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SHORT COMMUNICATION

A new antibacterial amino phenyl pyrrolidone derivative from a novel marine gliding bacterium *Rapidithrix thailandica*

Yutthapong Sangnoi · Anuchit Plubrukarn · Vullapa Arunpairojana · Akkharawit Kanjana-Opas

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Abstract A recently described marine gliding bacterium *Rapidithrix thailandica* strain TISTR 1741 was isolated from biofilm specimen collected from the Andaman Sea in Thailand. Four liters fermentation broth of *R. thailandica* TISTR 1741 cultivated in VY/2 medium were extracted with methanol to yield a novel amino phenyl pyrrolidone derivative compound (**1**) with antibacterial activities. The chemical structure and physico-chemical properties of **1** were investigated by spectrometry techniques. Compound **1** exhibited selective inhibition against vancomycin-resistant *Enterococcus faecalis* (VRE) with the MIC of 5.97 mM.

Keywords Marine gliding bacterium · *Rapidithrix thailandica* · Amino phenyl pyrrolidone derivative · Antibacterial activities · Vancomycin-resistant *Enterococcus faecalis* (VRE)

Y. Sangnoi
Department of Aquatic Science, Faculty of Natural Resources, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
e-mail: yutthapong.s@psu.ac.th

A. Plubrukarn
Marine Natural Products Research Unit, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

V. Arunpairojana
Thailand Institute of Scientific and Technological Research (TISTR), 35 Moo 3, Technopolis, Khlong 5, Khlong Luang, Pathum Thani 12120, Thailand

A. Kanjana-Opas (✉)
Department of Industrial Biotechnology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
e-mail: akkharawit.k@psu.ac.th

Introduction

Gliding bacteria are the Gram-negative, motile bacteria which use gliding motility instead of flagella. The most studied group of the gliding bacteria are terrestrial Myxobacteria which yielded more than 500 novel chemical structures exhibiting highly interesting biological activities such as antimicrobial and cytotoxic activities. Regardless of the high biological and chemical diversities, only a few reports of gliding bacteria isolated from marine environment have been known so far. However, the number of reports on newly isolated taxa of marine gliding bacteria has been increasing during the past few years i.e. *Olleya*, *Aureispira*, *Mariniflexile*, *Fulvivirga*, *Rapidithrix*, *Perexilibacter*, *Limibacter*, *Cellulophaga*, *Winogradskyella*, *Ekhidna*, *Salinimicrobium*, *Corallibacter*, *Tenacibaculum*, *Sunxiuqinia*, *Catalinimonas* and *Muricauda* (Nichols et al. 2005; Hosoya et al. 2006; Nedashkovskaya et al. 2006, 2007, 2009, 2010; Srisukchayakul et al. 2007; Yoon et al. 2007, 2008; Kahng et al. 2009; Alain et al. 2010; Kim et al. 2012; Piñeiro-Vidal et al. 2012; Takai et al. 2012; Choi et al. 2013; Kim et al. 2013).

Interestingly, the study of secondary metabolites produced by marine gliding bacteria is still limited. Fudou et al. (2001) firstly reported the isolation of haliangicin from *Haliangium luteum* which showed antifungal activity against *Pythium ultimum*. Spyere et al. (2003) isolated the neoverrucosane diterpenoids from *Saprospira grandis* when cultivated in RL 1 medium in seawater but no biological activity of these compounds were reported. Iizuka et al. (2006) reported the isolation of miuraenamides from a

novel *Paraliomyxa miuraensis* with antifungal activity against *Phytophthora capsici*. Our group recently reported the novel metabolites from *Rapidithrix thailandica* including marinoquinoline A (Kanjana-opas et al. 2006) and other pyrrole derivatives (Sangnoi et al. 2008). It is worth mentioning that marinoquinoline A is the first report of natural product obtained from the marine gliding bacterium *R. thailandica*. The compound exhibited a strong inhibiting effect against acetylcholinesterase (AChE) with an IC_{50} of 4.90 ± 0.9 μM without cytotoxicity against the human cancer cells i.e. MCF-7, HeLa, HT-29 and KB (Sangnoi et al. 2008). Recently, Oku et al. (2008) isolated polyketide-peptide metabolites, ariakemicins, from an un-identified species of *Rapidithrix* which showed antibacterial activity against *Staphylococcus aureus*. However, the compounds were not stable to maintain their antibacterial activities.

