral data with data already published.

2.6.1. Cyclo-(L-Prolyl-L-Valine) (**1**)

From the fractions eluted with AcOEt (9.4 g) of the chloroform: methanol extract of the biomass (99.8 g of dry biomass), 3.1 mg of a white powder was obtained (Mp 144–147°C) and TLC (1:9 n-Hex: AcOEt): Rf = 0.35. ^{1}H RMN ($CDCl_3$, 600 Hz) δ, ppm: 5.75 (1 H, sa, H-4); 4.08 (1 H, t, J = 7.81 Hz, H-6); 3.94 (1 H, sa, H-3); 3.6 (2 H, c, H-9); 2.64 (1 H, c, H-10); 2.36 (1 H, c, H-7); 2.01 (3 H, c, H-7′ and H-8); 1.05 (3 H, d, J = 7.24 Hz, H-11); 0.91 (3 H, d, J = 6.76 Hz, H-12) [19].

2.6.2. Cyclo-(L-Leucyl-L-Proline) (**2**)

From the fractions eluted with AcOEt (9.4 g) of the chloroform: methanol extract of the biomass (99.8 g of dry biomass), 3.6 mg of a colorless crystal was obtained (Mp 168–172°C) and TLC (1:9 n-Hex: AcOEt): Rf = 0.45. ^{1}H RMN ($CDCl_3$, 600 Hz) δ, ppm: 5.88 (1 H, sa, H-4); 4.16 (1 H, t, J = 7.82 Hz, H-6); 4.05 (1 H, dd, J = 3.74, 9.52 Hz, H-3); 3.59 (2H, c, H-9); 2.38 (1 H, c, H-7); 2.10 (3 H, c, H-10 y H-8); 1.96 (1 H, c, H-7′); 1.78 (1 H, c, H-11); 1.56 (1 H, c, H-10′); 1.01 (3 H, d, J = 5.9 Hz, H-12); 0.97 (3 H, d, J = 6.1 Hz, H-13) [10,19].

3. Results

3.1. Isolation and identification of bacterial strain

The bacterial strain was isolated from tumors located in the stem of *P. patula* samples, and when an HR test in *N. tabacum* was carried out, the strain caused a notorious necrotic effect in the leaves. This strain was cultivated in KB medium, and after 48 h, the bacterial strain showed light yellow colonies that were smooth and convex with regular margins and that produced a pigment that demonstrated a light green fluorescence when irradiated under UV light (λ = 360 nm) characteristic of the genus *Pseudomonas* [12]. When a biochemical LOPAT test was carried out, the strain showed production of levan, cytochrome oxidase, arginine dihydrolase, and pectolytic activity. All these biochemical characteristics are consistent with those described by Dabboussi et al. [20] for *P. cedrina*.

Additionally, the strain was genetically identified using the 16S rRNA, and the obtained sequence was compared against nucleotide entries in the databases of GenBank, NCBI. The strain shared a sequence similarity of 100% with *P. cedrina* (GenBank accession no. KT767911.1) and was placed in the *P. cedrina* clade (Fig. 1).

3.2. Antiproliferative activity

Using the *P. cedrina* strain, biomass and culture broth extracts were prepared. Following this, assays against five human solid tumor cell lines were carried out employing these extracts. The extract obtained from biomass exhibited 50% growth inhibition at concentrations below 50 μg mL^{-1} against three of the studied solid tumor cell lines: A-549 (44 μg mL^{-1}), HBL-100 (32 μg mL^{-1}), and HeLa (33 μg mL^{-1}) (Table 2).

Table 2
Values of growth inhibition 50% (GI50) (μg mL^{-1}) of the extract obtained from the biomass of *P. cedrina* against five solid tumor cell lines.

