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DIKETOPIPERAZINE PRODUCED BY PSYCHROPHILIC YEAST Glaciozyma antarctica PI12

(Diketopiperazin Dihasilkan oleh Yis Psikropilik Glaciozyma antarctica PI12)

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Abstract

Four diketopiperazine derivatives; cyclo(-Pro-Val) (1), (-)-cyclo(-Pro-Tyr) (2), (-)-cyclo(-Pro-Phe) (3) and (+)-cyclo(-Pro-Leu) (4) were isolated from the ethyl acetate extract of *Glaciozyma antarctica* PI12, a cold-adapted yeast that belongs to family Kriegeriales. The compounds were isolated by radial chromatography and thin layer chromatography techniques and the chemical structures were elucidated by infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry.

Keywords: Glaciozyma antarctica PI12, diketopiperazine, Kriegeriales

Abstrak

Empat terbitan diketopiperazin; siklo(-Pro-Val) (1), (-)-siklo(-Pro-Tir) (2), (-)-siklo(-Pro-Phe) (3) and (+)-siklo(-Pro-Leu) (4) yang dipencilkan daripada ektrak etil asetat *Glaciozyma antarctica* PI12, merupakan yis adaptasi sejuk yang terdiri dari famili Kriegeriales. Sebatian – sebatian terpencil telah diasingkan menggunakan teknik kromatografi pepejal dan kromatografi lapisan nipis dan struktur kimia dijelaskan oleh spektroskopi infra merah (IM), ultra lembayung (UL), resonans magnetik nuklear (RMN) dan spektrometri jisim.

Kata kunci: Glaciozyma antarctica PI12, diketopiperazin, Kriegeriales

Introduction

Antarctica is a continent with extreme low temperature and nutrients, with high UV radiation, low water availabilities and frequent freeze-thaw cycle [1]. Therefore, the biology of Antarctica more than other continents, is dominated by microorganisms [2], with a high level of adaptation and able to withstand extreme condition [3]. However, temperature is one of major factors affecting the survival of microorganisms in Antarctica [4]. Cold adapted fungi that grow optimally at temperatures less than 15 °C and cannot grow above 20 °C are called "psychrophilic fungi or cold-adapted fungi" [5]. Since the 1960s, bioactive secondary metabolites have been isolated and structurally characterised from psychrophiles [6] such as bioactive non-ribosomal peptides (NRPs),

compounds derived from the NRP-synthetase that were developed into antibiotics i.e. bacitracin, polymyxin B and E [7].

Glaciozyma antarctica PI12 belongs to the family Kriegeriales [8] and was isolated from a marine environment in Antarctica [9]. Recently, this psychrophilic yeast was reclassified from *Leucosporidium antarcticum* to *Glaciozyma antarctica* PI12 [10] and has also been isolated from various locations in Antarctica [11, 12]. *Glaciozyma antarctica* PI12 was widely investigated in recent yearsin most antifreeze protein production and cold-active enzyme, including an extracellular serine proteinase [13]. The main objective of this paper is to describe the isolation and structure characterisation from psychrophilic yeast *G. antarctica* PI12.

Materials and Methods

Biological materials

The *G. antarctica* PI12 sample used was a gift from Prof. Dr. Nazalan Najimudin from Universiti Sains Malaysia in Penang, Malaysia. It was identified based on the biochemical characteristics and the sequence of the internal transcribed spacer (ITS) as well as the LSU rRNA (Accession numbers JX89896955 and JX896956) by the National Collection of Yeast Cultures, Norwich, UK. The strain was cultured on Tryptic Soy Agar for 10 days at 4 °C until a single colony was obtained [14]. The culturing of *G. antarctica* PI12 was conducted in collaboration with the Malaysia Genome Institute.

Cultivation of yeast

 $10~\mu L$ of the *G. antarctica* PI12's glycerol stock was sub-cultured on the Yeast Extract-Peptone-Dextrose (YPD) agar with $50~\mu g/mL$ of ampicillin and $50~\mu g/mL$ of kanamycin at $12~^{\circ}C$ until a single colony was obtained. Next, a starter culture of *G. antarctica* PI12 was prepared by inoculating a single colony of *G. antarctica* PI12 from the agar plate into 10~mL of YPD medium (50~mL falcon tube) with $50~\mu g/mL$ of ampicillin and $50~\mu g/mL$ of kanamycin. It was then cultured at $12~^{\circ}C$ at 180~rpm until mid-log phase (OD600: 1.0~to~1.8) (5~days). Thirdly, a fixed amount ($10^6~yeast~cells/mL$) of starter culture was inoculated into 50~mL of YPD medium (three 250~mL of conical flask were used) with two antibiotics, and then cultured at $12~^{\circ}C$ at 180~rpm until stationary phase at day 14~[15].

