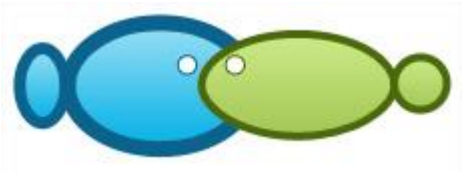

Bioactive compounds from a soft coral-derived *Bacillus* sp.

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Abstract. Marine bacteria are an interesting issue for searching of natural bioactive compounds when rising up of drug resistant bacteria and incurable diseases. This is the first report on pure bioactive compounds from a *Bacillus* sp. strain C-1-10 isolated from the soft coral *Alcyonium digitatum* in the Baltic Sea. The strain C-1-10 showed capable of yielding at least 7 natural compounds on both Glucose Yeast Malt medium and Bannett medium. The pure compounds were isolated with semi-preparative high-performance liquid chromatography (HPLC). Four purified natural substances are tested for antimicrobial activities, antifungal, cytotoxic and enzyme inhibition. All four compounds showed inhibition of *Staphylococcus epidermidis* and *Staphylococcus aureus* MRSA. The compound 5 showed rather weak inhibition of Gram-negative *Escherichia coli* and was inactive to phosphodiesterase 4, while the other compounds were inactive to *E. coli* and active against phosphodiesterase 4. However, all four pure compounds were inactive to other Gram-negative bacteria and fungi and exhibited no cytotoxic activity, and no inhibition to glycogen synthase kinase, and protein-tyrosine-phosphatase. A soft coral derived *Bacillus* sp. strain C-1-10 showed a promised strain for bioactive compound producing.
Keywords: antibacterial activity, *Bacillus* sp., bioactive compounds, natural products, secondary metabolites, soft coral.

Introduction. Sustainable development goal 14 (SDG 14: Life Below Water) emphasizes the conservation and sustainable use of oceans, seas, and marine resources in support of sustainable development (United Nations, 2015). Among its key targets is the sustainable exploitation of marine biological resources, including the promotion of innovation in the discovery and utilization of marine-derived bioactive compounds that contribute to human health, environmental protection, and economic growth. Marine ecosystems harbor extraordinary biological and chemical diversity, encompassing microorganisms, macroalgae, and invertebrates that have evolved specialized biochemical pathways to survive under extreme physicochemical conditions and intense ecological competition (Leal & Calado, 2014; Blunt et al., 2018). These evolutionary pressures frequently drive the biosynthesis of structurally novel secondary metabolites with potent biological activities, many of which have served as leads or templates for pharmaceuticals, nutraceuticals, and industrial products (Newman & Cragg, 2016; Carroll et al., 2021). Consequently, marine bioprospecting has emerged as a strategic research direction aligned with SDG 14, linking biodiversity conservation with sustainable innovation and blue economy development (Jaspars et al., 2016; Sreedharan et al., 2023; Yaraguppi et al., 2025).

Marine microorganisms, in particular, have attracted increasing attention because of their ability to produce structurally unique and biologically active compounds that are often difficult or impossible to synthesize chemically. Among these microorganisms, bacteria of the genus *Bacillus* are well recognized for their capacity to generate a wide range of bioactive metabolites, including antibacterial, antifungal, antiviral, and anticancer agents. Genomic analyses have revealed that approximately 8% of the *Bacillus* genome is devoted to gene clusters involved in the biosynthesis of secondary metabolites with potential bioactivity (Kunst et al., 1997; Kiesewalter et al., 2021).

Similarly, genomic screening of the marine *Bacillus subtilis* subsp. *spizisenii* strain gtP20b, isolated from the Indian Ocean, demonstrated an even higher proportion (8.5%) of genes associated with secondary metabolite biosynthesis (Fan et al., 2011).