A-549 (lung)	HBL-100 (breast)	HeLa (cervix)	T-47D (breast)	WiDr (colon)
44	32	33	64	55

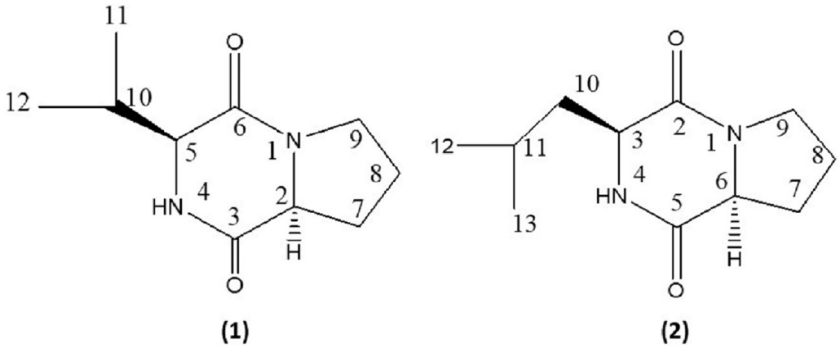

Fig. 2. Structure of the compounds isolated from *P. cedrina*. Figure created with ChemDraw Ultra 8.0 software.

3.3. Purification and structural elucidation of compounds

The biomass extract (9.4 g) was purified to identify the metabolites with antiproliferative activity, and the following compounds were isolated: (**1**) cyclo-(L-Prolyl-L-Valine) and (**2**) cyclo-(L-Leucyl-L-Proline) (Fig. 2). Both were identified by comparison with an authentic sample and with their previously reported ^{1}H-NMR spectroscopic data [10,19].

4. Discussion

There are reports of different bacterial genera that can be associated with plants, with *Pseudomonas* as the most abundant genus in the phyllosphere, a region that includes leaves, stems, and trunks. Usually, this genus is opportunistic and, in some cases, potentially phytopathogenic [5,21].

In addition, another characteristic of the genus *Pseudomonas* is their versatility to produce secondary metabolites depending on environmental conditions [22]. These metabolites show a wide variety of biological activities, such as siderophores [23], cellular signaling molecules [24], antimicrobial [10], cytotoxicity against some cancer cell lines [7,8,9,10,25], and toxins [5,26]. Some metabolites such as safracins and phenazines obtained from bacteria of the genus *Pseudomonas* have been identified to exhibit antitumor activity [7] or diketopiperazines, isolated from *P. fluorescens* H40, which exhibited cytotoxicity against Hep-2 cell lines [10].

According to the methodology for evaluating *in vitro* anticancer drug discovery screen suggested by the National Cancer Institute (NCI) of the USA [18], the biomass extract from *P. cedrina* evaluated in this study exhibited antiproliferative properties against three of the studied solid tumor cell lines: A-549, HBL-100, and HeLa.

On the other hand, the compounds (1, 2) were identified by analyzing their ^{1}H NMR spectroscopic data as two diketopiperazines (DKPs). These compounds are cyclic dipeptides that are obtained by the condensation of two α-amino acids and are produced by both bacteria and fungi [27]. Previously, it was thought that they were "artifacts" of the microorganisms that were produced as part of the assimilation of the culture medium. However, recent studies have shown that these compounds are synthesized by the action of the cyclodipeptide synthases, a family of enzymes involved in the synthesis of non-ribosomal peptides [27,28]. Some biological activities that have been identified for this type of compounds are antifungal [29,30,31], antibacterial [32,33], inhibition of biofilm formation [34], inhibition of fouling [35], antilarval [33], antiproliferative against glioblastoma cells [36], and cytotoxic against some cancer cell lines [10].

Particularly, it has been reported that DKPs and their derivate molecules exhibit biological activities against human carcinoma cells through different mechanisms such as DNA-binding agents [37], inhibition of cell cycle [38], inhibition of the (BCRP/ABCG2) multidrug transporter [39], depolymerization of tubuline [40], and inactivation of the antiproteolytic activity of the serpin plasminogen activator inhibitor-1 (PAI-1) [41]. These biological activities of the 2,5-diketopiperazines and their derivate compounds are associated with their scaffold that provides them with different chemical properties such as conformational rigidity, resistance to proteolysis, and mimicking peptidic pharmacophoric groups and donor or acceptor groups for hydrogen bonding essential for interaction with biological targets [42].

Finally, the reports related to *P. cedrina* describe only new isolations of this species from different samples such as grasses [21], spring water [20], herbal plants [22], and desert soil [43]. Although it is known that bacteria of the genus *Pseudomonas* produce a wide variety of bioactive metabolites [5], for the species *P. cedrina*, it has not been reported whether this species produces this type of metabolite, and has antiproliferative activity against human tumor cell lines of the cervix (HeLa), lung (A-549), and breast (HBL-100).