In this study, we report the isolation of additionally novel metabolite of *R. thailandica* (TISTR 1741) by using an antibacterial bioassay guided fractionation and spectroscopic methods.

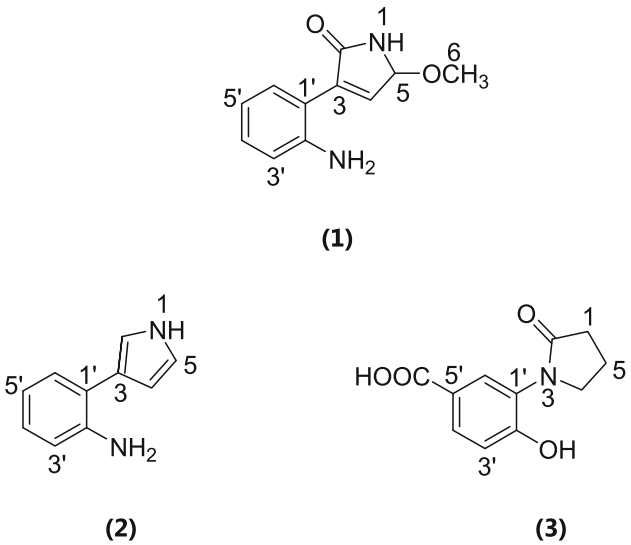

Fig. 1 Structures of new amino phenyl pyrrolidone derivative [3-(2-amino-phenyl)-5-methoxy-1,5-dihydro-pyrrol-2-one] (**1**), 3-(2′-aminophenyl)-pyrrole (**2**) and pistaciamide (**3**)

Materials and methods

Microorganisms

Rapidithrix thailandica strain TISTR 1741 (AB265183) was isolated from the biofilm on a shell sample collected from the Yong Ling beach, Trang province, Thailand. The biofilm sample was rinsed with sterile seawater and inoculated on to a modified seawater glutamate agar medium (SWG) [L-glutamic acid monosodium salt, 1.0 g; NH_4NO_3, 0.01 g; K_2HPO_4, 0.01 g; agar 15 g and seawater, 1/L] (Hosoya et al. 2006) and the pure culture was obtained by a subculturing technique followed by a single cell isolation with a micromanipulator. The isolate was maintained on modified SAP2 agar medium [tryptone, 1 g; yeast extract, 1 g; agar, 15 g; sea water, 1/L] (Reichenbach 1991) at 25 °C. The identification of the isolate was completed by 16S rRNA gene sequence and chemotaxonomic analyses described in Srisukchayakul et al. (2007).

Fermentation of TISTR 1741 isolate

The TISTR 1741 isolate was cultivated into 40 × 250-ml Erlenmeyer flasks each containing 100 ml of modified VY/2 medium [Baker's yeast paste, 5 g; seawater, 1/L] and 2 g of amberlite XAD-16 resins (Spyere et al. 2003) at 25 °C, 200 rpm for 7 days. The resins were separated by a filtration and rinsed twice with deionized water in order to remove salt and remaining culture medium. The resins were combined and left to dry at room temperature for 10 min before soaked twice with 2/l of methanol for 6 h at room temperature. Crude extract was obtained after filtering the resins and evaporating of the solvent phase with a rotary evaporator.