Marine *Bacillus* species are prolific producers of diverse classes of bioactive compounds, including polypeptides, lipopeptides, macrolactones, fatty acids, polyketides, lipoamides, and isocoumarins (Kiesewalter et al., 2021). These metabolites have been reported to exhibit antimicrobial, anticancer, and antialgal activities (Hamdache et al., 2011). Investigations of antimicrobial-producing bacteria associated with four sympatric gorgonian corals in the China Sea and the black coral *Antipathes dichotoma* from the South China Sea identified members of the genus *Bacillus* as dominant and highly productive sources of antimicrobial compounds (Zhang et al., 2012; Peng et al., 2013).

Within mutualistic associations with corals, *Bacillus megaterium* and *Exiguobacterium* sp. have been shown to produce antibiotic compounds that contribute to host defense. For instance, *B. megaterium* exhibited antimicrobial activity against *B. subtilis*, while *Exiguobacterium* sp. inhibited *Serratia marcescens* PDL100, the causative agent of coral white pox disease in *Acropora palmata* (Ritchie, 2006). In addition, *Bacillus cereus* M15, isolated from soil, displayed inhibitory activity against both Gram-positive and Gram-negative bacteria (Yilmaz et al., 2006), and sediment-derived *Bacillus* strains have likewise been identified as potent producers of antimicrobial metabolites.

Bacillus species are generally fast-growing bacteria with high nutrient and spatial demands, leading to intense competitive interactions with neighboring microorganisms within their habitats. Notably, *Bacillus* spp. are among the most abundant bacterial taxa associated with marine invertebrates, where they play important roles in host defense and health (Wahl et al., 2012). *Bacillus licheniformis* isolated from marine sponges produces an exopolysaccharide with antibiofilm activity, potentially enhancing host resistance to microbial colonization (Sayem et al., 2011). Moreover, the genus exhibits high diversity within tropical sponge microbiomes and has been repeatedly reported as a rich source of antimicrobial compounds (Mien et al., 2020).

Marine *Bacillus* strains are therefore considered genetically and chemically rich resources for the discovery of novel bioactive metabolites, particularly those with pharmaceutical potential (Mondol et al., 2013). *Bacillus* sp. strain SEB32, isolated from the marine sponge *Dysidea fragilis*, demonstrated broad-spectrum antimicrobial activity against several fish pathogens, including *Vibrio alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *Aeromonas salmonicida*, *Flavobacterium* sp., *Edwardsiella* sp., and *Proteus mirabilis* (Mohan et al., 2016). Similarly, *B. subtilis* strains isolated from marine sediments have been reported to produce gageopeptins A and B, novel compounds that inhibit zoospore motility in the plant pathogen *Phytophthora capsici* (Tareq et al., 2014).

Furthermore, ethyl acetate extracts of *B. subtilis* subsp. *subtilis* RG, isolated from mangrove ecosystems, exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, antifungal activity, antioxidant capacity at 1000 μg mL^{-1}, and anticancer effects against human breast adenocarcinoma cells (Ramasubburayan et al., 2015). Collectively, these findings highlight the considerable potential of marine-derived *Bacillus* strains as sources of novel bioactive compounds for therapeutic development.

Aligned with the objectives of SDG 14, the present study supports sustainable bioprospecting by isolating pure compounds from a marine-derived *Bacillus* sp., evaluating their biological activities, and discussing their potential pharmaceutical applications.

Material and Method

Origin, cultivation and identification of the strain C-1-10. The strain C-1-10 was originally isolated from a soft coral *Alcyonium digitatum* in the Baltic Sea in Tropic Marine medium and was identified as *Bacillus* sp., based on 16S rRNA sequence analysis (Pham et al., 2016). Strain C-1-10 showed 99.8% sequence similarity with *Bacillus amyloliquefaciens* NBRC15535^T (GenBank accession number AB255669), and shared 99.7% sequence similarity with *Bacillus methylotrophicus* CBMB205^T (GenBank accession

number EU194897) and 99.5% sequence similarity with *Bacillus vallismortis* DSM11031^T (GenBank accession number AB021198) respectively.