5. Conclusions

This is the first report on the association of *P. cedrina* with the stems of *P. patula* in Mexico and the antiproliferative activity of extracts from this species of bacteria against human solid tumor cell lines of the cervix (HeLa), lung (A-549), and breast (HBL-100). In addition, we isolated the bioactive diketopiperazines cyclo-(L-Prolyl-L-Valine) and cyclo-(L-Leucyl-L-Proline), which could be responsible for antiproliferative activity.

Conflict of interest

None.

Financial support

The authors offer special thanks to Consejo Nacional de Ciencia y Tecnología (CONACYT), México for the scholarship 320366 N° Reg. 259584 and the financial support through project UV-CA-354 PRODEP 2015 thematic network.

References

[1] Gutiérrez RM, González AM, Ramírez AM. Compounds derived from endophytes: A review of phytochemistry and pharmacology. Curr Med Chem 2012;19(18): 2992–3030. https://doi.org/10.2174/092986712800672111 PMID: 22489725.

[2] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 2012;75(3):311–35. https://doi.org/10.1021/np200906s PMid:22316239.

[3] Mangamuri UK, Vijayalakshmi M, Poda S, et al. Bioactive metabolites produced by *Pseudonocardia endophytica* VUK-10 from mangrove sediments: Isolation, chemical structure determination and bioactivity. J Microbiol Biotechnol 2015;25(5): 629–36. https://doi.org/10.4014/jmb.1407.07041 PMID: 25418482.

[4] Mavrodi DV, Parejko JA, Mavrodi OV, et al. Recent insights into the diversity, frequency and ecological roles of phenazines in fluorescent *Pseudomonas* spp. Environ