Isolation of amino phenyl pyrrolidone derivative (**1**)

The crude extract obtained from 4-L fermentation (0.9 g) was subjected to a size exclusion chromatography using Sephadex LH-20 as a stationary phase and 100 % methanol as a mobile phase yielded seven fractions. After the antibacterial assay, an active fraction (fraction 3; 61.5 mg) was further separated and purified by a reversed phase C-18 HPLC (Phenomenex, 250 × 10 mm, 10 μm, 30:70 MeOH in H_2O, 4.5 ml/min, 210 nm) to yield compound **1** (Fig. 1) (1.2 mg, $t_R = 15$ min).

Physicochemical properties

UV spectra were recorded on a Hewlett Peckard® 8452A diode array spectrophotometer (France). IR spectra were recorded on a Jasco® IR-810 infrared spectrophotometer (Japan). Mass spectra, both low and high resolutions, were operated on an MAT 95 XL mass spectrometer (Germany). NMR spectra were recorded on an FT-NMR, Varian Unity® Inova 500 spectrometer (Germany) at 500 MHz for ^1H. The NMR chemical shifts for ^1H and ^{13}C, respectively, were referenced to the solvent peaks of δ_H 7.24 and δ_C 77.0 ppm for $CDCl_3$ solution.

Antimicrobial activities

Compound **1** was tested for antimicrobial activities against nine microorganisms including methicillin-resistant *S.*

aureus (MRSA) ATCC 43300, vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299, *S. aureus* TISTR 517, *E. faecalis* TISTR 459, *Bacillus subtilis* ATCC 6633, *Salmonella typhi* (clinical strain), *Salmonella sonei*, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231. The antimicrobial assay was performed by a colorimetric microdilution broth technique using RPMI1640 medium and AlamarBlue as an indicator as described elsewhere (Boonsri et al. 2006). In brief, the tested microorganisms were prepared as suspension in RPMI1640 medium and mixed with AlamarBlue indicator at the final concentration of 1 %. The cell suspension was then transferred into a 96-well microtiter plate (100 µl/well except for first row which contained 190 µl/well). A 10 µl of the tested compound dissolved in DMSO at a final concentration of 50 µg/ml was added to each well of the first row and mixed well with a micropipette. Half of the mixtures in the first row were then transferred to the next well in the second row to perform a half-fold dilution. The dilution process was repeated until the extracts were diluted 128 times in the last row. The excess 100 µl of the mixture in the last row was discarded. The microtiter plates were incubated at 37 °C for 8–12 h and 24 h for antibacterial and antifungal assays, respectively. The antimicrobial activity was determined as the minimal inhibitory concentration (MIC) value which was the least concentration of the extract that could inhibit the growth of tested microorganisms.

Results

Isolation of amino phenyl pyrrolidone derivative (1) and its physicochemical properties