Cultivation and extraction of the strain C-1-10. Chemical analyses of metabolites after growth in Glucose Yeast Malt (GYM) and Bannett (BM) media (100 mL cultures) showed that the strain C-1-10 produced 7 compounds in both media. Beside those present in BM medium, additional metabolites were produced in GYM. There were 8 interesting compounds found in 100 mL cultures in GYM medium with rather large amounts and also good UV signals. Therefore, the strain C-1-10 was scaled up to 1 L in GYM medium. Broth of 10 L cultures in total was extracted with ethyl acetate and applied to preparation.

Compound purification. Semi-preparative high-performance liquid chromatography (HPLC) was used for separation of the metabolites which were produced by C-1-10. Preparation of 2.2 g crude extract obtained from 10 L cultures was carried out with Merck Hitachi Elite LaChrom system 2 with a gradient from 10% B at 0 min increasing to 100% B from 25 min to 27 min (flow 14 mL min^{-1}), and yielded 430 mg fraction 1 at 9 to 16 min, 50 mg fraction 2 at 16 to 18 min, 40 mg fraction 3 at 18 to 22 min, 60 mg fraction 4 at 23 min, 160 mg fraction 5 at 24 min, and 120 mg fraction 6 at 24.5 min.

To purify compounds 4 (*m/z* 993) and 5 (*m/z* 1007), preparation of 60 mg fraction 4 was carried out by using Merck Hitachi Elite LaChrom system 2 with a gradient from 70% B at 0 min increasing to 100% B at 13 min to 14.5 min, (flow 14 mL min^{-1}). The pure compound 4 was yielded 3.5 mg and the pure compound 5 was yielded 6.5 mg. To purify compounds 6 and 7, 160 mg of fraction 5 were applied to preparation with Merck Hitachi Elite LaChrom system 2 using the same gradient as for the purification of fraction 4. The pure compound 6 (*m/z* 1021) was yielded 60 mg and the pure compound 7 (*m/z* 1035) was yielded 4 mg.

Antimicrobial test of pure compounds. The *B. subtilis* (DSM 347), *Staphylococcus epidermidis* (DSM 20044), *Staphylococcus aureus* MRSA (DSM 18827), and *Propionibacterium acnes* (DSM 1897) were representatives of Gram-positive bacteria, in addition *Escherichia coli* (DSM 498) and *Klebsiella pneumoniae* (DSM 30104) were used for Gram-negative test. *Candida albicans* (DSM 1386), *Septoria tritici* and *Trichophyton rubrum* were employed for antifungal tests. G. Rimbach (University of Kiel, Germany) kindly provided the mouse fibroblast cell line (NIH-3T3). The human hepatocellular carcinoma cell line (HepG2) was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Those cell lines were implemented for cytotoxic assays. Four enzymes acetylcholinesterase (AchE), glycogen synthase kinase, phosphodiesterase 4 (PDE-4β2) and protein-tyrosine-phosphatase were used for enzyme inhibition assays.

Antimicrobial activity test against *B. subtilis*, *E. coli* and *P. acnes* were performed as described by Schneemann et al. (2010). Antimicrobial testing in the same manner as *S. epidermidis* were applied for *S. aureus* MRSA and *K.* pneumoniae according to Silber et al. (2013). A phytopathogenic *S. tritici* and dermatophyte fungus *T. rubrum* were tested according to Jansen et al. (2013). The cytotoxic activity against NIH-3T3, HepG2, and inhibitory against phosphodiesterase (PDE-4β2) were determined according to Schulz et al. (2011). Inhibitory activity against acetylcholinesterase (AchE) and the human pathogenic yeast *C. albicans* were performed according to Ohlendorf et al. (2012). The enzyme inhibition test for glycogen synthase kinase-3β was performed according to Baki et al. (2007) and the test for protein tyrosine phosphatase was implemented following to Helaly et al. (2009). The final concentration of the pure compounds used in the assays was 100 µM for antibacterial and antifungal activity, 50 µM for cytotoxic activity, and 10 µM for enzymatic inhibition. All experiments were performed at GEOMAR.