Extraction and isolation

The production culture was centrifuged (10,000 rpm, 4 °C, 5 min). The supernatant was filtered under vacuum to remove any cells that were not pelleted out and its pH was adjusted to 4.0. The supernatant was extracted thrice with ethyl acetate, and concentrated using a rotary evaporator. The ethyl acetate (EtOAc) extract (200.7 mg) of *G. antarctica* PI12 was subjected to radial chromatography (RC) with 1 mm thickness silica gel on a round glass plate and eluted with the mixtures of dichloromethane (DCM) and methanol (MeOH) with increasing polarity (started with DCM/MeOH, 9.6: 0.4). The eluates showing the same profile on thin layer chromatography (TLC) were combined to give three fractions (I-III). Purification of Fraction I (1-4) (29.7 mg) was carried out using RC with a silica gel plate of 0.5 mm thickness eluted with DCM and MeOH (9.2: 0.8) in 5% polarity increment to yield Compound 1 (1 mg). Purification of Fraction III (5-7) (48.8 mg) was conducted by utilising another RC with silica gel plate of 0.5 mm thickness. Elution with DCM and MeOH (8.4: 1.6) produced Compound 2 (1 mg), Compound 3 (1.5 mg), and Compound 4 (2 mg).

Results and Discussion

Characterization study

Cyclo(-Pro-Val) (1) is a white amorphous solid. ESI-MS [M + H]⁺ at m/z: 197. IR $\bar{\nu}_{max}$ (ATR) cm⁻¹: 3191, 2956, 1640, 1453, 1110, 922. ¹H NMR (MeOD, 700 MHz) δ_{H} (ppm) and ¹³C NMR (MeOD, 175 MHz) δ_{C} (ppm) data are tabulated in Table 1.

(-)-Cyclo(-Pro-Tyr) (2) is a white amorphous solid. $[\alpha]_D^{20}$ -43.1 (c 0.14, ethanol). ESI-MS $[M + H]^+$ ion at m/z: 261. IR $\bar{\nu}_{max}$ (ATR) cm⁻¹: 3248, 2927, 2853, 1747, 1658, 1449, 1252, 1174, 1114, 1017, 858. ¹H NMR (MeOD, 700 MHz) δ_H (ppm) and ¹³C NMR (MeOD, 175 MHz) δ_C (ppm) data are tabulated in Table 1.

(-)-Cyclo(-Pro-Phe) (3) is a white amorphous solid. $[\alpha]_D^{20}$ -72 (c 0.7, methanol). ESI-MS $[M+H]^+$ ion at m/z: 169. IR $\bar{\nu}_{max}$ (ATR) cm⁻¹: 3357, 2956, 2108, 1647, 1449, 1318 and 1087. ¹H NMR (MeOD, 700 MHz) δ_H (ppm) and ¹³C NMR (MeOD, 175 MHz) δ_C (ppm) data are tabulated in Table 1.

(+)-Cyclo(-Pro-Leu) (**4**) is a white powder. $[\alpha]_D^{20}$ +28.1 (*c* 0.032, ethanol). ESI-MS $[M+H]^+$ ion at m/z: 211. IR $\bar{\nu}_{max}$ (ATR) cm⁻¹: 3222, 2958, 2930, 2872, 1686, 1676, 1426, 1302, 1275, 1235, 1157, 1102, 1032, 996-919. ¹H NMR (MeOD, 700 MHz) δ_H (ppm) and ¹³C NMR (MeOD, 175 MHz) δ_C (ppm) data are tabulated in Table 1.