Microbiol 2013;15(3):675–86. https://doi.org/10.1111/j.1462-2920.2012.02846.x PMID: 22882648.
[5] Gross H, Loper JE. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Prod Rep 2009;26:1408–46. https://doi.org/10.1039/b817075b PMID: 19844639.
[6] Müller T, Behrendt U, Ruppel S, et al. Fluorescent pseudomonads in the phyllosphere of wheat: potential antagonists against fungal phytopathogens. Curr Microbiol 2016; 72(4):383–9. https://doi.org/10.1007/s00284-015-0966-8 PMID: 26687461.
[7] Ikeda Y, Idemoto H, Hirayama F, et al. Safracins, new antitumor antibiotics. I. Producing organism, fermentation and isolation. J Antibiot 1983;36(10):1279–83. https://doi.org/10.7164/antibiotics.36.1279 PMID: 6417094.
[8] Kamal A, Shaik AB, Kumar CG, et al. Metabolic profiling and biological activities of bioactive compounds produced by *Pseudomonas* sp. strain ICTB-745 isolated from Ladakh, India. J Microbiol Biotechnol 2012;22(1):69–79. https://doi.org/10.4014/jmb.1105.05008 PMID: 22297221.
[9] Mavrodi DV, Blankenfeldt W, Thomashow L. Phenazine compounds in fluorescent *Pseudomonas* spp. biosynthesis and regulation. Annu Rev Phytopathol 2006;44: 417–45. https://doi.org/10.1146/annurev.phyto.44.013106.145710 PMID: 16719720.
[10] Santos OC, Soares AR, Machado FL, et al. Investigation of biotechnological potential of sponge-associated bacteria collected in Brazilian coast. Lett Appl Microbiol 2015;60(2):140–7. https://doi.org/10.1111/lam.12347 PMID: 25355062.
[11] World Health Organization (WHO). Cancer. Fact sheet N° 297. Available from Internet: http://www.who.int/en/news-room/fact-sheets/detail/cancer. [cited August 15, 2018].
[12] Schaad NW, Jones JB, Chun W. Gram-negative bacteria, *Pseudomonas*. In: Schaad NW, Jones JB, Chun W, editors. Laboratory guide for identification of plant pathogenic bacteria. 3rd ed. Minnesota: APS Press; 2001. p. 84–120.
[13] Nissinen R, Lai FM, Laine MJ, et al. *Clavibacter michiganensis* subsp. *Sepedonicus* elicits a hypersensitive response in tobacco and secretes hypersensitive response-inducing protein(s). Phytopathology 1997;87(7):678–84. https://doi.org/10.1094/PHYTO.1997.87.7.678 PMID: 18945088.
[14] Cheng HR, Jiang N. Extremely rapid extraction of DNA from bacteria and yeast. Biotechnol Lett 2006;28(1):55–9. https://doi.org/10.1007/s10529-005-4688-z PMID: 16369876.
[15] Kondo R, Imai I, Fukami K, et al. Phylogenetic analysis of algicidal bacteria (family *Flavobacteriaceae*) and selective detection by PCR using a specific primer set. Fish Sci 1999;65(3):432–5. https://doi.org/10.2331/fishsci.65.432.
[16] Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33(7):1870–4. https://doi.org/10.1093/molbev/msw054 PMID: 27004904.
[17] Monks A, Scudeiro D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. J Natl Cancer Inst 1991;83 (11):757–66. https://doi.org/10.1093/jnci/83.11.757 PMID: 2041050.
[18] Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82(13):1107–12. https://doi.org/10.1093/jnci/82.13.1107 PMID: 2359136.
[19] Trigos A, Reyna S, Cervantes L. Three diketopiperazines from the cultivated fungus *Fusarium oxysporum*. Nat Prod Lett 1995;6(4):241–6. https://doi.org/10.1080/10575639508043166.
[20] Dabboussi F, Hamze M, Elomari M, et al. Taxonomic study of bacteria isolated from Lebanese spring waters: Proposal for *Pseudomonas cedrella* sp. nov. and *P. orientalis* sp. nov. Res Microbiol 1999;150(5):303–16. https://doi.org/10.1016/S0923-2508(99)80056-4 PMID: 10422691.
[21] Behrendt U, Schumann P, Meyer JM, et al. *Pseudomonas cedrina* subsp. *fulgida* subsp. nov., a fluorescent bacterium isolated from the phyllosphere of grasses; emended description of *Pseudomonas cedrina* and description of *Pseudomonas cedrina* subsp. *cedrina* subsp. nov. Int J Syst Evol Microbiol 2009;59:1331–5. https://doi.org/10.1099/ijs.0.005025-0 PMID: 19502311.
[22] Goryluk-Salmonowicz A, Piórek M, Rekosz-Burlaga H, et al. Endophytic detection in selected European herbal plants. Pol J Microbiol 2016;65(3):369–75. https://doi.org/10.5604/17331331.1215617 PMID: 29334055.
[23] Meyer JM, Stintzi A. Iron metabolism and siderophores in *Pseudomonas* and related species. In: Montie TC, editor. Biotechnology handbooks. PseudomonasNew York: Plenum Publishing Co.; 1998. p. 201–43. https://doi.org/10.1007/978-1-4899-0120-0_7.
[24] Fukuchi N, Isogai A, Nakayama J, et al. Structure and stereochemistry of three phytotoxins, syringomycin, syringotoxin and syringostatin, produced by *Pseudomonas syringae* pv. *syringae*. J Chem Soc Perkin Trans 1 1992;9:1149–57. https://doi.org/10.1039/p19920001149.