Results

Metabolic profiles. The substances identified in strain C-1-10 in 100 mL cultures in GYM and BM media are shown in Table 1 and Figure. 1. This strain produced at least 7 compounds (compound 1-7) in both media. One out of those compounds was unknown (compound 1, *m/z* 686). The other compounds might be all known.

Table 1

Chemical profiles of the strain C-1-10 in screening cultural media

Peak	*Media (100 mL)*	*m/z*	*Rt [min]*	*UV [nm] maxima*	*CN/comment*	*References*
1	GYM BM	686	3.7	233, 271, 323	Unknown	This study
2	GYM BM	772	5.2	231,275, 328	0-demethylpaulomacin A from *Streptomyces paulus*	(Argoudelis et al., 1988b)
3	GYM BM	502	6.0	230	Bistratamide C from ascidian *Lissoclinum bistratum*	(Foster et al., 1992)
4	GYM BM	993	6.2	226, 279	Surfactin by *B. subtilis* OKB105. Compound purification	(Kowall et al., 1998) This study
5	GYM BM	1007	6.3	226, 280	Surfactin (no information about UV maxima) Compound purification	(Kowall et al., 1998) This study
6	GYM BM	1021	6.4	225, 278	Bacillopeptin A (UV_{max} 228, 278; *m/z* 1021) Compound purification	(Kajimura et al., 1995) This study
7	GYM BM	1035	6.5	226, 278	Bacillopeptin B (UV_{max} 228, 278; *m/z* 1035) Compound purification	(Kajimura et al., 1995) This study

Rt: retention time; CN: compound name.