Table 1. ¹H and ¹³C NMR data (700 and 175 MHz, MeOD) for compounds **1-4**

Position	APT (ppm)				¹ H (mult., ΣH))			
	1	2	3	4	1	2	3	4
1-N	-	-	-	-	-	-		-
2	165.8	165.7	165.5	167.1	-	-	_	_
3	59.9	56.6		53.1	3.92 (d, 1H)	4.37 (m, 1H)	4.25 (t, 1H)	4.00 (t, 1H)
4-NH	-	-	-	-	7.93 (<i>br-s</i> , 1H)	7.65 (s, 1H)	7.62 (s, 1H)	7.99 (<i>br-s</i> , 1H)
5	170.9	169.6	169.3	171.1	-	-	_	-
6	58.7	58.7	58.6	58.9	4.11 (<i>t</i> , 1H)	4.05 (m, 1H)	4.14 (t, 1H)	4.18 (t, 1H)
7	28.3	28.0	28.0	27.9	1.82 (<i>m</i> , 1H)	2.33 (q, 1H)	2.29 (<i>m</i> , 1H)	2.12 (q, 1H)
					2.14 (<i>m</i> , 1H)	1.99(q, 1H)	1.92(q, 1H)	1.89(q, 1H)
8	22.4	21.4	21.3	22.9	1.80 (<i>m</i> , 1H)	2.01 (m,1H)	2.01 (<i>m</i> ,1H)	1.91 (m, 1H)
					1.86 (<i>m</i> , 1H)	1.93 (m, 1H)	1.93 (m, 1H)	1.80 (m, 1H)
9	45.1	44.6	44.5	45.3	3.33 (t, 1H)	3.63 (<i>m</i> , 1H)	3.68 (m, 1H)	3.36 (t, 1H)
					3.38 (t, 1H)	3.57 (m, 1H)	3.58 (m, 1H)	3.37 (t, 1H)
10	28.2	36.3	36.3	38.1	2.32 (m, 1H)	3.46 (<i>dd</i> , 1H)	3.47 (dd, 1H)	1.75 (t, 1H)
						2.79 (dd, 1H)	2.79 (dd, 1H)	1.36 (t, 1H)
11	18.7	-	-	24.5	0.99 (d, 3H)	-	-	1.85 (m, 1H)
12	16.7	-	-	23.2	0.83 (<i>d</i> , 3H)	-	-	0.86(d, 3H)
13	-	-	-	22.3	-	-	-	0.85(d, 3H)
1`	-	126.3	126.2	-	-	-	-	-
2`/6`	-	130.7	130.7	-	-	7.05 (dd, 2H)	7.02 (dd, 2H)	-
3`/5`	-	115.0	130.3	-	-	6.79 (dd, 2H)	6.73 (dd, 2H)	-
4`	-	156.1	128.2	-	-	5.35 (<i>br-s</i> ,1H), -OH	7.24 (d, 1H)	-

Compound 1 was isolated as white amorphous solid. Its molecular formula was determined as $C_{10}H_{16}N_2O_2$, obtained by ESI–MS [M + H]⁺ m/z 197. The IR spectrum showed the presence of carbonyl groups from amide (1640 cm⁻¹), and also saturated methylene groups at the range of 2956-2889 cm⁻¹. The ¹³C-APT NMR spectrum showed the location of ten signals representing two quaternary, three methines, three methylenes and two methyls carbons. The double bond equivalent (DBE) value of four indicated that this compound contained two double bonds and two cyclic aliphatic. The most deshielded carbons at δ_C 170.9 and 165.8 ppm were indicated as an asymmetrical carbonyl group. Three saturated non-equivalent methylenes signals at δ_C 28.3 (C-7), 22.4 (C-8) and 45.1 (C-9) were attributed to a pyrrolidine ring. A methine carbon at δ_C 58.7 ppm (C-6) was the specific of methine signal of diketopiperazine alkaloid and another one methine at δ_C 59.9 ppm (C-3) indicated the presence of a methine existed

between carbonyl and secondary amide while the two non-equivalent gem-dimethyls at δ_C 18.7 ppm (C-11) and δ_C 18.7 ppm (C-12) bound a saturated methine at δ_C 28.2 ppm (C-10).