The methanolic-extract (0.9 g) of *R. thailandica* TISTR 1741 yielded 1.2 mg of **1** after consecutive chromatography on Sephadex LH-20 and preparative RP C-18 HPLC, respectively. The molecular formula of **1** was proposed as $C_{11}H_{12}O_2N_2$, according to the pseudomolecular signal at *m/z* 205.1 $[M + H]^+$ which was confirmed by the pseudomolecular signal at *m/z* 205.0970 $[M + H]^+$ (calc for $C_{11}H_{12}O_2N_2$ 205.0977) in ESIMS spectrum. The other properties are summarized in Table 1. The ^{13}C NMR spectrum exhibited the signals of four sp^2 methines (δ 117.1, C-3′; 130.5, C-4′; 118.7, C-5′ and 130.6, C-6′) and two quarternary carbons (δ 116.9, C-1′; and 145.4, C-2′). Among these, six belonged to a phenyl moiety. From the ^1H NMR spectrum (500 MHz, $CDCl_3$) (Table 2), series of resonances assigned to an *o*-disubstituted phenyl moiety were observed at δ 6.73 (d; $J = 8.0$ Hz; H-3′), 7.18 (dd; $J = 7.0$, 8.0 Hz; H-4′), 6.78 (dd; $J = 7.0$, 7.5 Hz; H-5′) and 7.28 (m; H-6′). The evidence of primary amino group was confirmed from the IR absorption band at ν_{max} 3,300 cm^{-1} and from the exchangeable proton resonance at δ 4.36 (br s). A primary amino group was proposed to attach to C-2′ (δ 145.4) of a phenyl ring moiety due to the low-fielded chemical shift. Another moiety, ^{13}C NMR spectrum demonstrated the signal at δ 171.7 (C-2) indicated the amide carbonyl group. This was clearly confirmed from the IR absorption band at ν_{max} 1,700 cm^{-1} and the broad signal at δ 6.22 (br s) indicating the presence of carbonyl and an exchangeable proton, respectively. The remaining resonances of one sp^2 methine (δ 6.96 s; H-4), one oxygenated methine (δ 5.59 s; H-5) and an O-methoxyl group (δ 3.35 s; H-6) were put together and established the presence of pyrrolidone moiety which clearly confirmed by HMBC correlations from H-4 (δ 6.96) to C-2 (δ 171.7) and C-5 (δ 84.0), from H-5 (δ 5.59) to C-4 (δ 140.9) and C-6 (δ 52.3) and from H-6 (δ 3.35) to C-5 (δ 84.0). Connection of phenyl and pyrrolidone moieties was observed from the correlation between H-6′ (δ 7.28) and C-3 (δ 140.5). Compound **1** was therefore proposed as a new amino phenyl pyrrolidone derivative [3-(2-Amino-phenyl)-5-methoxy-1,5-dihydro-pyrrol-2-one].

Table 1 Physico-chemical properties of 3-(2-amino-phenyl)-5-methoxy-1,5-dihydro-pyrrol-2-one

Appearance	Brownish orange glass
Molecular formula	$C_{11}H_{12}O_2N_2$
EI-MS *m/z* (rel. int)	205 (89), 173 (14) $[M + H]^+$
HR-EIMS found	205.0970 $[M + H]^+$
calcd	205.0977 ($C_{11}H_{12}O_2N_2$)
$[\alpha]_D^{25}$	+11.4 (*c* 0.2; MeOH)
UV (MeOH) λ_{max} nm (log ε)	208 (3.23), 230 (3.10)
IR (thin film) ν_{max} cm^{-1}	3,300, 2,925, 1,700, 1,620, 1,450

Table 2 ^1H and ^{13}C NMR spectral data of 3-(2-Amino-phenyl)-5-methoxy-1,5-dihydro-pyrrol-2-one (500 MHz for ^1H; $CDCl_3$)

Position	δ_H (mult.; *J* in Hz)	δ_C (mult.)	HMBC correlation
1	6.22 (br s)	–	–
2	–	171.7 (C)	H-4
3	–	140.5 (C)	H-6′
4	6.96 (s)	140.9 (CH)	H-5
5	5.59 (s)	84.0 (CH)	H-4, H-6
6	3.35 (s)	52.3 (CH_3)	H-5
1′	–	116.9 (C)	H-3′
2′	–	145.4	H-4′, H-6′
3′	6.73 (d; 8.0)	117.1 (CH)	H-5′
4′	7.18 (dd; 7.0, 8.0)	130.5 (CH)	H-6′
5′	6.78 (dd; 7.0, 7.5)	118.7 (CH)	H-3′
6′	7.28 (m)	130.6 (CH)	H-4′, H-5′
2′-NH_2	4.36 (br s; 2H)	–	–

Table 3 Antimicrobial spectrum of the 3-(2-amino-phenyl)-5-methoxy-1,5-dihydro-pyrrol-2-one

Tested microorganisms	MIC (mM)
Gram-positive	
Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300	>24.37
Staphylococcus aureus	12.19
Bacillus subtilis ATCC 6633	>24.37
Vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299	5.97
Enterococcus faecalis TISTR459	>50
Gram-negative	
Pseudomonas aeruginosa ATCC 27853	24.37
Salmonella sonei	>24.37
Salmonella typhi	>24.37
Yeast	
Candida albicans ATCC10231	>24.37