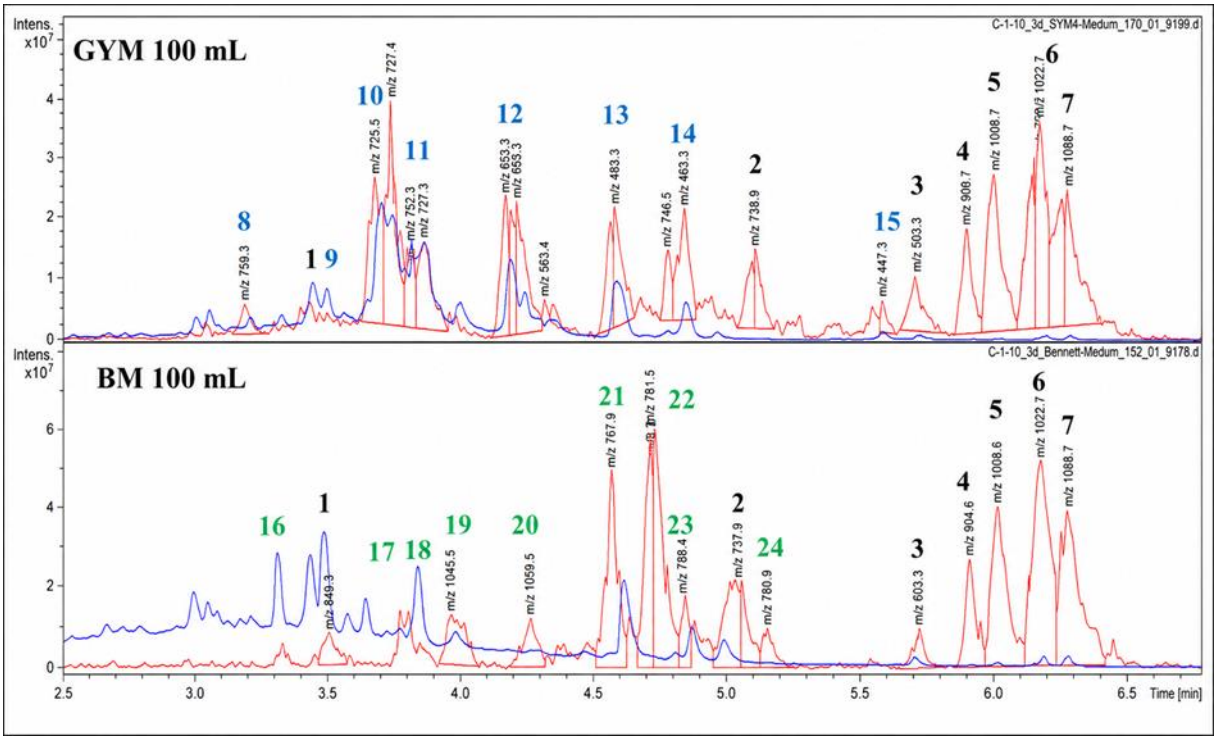

Figure 1. Chemical profiles of C-1-10 in 100 mL cultures (red = mass, blue = UV).

The compound 2 (UV_{max} 231, 275, 328; *m/z* 772) might be an O-demethylpaulomycin A (M/Z 772, UV max 236, 274, 321) which was isolated from *S. paulus* [23]. 1988).

Compound 3-7 might be known cyclic peptides. The compound 3 (UV_{max} 233; *m/z* 502) might be a cyclic hexapeptide bistratamide C (UV_{max} 233; *m/z* 502) which was isolated previously from the ascidian *L. bistratum* [24].

The known cyclic peptides [compound 4 (*m/z* 993), compound 5 (*m/z* 1007), compound 6 (*m/z* 1021) and compound 7 (*m/z* 1035) were major compounds produced by the strain C-1-10. Those main produced cyclic peptides were successful isolated. Searching of molecular weight and biological sources revealed that *Bacillus* spp. were sources of lipopeptides including members of surfactin class and iturin class. Compounds with molecular weight 993, 1007, 1021 and 1035 were surfactins produced by *B. subtilis* OKB105 (Kowall et al., 1998). But no UV information was available for those surfactins. The compounds 4 (UV_{max} 226, 279; *m/z* 993) and 5 (UV_{max} 226, 280; *m/z* 1007) which were produced by *Bacillus* C-1-10 in this study might be surfactin relative substances. Compound 6 (UV_{max} 225, 278; *m/z* 1021) and compound 7 (UV_{max} 226, 278; *m/z* 1035) might be bacillopeptin A (UV_{max} 228, 278; *m/z* 1021) and B (UV_{max} 228, 278; *m/z* 1035), respectively (Kajimura et al., 1995).

In addition to these compounds produced in both media (black numbers), this strain produced compounds 8-15 (blue numbers) in 100 mL cultures in GMY medium and compounds 16-24 (green numbers) in 100 mL cultures in BM medium (Table 2, Figure 1).

Table 2

The various chemical profiles of the strain C-1-10 from two media

Peak	*Media 100 mL*	*m/z*	*Rt [min]*	*UV [nm] maxima*	*CN/comment*
8	GYM	758	3.4	227, 284, 305 320, 337, 355	This study
9	GYM	742	3.8	232, 270, 306 322, 338, 357 387	This study
10*	GYM	742 744	4.0	226, 286, 324 341, 362, 384	This study
11	GYM	726	4.1	235, 273, 322 343, 350, 362 384	This study
12	GYM	580	4.5	223, 285, 327 345, 349, 363 383	This study
13*	GYM	745 462	4.8	236, 274, 286	This study
14	GYM	462	5.1	235, 273, 286	This study
15	GYM	446	5.8	233, 272, 286	This study
16	BM	586	3.6	235, 270, 309 324	This study
17	BM	1030	3.9	227, 276	Bacillomycin D1
18	BM	366	4.1	235, 268, 311	Paecilomycin E/F
19	BM	1044	4.2	227, 275	Bacillomycin D2
20	BM	1058	4.3		Bacillomycin D4/5
21	BM	766	4.7	231, 275, 287	This study
22	BM	780	4.9	235, 273, 287	This study
23	BM	787	5.1	233, 274, 287	This study
24	BM	779	5.2		This study

* Each of peaks 10 and 13 consist of 2 compounds *m/z* 742, *m/z* 744 and *m/z* 745, *m/z* 462, respectively; Rt: retention time; CP: compound purification; CN: compound name.