Meanwhile, 12 proton signals representing 16 protons appeared in the 1 H-NMR spectrum as shown in Table 1. Six of the signals came from the three non-equivalent methylenes proton at H-7, H-8 and H-9, forming a basic pyrrolidine ring. An aminated methine proton, H-6 (δ_{H} 4.11 ppm) resonated as a triplet, indicating that C-6 was adjacent to a methylene group (C-7). Another methine proton at H-10 (δ_{H} 2.32 ppm) was resonated as a multiplet, because it was bound to two non-equivalent gem-dimethyls at C-11 and C-12. Comparison of the 1 H and 13 C-APT NMR spectra of the compound suggested that it was Cyclo(-Pro-Val) (Fig. 1) [16]. Thus, the structure of Cyclo(-Pro-Val) (1) was previously isolated from *Aspergillus fumigatus* [17], *Pseudomonas aeruginosa* [18], and *Stenotrophomonas* sp. [19].

Figure 1. Chemical structure of compound 1

Compounds 2 and 3 (Figure 2) were found in Fraction III as a UV absorbing spot, which stained to violet with anisaldehyde/sulphuric acid. The 1H NMR spectrum of Compound 2 showed two *ortho*-coupled signals at δ_H 7.05 (H-2', 6') and 6.79 (H-3', 5'), which pointed to an AA'-BB' system of a 1,4-disubstituted aromatic ring, as well as two signals at δ 4.37 and 4.05 for two methines attached to electron withdrawing substituents. The spectrum proton of Compound 2 showed also two doublet of doublets for an ABX system of a methylene group at δ_H 3.46 and 2.79 (CH₂-10), as well as three methylene multiplets (CH₂-7) (δ_H 2.33, 1.99) attached to a heteroatom and CH₂-8, 9 (δ_H 2.01, 1.93, 3.63, 3.57). The ESI mass spectra determined the molecular weight of Compound 2 as 261 Dalton by (+)-ESI mode. The structure was further confirmed as (-)-cyclo(-Pro-Tyr) (2) by comparison with an authentic sample and spectra from our collection [20]. (-)-cyclo(-Pro-Tyr) (2) was previously isolated from *Pseudomonas fluorescens* GcM5-1A [21], *Streptomyces* sp. H7372 and ML 1532 [22, 23], *Pseudomonas aeruginosa* [18] and *Lysobacter capsici* AZ78 [24]. The difference between Compound 2 and Compound 3 lay on C-4`, where Compound 2 had a hydroxy at C-4` (Figure 2).

Figure 2. Structure of compounds 2 and 3

From the spectroscopy data and comparison with the literature, compound **3** was confirmed as (-)-cyclo(-Pro-Phe) (**3**) [17] previously isolated from microbial sources, including *Streptomyces rochei* 87051-3 [16], *Streptomyces sudanensis*. A4.4 [25], *Tyridiomyces formicarum* [26], and *Alternaria alternata* [27].

(+)-Cyclo(-Pro-Leu) (4), $[\alpha]_D^{20}$ +28.1 (c 0.032, ethanol), was isolated as a white amorphous material with molecular formula $C_{11}H_{18}N_2O_2$, obtained by ESI–MS $[M+H]^+$ m/z 211. The IR spectrum showed bands attributed to the aliphatic carbons (2958, 2930, 2872 cm⁻¹) and amide (1686, 1676 cm⁻¹) functional groups. The ¹H NMR spectrum

of compound **4** indicated the presence of three methines at H-3, H-6 and H-11; four methylenes at H-7, H-8, H-9 and H-10; and two non-equivalent dimethyls at H-12 and H-13 (Table 1). 13 C NMR spectrum exhibited the presence of 11 carbon signals corresponding to two methyls, four methylenes, three methines and two quaternary carbons. The observation of the quaternary carbons at δ_C 167.1 (C-2) and 171.1 (C-5) suggested that these carbon signals were due to the carbonyl carbon of the amide groups. The DBE value of four indicated that this compound was containing two double bonds and two cyclic aliphatic. (+)-cyclo(Pro-Leu) (**4**) previously isolated from the Gramnegative Proteobacteria of *Burkholderia cenocepacia* and *Serratia marcescens* [28], *Alternaria alternata* [29], the bacteria *Pseudoalteromonas* sp. [30] and *Vibrio alginolyticus* [31].

Figure 3. Structure of compounds 4

Conclusion

Four diketopiperazine derivatives, i.e. cyclo(-Pro-Val) (Compound1), (-)-cyclo(-Pro-Tyr) (Compound 2), (-)-cyclo(-Pro-Phe) (Compound3) and (+)-cyclo(-Pro-Leu) (Compound4) were isolated and reported for the first time from psychrophilic yeast *G. antarctica* PI12. All the compounds reported in the present study were not subjected to further bioactivity studies due to insufficient amounts.