Biological activities

Antimicrobial assay of **1** against a panel of microbial strains including five Gram-positive bacteria (MRSA, VRE, *B. subtilis*, *E. faecalis* and *S. aureus*), three Gram-negative bacteria (*P. aeruginosa*, *S. sonei* and *S. typhi*) and yeast (*C. albicans*) showed selective inhibitory activity against VRE (MIC 5.97 mM) followed by *S. aureus* (MIC 12.19 mM) as shown in Table 3. However, compound **1** showed no an inhibitory activity against neither Gram-negative bacteria nor yeast used in this study.

Discussion

Compound **1** isolating from the marine gliding bacterium *R. thailandica* TISTR 1741 showed a similar structure to 3-(2′-aminophenyl)-pyrrole (**2**) which was isolated from another strain of *R. thailandica* as previously reported by our group (Sangnoi et al. 2008). In addition, compound **1** exhibited a relative structure with pistaciamide (**3**) which was isolated from plant, *Pistacia chinensis* (Lui et al. 2008). Sangnoi et al. (2008) reported the acetylcholinesterase (AChE), which had regulated the brain's cognitive functions, inhibitory activity and cytotoxicity of **2**. It was inactive in the AChE inhibition and in the cytotoxicity bioassays (enzyme inhibition <30 % at 0.1 g/l, > 80 % cell viability at 20 g/l, respectively). Compound **2** showed no antimicrobial activities (data not shown) whereas no biological activities of **3** were reported. Unfortunately, we could not determine the AChE inhibition and the cytotoxic activity of **1** due to the scantiness of the compound available. The AChE inhibitory activities of **1** and **3** may be similar to that of **2** because of the structural similarity. However, the pyrrolidone derivatives belong to a racetam family which is known to have important value in pharmaceutical industry (Lui et al. 2008). A limited number of racetam (pyrrolidone derivatives) have been developed for clinical use as nootropic drugs that mean enhancement of learning and memory (Shorvon 2001). Most drugs in the pyrrolidone (racetam) class stimulate acetylcholine production and turnover. The examples of drugs in this class that go to a clinical development are such as aniracetam, fasoracetam and nefiracetam were used as anti-Alzheimer's disease agents (Fujita et al. 2002; Shorvon 2001). Piracetam, oxiracetam, nebracetam and pramiracetam were used for cognition enhancer whereas brivaracetam, seletracetam and levetiracetam were used for antiepilepsy (Kenda et al. 2004; Matagne et al. 2009; Shorvon 2001). The further investigation on biological activities like racetam family of **1** should also be considered.

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References

Alain K, Tindall BJ, Catala P, Intertaglia L, Lebaron P (2010) *Ekhidna lutea* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from the South East Pacific Ocean. Int J Syst Evol Microbiol 60:2972–2978. doi:10.1099/ijs.0.018804-0

Boonsri S, Karalai C, Ponglimanont C, Kanjana-opas A, Chantrapromma K (2006) Antibacterial and cytotoxic xanthones from the roots of *Cratoxylum formosum*. Phytochemistry 67:723–727. doi:10.1016/j.phytochem.2006.01.007

Choi EJ, Beatty DS, Paul LA, Fenical W, Jensen PR (2013) *Mooreia alkaloidigena* gen. nov., sp. nov. and *Catalinimonas alkaloidigena* gen. nov., sp. nov., alkaloid-producing marine bacteria in the proposed families *Mooreiaceae* fam. nov. and *Catalimonadaceae* fam. nov. in the phylum *Bacteroidetes*. Int J Syst Evol Microbiol 63:1219–1228. doi:10.1099/ijs.0.043752-0