[25] Reszka KJ, Bilski PJ, Britigan BE. Quenching of singlet oxygen by pyocyanin and related phenazines. Photochem Photobiol 2010;86:742–6. https://doi.org/10.1111/j.1751-1097.2010.00728.x PMID: 20408986.
[26] Narquizian R, Kocienski PJ. The pederin family of antitumor agents: Structure, synthesis and biological activity. In: Mulzer J, Bohlmann R, editors. The role of natural products in drug discovery. Germany: Springer-Verlag; 2000. p. 25–56. https://doi.org/10.1007/978-3-662-04042-3_2 PMid: 11077605.
[27] Gondry M, Sauguet L, Belin P, et al. Cyclodipeptide synthases are a family of tRNA-dependent peptide bond-forming enzymes. Nat Chem Biol 2009;5(6):414–20. https://doi.org/10.1038/nchembio.175 PMid: 19430487.
[28] Sauguet L, Moutiez M, Li Y, et al. Cyclodipeptide synthases, a family of class-I aminoacyl-tRNA synthetase-like enzymes involved in non-ribosomal peptide synthesis. Nucleic Acids Res 2011;39:4475–89. https://doi.org/10.1093/nar/gkr027 PMID: 21296757.
[29] Byun HG, Zhang H, Mochizuki M, et al. Novel antifungal diketopiperazine from marine fungus. J Antibiot (Tokyo) 2003;56(2):102–6. https://doi.org/10.7164/antibiotics.56.102 PMID: 12715868.
[30] Kwak MK, Liu R, Kim MK, et al. Cyclic dipeptides from lactic acid bacteria inhibit the proliferation of pathogenic fungi. J Microbiol 2014;52(1):64–70. https://doi.org/10.1007/s12275-014-3520-7 PMID: 24390839.
[31] Ström K, Sjögren J, Broberg A, et al. *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-*trans*-4-OH-L-Pro) and 3-phenyllactic acid. Appl Environ Microbiol 2002;68:4322–7. https://doi.org/10.1128/AEM.68.9.4322-4327.2002 PMid: 12200282.
[32] Kohn H, Widger W. The molecular basis for the mode of action of bicyclomycin. Curr Drug Targets Infect Disord 2005;5(3):273–95. https://doi.org/10.2174/1568005054880136 PMID: 16181146.
[33] Qi SH, Xu Y, Gao J, et al. Antibacterial and antilarval compounds from marine bacterium *Pseudomonas rhizosphaerae*. Ann Microbiol 2009;59:229–33. https://doi.org/10.1007/BF03178321.
[34] Wang JH, Yang CY, Fang ST, et al. Inhibition of biofilm in *Bacillus amyloliquefaciens* Q-426 by diketopiperazines. World J Microbiol Biotechnol 2016;32(143). https://doi.org/10.1007/s11274-016-2106-4 PMID: 27430510.
[35] Li X, Dobretsov S, Xu Y, et al. Antifouling diketopiperazines produced by a deep-sea bacterium, *Streptomyces fungicidicus*. Biofouling 2006;22(3):187–94. https://doi.org/10.1080/08927010600780771 PMID: 17290864.
[36] Ye X, Chai W, Lian XY, et al. Novel propanamide analogue and antiproliferative diketopiperazines from mangrove *Streptomyces* sp. Q24. Nat Prod Res 2016;31 (12):1390–6. https://doi.org/10.1080/14786419.2016.1253079 PMID: 27806640.
[37] Gomez-Monterrey I, Campiglia P, Carotenuto A, et al. Design, synthesis, and cytotoxic evaluation of a new series of 3-substituted spiro[(dihydropyrazine-2,5-dione)-6,3′-(2′,3′-dihydrothieno[2,3-*b*]naphtho-4′,9′-dione)] derivatives. J Med Chem 2007;50(8):1787–98. https://doi.org/10.1021/jm0612158.
[38] Zhao S, Smith KS, Deveau AM, et al. Biological activity of the tryprostatins and their diastereomers on human carcinoma cell lines. J Med Chem 2002;45(8):1559–62. https://doi.org/10.1021/jm0155953 PMID: 11931609.
[39] van Loevezijn A, Allen JD, Schinkel AH, et al. Inhibition of BCRP-mediated drug efflux by fumitremorgin-type indolyl diketopiperazines. Med Chem Lett 2001;11(1): 29–32. https://doi.org/10.1016/S0960-894X(00)00588-6 PMID: 11140726.
[40] Arunrattiyakorn P, Ikeda B, Nitoda T, et al. Enzymatic synthesis of dehydroderivatives from proline-containing cyclic dipeptides and their effects toward cell division. Biosci Biotechnol Biochem 2007;71(3):830–3. https://doi.org/10.1271/bbb.60651 PMID: 17341821.
[41] Brooks TD, Wang SW, Brünner N, et al. XR5967, a novel modulator of plasminogen activator inhibitor-1 activity, suppresses tumor cell invasion and angiogenesis *in vitro*. Anticancer Drugs 2004;15(1):37–44. https://doi.org/10.1097/00001813-200401000-00007 PMid: 15090742.
[42] Martins M, Carvalho I. Diketopiperazines: Biological activity and synthesis. Tetrahedron 2007;63(40):9923–32. https://doi.org/10.1016/j.tet.2007.04.105.
[43] Yadav AN, Sachan SG, Verma P, et al. Prospecting cold deserts of north western Himalayas for microbial diversity and plant growth promoting attributes. J Biosci Bioeng 2015;119(6):683–93. https://doi.org/10.1016/j.jbiosc.2014.11.006 PMID: 25575970.