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References

- 1. Ruisi, S., Barreca, D., Selbmann, L., Zucconi, L. and Onofri, S. (2007). Fungi in Antarctica. *Reviews in Environmental Science and Biotechnology*, 6: 127 141.
- 2. Nienow, J. A. and Friedmann, E. I. (1993). Terrestrial litophytic (rock) communities. In: Friedmann, E. I. (ed). Antarctica microbiology. Wiley-Liss, New York, pp. 343 412.
- 3. Friedmann, E. I. (1993). Antarctic microbiology. Wiley-Liss, New York, pp. 634.
- 4. Morgan-Kiss, R. M., Priscu, J. C., Pocock, T., Gudynaite-Savitch, L. and Humer, N. P. (2006). Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology Reviews*, 70: 222 252.
- 5. Morgan, R. Y. (1975). Psychrophilic bacteria. *Bacteriological Reviews*, 39: 144 167.
- Giddings, L. A. and Newman, D. J. (2015). Bioactive compounds from terrestrial extremophiles. Springer, New York, pp. 1.
- 7. Sewald, N. and Jakubke, H. D. (2002). Peptides: Chemistry and biology. NJ: Wiley, Berlin, pp. 119 –124.
- 8. Di Menna, M. E. (1960). Yeast from Antarctica. *Journal of General Microbiology*, 7: 295 300.
- 9. Fell, J. W., Statzell, A., Hunter, I. L. and Phaff, H. J. (1969). *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida*. *Antonie van Leeuwenhoek*, 35: 433 462.
- 10. Turchetti, B., Thomas-Hall, S. R., Connell, L. B., Branda. E., Buzzini, P., Theelen, B., Müller, W. H. and Boekhout, T. (2011). Psychrophilic yeasts from Antarctica and European glaciers: description of *Glaciozyma* gen. nov., *Glaciozyma martinii* sp. nov. and *Glaciozyma watsonii* sp. nov. *Extremophiles*, 15: 573 586.