Fudou R, Iizuka T, Yamanaka S (2001) Haliangicin, a novel antifungal metabolite produced by a marine myxobacterium: 1. Fermentation and biological characteristics. J Antibiot 54:149–152

Fujita R, Takayama N, Ueda H (2002) The cognition-enhancer nefiracetam is protective in BDNF-independent neuronal cell death under the serum-free condition. Neurochem Int 40:139–143. doi:10.1016/S0197-0186(01)00080-8

Hosoya S, Arunpairojana V, Suwannachart C, Kanjana-Opas A, Yokota A (2006) *Aureispira marina* gen. nov., sp. nov., a gliding, arachidonic acid-containing bacterium isolated from the southern coastline of Thailand. Int J Syst Evol Microbiol 56:2931–2935. doi:10.1099/ijs.0.64504-0

Iizuka T, Fudou R, Jojima Y, Ogawa S, Yamanaka S, Inukai Y, Ojika M (2006) Miuraenamides A and B, novel antimicrobial cyclic depsipeptides from a new slightly halophilic myxobacterium: taxonomy, production, and biological properties. J Antibiot 59:385–391. doi:10.1038/ja.2006.55

Kahng H, Chung BS, Lee D, Jung JS, Park JH, Jeon CO (2009) *Cellulophaga tyrosinoxydans* sp. nov., a tyrosinase-producing

bacterium isolated from seawater. Int J Syst Evol Microbiol 59:654–657. doi:10.1099/ijs.0.003210-0

Kanjana-opas A, Panphon S, Fun H, Chantrapromma S (2006) 4-Methyl-3H-pyrrolo[2,3-c]quinoline. Acta Cryst E62:2728–2730. doi:10.1107/S1600536806021088

Kenda BM, Matagne AC, Talaga PE, Pasau PM, Differding E, Lallemand BI, Frycia AM, Moureau FG, Klitgaard HV, Gillard MR, Fuks B, Michel P (2004) Discovery of 4-substituted pyrrolidone butanamides as new agents with significant antiepileptic activity. J Med Chem 47:530–549. doi:10.1021/jm030913e

Kim SB, Nedashkovskaya OI, Zhukova NV, Kuchlevskiy AD, Mikhailov VV (2012) *Corallibacter vietnamensis* gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae*. Int J Syst Evol Microbiol 62:569–574. doi:10.1099/ijs.0.028928-0

Kim JM, Jin HM, Jeon CO (2013) *Muricauda taeanensis* sp. nov., isolated from a marine tidal flat. Int J Syst Evol Microbiol 63:2672–2677. doi:10.1099/ijs.0.047647-0

Lui JJ, Geng CA, Lui XK (2008) A new pyrrolidone derivative from *Pistacia chinensis*. Chinese Chem Lett 19:65–67. doi:10.1016/j.cclet.2007.10.037

Matagne A, Margineanu D, Potschka H, Loscher W, Michel P, Kenda B, Klitgaard H (2009) Profile of the new pyrrolidone derivative seletracetam (ucb 44212) in animal models of epilepsy. Eur J Pharmacol 614:3–37. doi:10.1016/j.ejphar.2009.04.024

Nedashkovskaya OI, Kim SB, Kwak J, Mikhailov VV, Bae KS (2006) *Mariniflexile gromovii* gen. nov., sp. nov., a gliding bacterium isolated from the sea urchin *Strongylocentrotus intermedius*. Int J Syst Evol Microbiol 56:1635–1638. doi:10.1099/ijs.0.64293-0

Nedashkovskaya OI, Kim SB, Shin DS, Beleneva IA, Mikhailov VV (2007) *Fulvivirga kasyanovii* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from seawater in a mussel farm. Int J Syst Evol Microbiol 57:1046–1049. doi:10.1099/ijs.0.64641-0

Nedashkovskaya OI, Vancanneyt M, Kim SB, Zhukova NV (2009) *Winogradskyella echinorum* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from the seaurchin *Strongylocentrotus intermedius*. Int J Syst Evol Microbiol 59:1465–1468. doi:10.1099/ijs.0.005421-0