The mass analysis (Figure 1) indicated that at 4.0 min in GYM 100 mL was *m/z* 725 and *m/z* 727 in positive mode. It was found *m/z* 725 $[M+H-H_20]^+$, *m/z* 743 $[M+H]^+$, and *m/z* 765 $[M+H+Na]^+$ together with *m/z* 727 $[M+H-H_20]^+$, *m/z* 745 $[M+H]^+$, and *m/z* 767 $[M+H+Na]^+$. Therefore, the true molecular weights for these compounds were 742 and 744. Because the compounds *m/z* 742 and *m/z* 744 existed in the same retention time and had similar UV maxima, these compounds were listed in peak 10. Also, the mass of the compound 12 which was given by automatic analysis in Figure 1 at 4.5 min was 563,

the true molecular weight of this compound was decided as 580 because of the *m/z* 563 $[M+H-H_20]^+$, *m/z* 581 $[M+H]^+$, and 603 $[M+H+Na]^+$.

Beside the major cyclic peptide products, the compound 10 (*m/z* 742, *m/z* 744), the compound 11 (*m/z* 726), the compound 12 (*m/z* 580), and two interesting isomer compounds (13, 14) with molecular weight 462 were presented in rather high amounts and showed good UV signals (Rt at 4.0 to 5.1 min) in GYM 100 mL cultures (Table 2, Figure 1). At the similar retention time of compounds 10 to 12, known cyclic peptides were found. These were compound 19 (*m/z* 1044) and 20 (*m/z* 1058) either present at rather low amounts and with weak or no UV signals (Table 1, Figure 1). At similar retention time and UV maxima, compounds 21 (*m/z* 767), 22 (*m/z* 780), and 23 (*m/z* 787) were found as major compounds in screening BM, while two isomers (compound 13 and 14) were found in 100 mL cultures in GYM medium (Table 1, Figure 1). Other compounds were produced in 100 mL cultures in both media either at low amounts or revealed weak UV signals. These were the compounds 8, 9, and 15 in GYM, and the compounds 16, 17, and 24 in BM.

The metabolites of the strain C-1-10 in 1 L cultures in GYM medium are shown in Table 3 and Figures 2 and 3.

This strain produced 7 compounds [compound 1 (*m/z* 686), compound 2 (*m/z* 772), and 5 cyclic peptides (compounds 3-7)]. In addition, compound 13, 14 which were produced in GYM 100 mL cultures and compound 17, 18, 19, 20 and 22 which were found in BM 100 mL cultures were available in 1 L cultures in GYM medium. The known cyclic peptides (compound 4-7) which were originally isolated from *Bacillus* species were major products with high amounts in mass and rather good UV signals, whilst compound 3 which is considered as hexapeptide from the ascidian *L. bistratum* and others existed in very low amounts. The known cyclic peptides which were produced in 100 mL cultures in BM medium were also found in big scale cultivation in 1 L in GYM medium. These were compounds 17 (*m/z* 1030), 19 (*m/z* 1044), and 20 (*m/z* 1058) with rather low amounts and weak UV signals. The isomer compounds (compounds 13 and 14 with *m/z* 462) were found in rather low amounts in GYM 1 L cultures. The compound 14 with *m/z* 745 (100 mL cultures in GYM) and compound 22 with *m/z* 780 (100 mL cultures in BM) were also found in GYM 1 L cultures. They shared the same UV maxima and retention time to one out of two isomers (*m/z* 462).