- 11. Donachie, S. P. (1995). Ecophysiological description of marine bacteria from Admiralty Bay (Antarctica), and the digestive tracts of selected *Euphausiidae*. Ph.D. Thesis, Department of Antarctic Biology, Polish Academy of Sciences, Warsaw.
- 12. Connell, L. B., Redman, R., Craig, S., Scorzetti, G., Iszard, M. and Rodriguez, R. (2008). Diversity of soil yeasts isolated from South Victoria Land, Antarctica. *Microbial Ecology*, 56: 448 459.
- 13. Pazgier, M., Turkiewicz, M., Kalinowska, H. and Bielecki, S. 2003. The unique cold-adapted extracellular subtilase from psychrophilic yeast *Leucosporidium antarcticum*. *Journal of Molecular Catalysis B: Enzymatic*, 21: 39 42.
- 14. Hashim, N. H. F., Sulaiman, S., Bakar, F. D. A., Illias, R. M., Kawahara, H., Najimudin, N., Mahadi, N. M. and Murad, A. M. A. (2014). Molecular cloning, expression and characterisation of Afp4, an antifreeze protein from *Glaciozyma antarctica*. *Polar Biology*, 37: 1495 1505.
- 15. Hashim, N. H. F., Bharudin, I., Nguong, D. L. S., Higa, S., Bakar, F. D. A., Nathan, S., Rabu, A., Kawahara, H., Illias. R. M., Najimudin, N., Mahadi, N. M. and Murad, A. M. A. (2013). Characterization of Afp1, an antifreeze protein from the psychrophilic yeast *Glaciozyma antarctica* PI12. *Extremophiles*, 17: 63 73.
- 16. Lee, H. B., Choi, Y. C. and Kim, S. U. (1994). Isolation and identification of Maculosins from *Streptomyces rochei* 87051-3. *Agricultural Chemistry and Biotechnology*, 37(5): 339 342.
- 17. Furtado, N. A. J. C., Pupo, M. T., Carvalho, I., Campo, V. L., Duarte, M. C. T., and Bastos, J. K. (2005). Diketopiperazines produced by an *Aspergillus fumigatus* Brazilian strain. *Journal of the Brazilian Chemical Society*, 16(6B): 1448 1453.
- 18. Vasquez-Rivera, D., González, O., Guzmán-Rodríguez, J., Diaz-Pérez, A. L., Ochoa-Zarzosa, A., Lopez-Bucio, J., Meza-Carmen, V. and Campos-García, G. (2015). Cytotoxicity of cyclodipeptides from *Pseudomonas aeruginosa* PAO1 leads to apoptosis in human cancer cell lines. *BioMed Research International*, 2015: 1 9.
- 19. Jermnak, U., Chinaphuti, A., Poapolathep, A., Kawai, R., Nagasawa, H. and Sakuda, S. (2013). Prevention of aflatoxin contamination by a soil bacterium of *Stenotrophomonas* sp. that produces aflatoxin production inhibitors. *Microbiology*, 159: 902 912.
- 20. Laatsch, H. (2010). AntiBase a data base for rapid dereplication and structure determination of microbial natural products. Wiley-VCH. Germany, Wein-heim.
- 21. Guo, Q., Guo, D., Zhao, B., Xu, J. and Li, R. (2007). Two cyclic dipeptides from *Pseudomonas fluorescens* GcM5-1A carried by the pine wood nematode and their toxicities to Japanese black pine suspension cells and seedlings in vitro. *Journal of Nematology*, 39(3): 243 247.
- 22. Cheenpracha, S., Borris, R. P., Tran, T. T., Jee, J. M., Seow, H. F., Cheah, H. Y., Ho, C. C. and Chang, L. C. (2011). Three new amides from *Streptomyces* sp. H7372. *Journal of the Brazilian Chemical Society*, 22(2): 223 229.
- 23. Munekata, M. and Tamura, G. (1981). Selective inhibition of SV40-transformed cell growth by diketopiperazines. *Agricultural and Biological Chemistry*, 45(11): 2613 2618.
- 24. Puopolo, G., Cimmino, A., Palmieri, M. C., Giovannini, O., Evidente, A. and I. Pertot, I. (2014). *Lysobacter capsici* AZ78 produces cyclo(L-Pro-L-Tyr), a 2,5-diketopiperazine with toxic activity against sporangia of *Phytophthora infestans* and *Plasmopara viticola*. *Journal of Applied Microbiology*, 117: 1168-1180.
- 25. Hamza, A. A., Ali, H. A., Clark, B. R., Murphy, C. D. and Elobied, E. A. (2013). Cyclo (L-phenyl, L-prolyl). Diketopiperazines from a newly isolated *Streptomyces sudanensis*. A4.4. *Journal of Biotechnology and Pharmaceutical Research*, 4(1): 1 7.
- 26. Wang, Y., Mueller, U. G. and Clardy, J. (1999). Antifungal diketopiperazines from symbiotic fungus of fungus-growing ant *Cyphomyrmex minitus*. *Journal of Chemical Ecology*, 25(4): 935 941.
- 27. Stierle, A. C., Cardellina, J. H. and Strobelt, G. A. (1988). Maculosin, a host-specific phytotoxin for spotted knapweed from *Alternatia Alternata*. *Proceedings of the National Academy of Sciences of the United States of America*, 85: 8008 8011.
- 28. Yap, A. C., Kok-Gan Chan, K. G. and Choo, Y. M. (2016). Isolation and identification of metabolites from the Gram-negative proteobacteria of *Burkholderia cenocepacia* and *Serratia marcescens*. *Sains Malaysiana*, 45(7): 1073 1077.
- 29. Musetti, R., Polizzotto, R., Vecchione, A., Borselli, S., Zulini, L., D'Ambrosio, M., di Toppi, L. S. and Pertot, I. (2007). Antifungal activity of diketopiperazines extracted from *Alternaria alternata* against *Plasmopara viticola*: An ultrastructural study. *Micron*, 38: 643 650.

- 30. Luis, S. M., Ballesteros, J. and Gutiérrez, M. (2011). Antibacterial constituents from the octocoral associated bacterium *Pseudoalteromonas* sp. *Revista Latinoamericana de Química*, 39: 1 2.
- 31. Wang, X., Huang, Y., Sheng, Y., Su, P., Qiu, Y., Ke, C. and Feng, D. (2017). Antifouling activity towards mussel by small-molecule compounds from a strain of *Vibrio alginolyticus* bacterium associated with Sea Anemone *Haliplanella* sp. *Journal of Microbiology and Biotechnology*, 27(3): 460–470.