Nedashkovskaya OI, Vancanneyt M, Kim SB, Han J, Zhukova NV, Shevchenko LS (2010) *Salinimicrobium marinum* sp. nov., a halophilic bacterium of the family *Flavobacteriaceae*, and emended descriptions of the genus *Salinimicrobium* and *Salinimicrobium catena*. Int J Syst Evol Microbiol 60:2303–2306. doi:10.1099/ijs.0.019166-0

Nichols CM, Bowman JP, Guezennec J (2005) *Olleya marilimosa* gen. nov., sp. nov., an exopolysaccharide-producing marine bacterium from the family *Flavobacteriaceae*, isolated from the Southern Ocean. Int J Syst Evol Microbiol 55:1557–1561. doi:10.1099/ijs.0.63642-0

Oku N, Adachi K, Matsuda S, Kasai H, Takatsuki A, Shizuri Y (2008) Ariakemicins A and B, novel polyketide-peptide antibiotics from a marine gliding bacterium of the genus *Rapidithrix*. Org Lett 10:241–248. doi:10.1021/ol8007292

Piñeiro-Vidal M, Gijón D, Zarza C, Santos Y (2012) *Tenacibaculum dicentrarchi* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from European sea bass. Int J Syst Evol Microbiol 62:425–429. doi:10.1099/ijs.0.025122-0

Reichenbach H (1991) The Order cytophagales. In: Starr MP, Stolp H, Triiper HG, Balows A, Schlegel HG (eds) The prokaryotes, 2nd edn. Springer, Berlin, pp 3631–3675

Sangnoi Y, Sakulkeo O, Yuenyongsawad S, Kanjana-opas A, Ingkaninan K, Plubrukarn A, Suwanborirux K (2008) Acetylcholinesterase-inhibiting activity of pyrrole derivatives from a novel marine gliding bacterium, *Rapidithrix thailandica*. Mar Drugs 6:578–586. doi:10.3390/md20080029

Shorvon S (2001) Pyrrolidone derivatives. Lancet 358:1885–1892. doi:10.1016/S0140-6736(01)06890-8

Spyere A, Rowley DC, Jensen PR, Fenical W (2003) New neoverrucosane diterpenoids produced by the marine gliding bacterium *Saprospira grandis*. J Nat Prod 66:818–822. doi:10.1021/np0205351

Srisukchayakul P, Suwannachart C, Sangnoi Y, Kanjana-Opas A, Hosoya S, Yokota A, Arunpairojana V (2007) *Rapiditrix thailandica* gen. nov., sp. nov., a marine gliding bacteria isolated from Andaman sea, the southern coastline of Thailand. Int J Syst Evol Microbiol 57:2275–2279. doi:10.1099/ijs.0.65087-0

Takai K, Abe M, Miyazaki M, Koide O, Nunoura T, Imachi H, Inagaki F, Kobayashi T (2012) *Sunxiuqinia faeciviva* sp. nov., a facultatively anaerobic organoheterotroph of the *Bacteroidetes* isolated from deep subseafloor sediment. Int J Syst Evol Microbiol 63:1602–1609. doi:10.1099/ijs.0.044065-0

Yoon J, Ishikawa S, Kasai H, Yokota A (2007) *Perexilibacter aurantiacus* gen. nov., sp. nov., a novel member of the family '*Flammeovirgaceae*' isolated from sediment. Int J Syst Evol Microbiol 57:964–968. doi:10.1099/ijs.0.64845-0

Yoon J, Matsuo Y, Kasai H, Yokota A (2008) *Limibacter armeniacum* gen. nov., sp. nov., a novel representative of the family '*Flammeovirgaceae*' isolated from marine sediment. Int J Syst Evol Microbiol 58:982–986. doi:10.1099/ijs.0.65595-0