Table 3

The metabolites of the strain C-1-10 in 1 L cultures in GYM medium

Peak	*Media 1000 mL*	*m/z*	*Rt [min]*	*UV [nm] maxima*	*CN/comment*
1	GYM	686	3.7	233, 271, 323	Unknown
17	GYM	1030	3.9	227, 276	Bacillomycin D1
18	GYM	366	4.1	235, 268, 311	Paecilomycin E/F
19	GYM	1044	4.2	227, 275	Bacillomycin D2
20	GYM	1058	4.3		Bacillomycin D4/5
13*	GYM	745 462	4.8	236, 274, 286	Unknown
22	GYM	780	4.9	235, 273, 287	Unknown
14	GYM	462	5.1	235, 273, 286	Unknown
2	GYM	772	5.2	231,275, 328	0-demethylpaulomacin A
3	GYM	502	6.0	230	Bistratamide C
4	GYM	993	6.2	226, 279	Surfactin
5	GYM	1007	6.3	226, 280	Surfactin
6	GYM	1021	6.4	225, 278	Bacillopeptin A
7	GYM	1035	6.5	226, 278	Bacillopeptin B

Peak 13 consists of 2 compounds, *m/z* 745, *m/z* 462, respectively; Rt: retention time; CN: compound name.

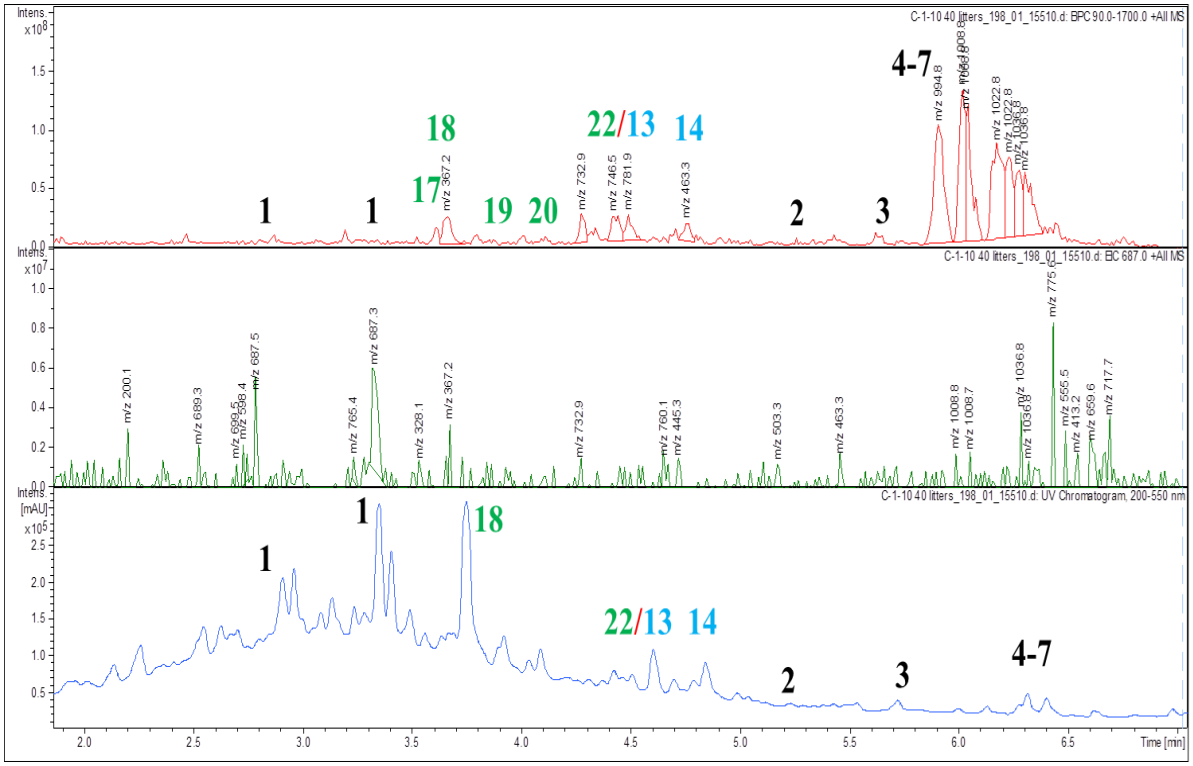

Figure 2. Chemical profiles of C-1-10 in 1 L cultures GYM medium
(red-mass, green-presented of mass 687 in two different times, blue-UV).

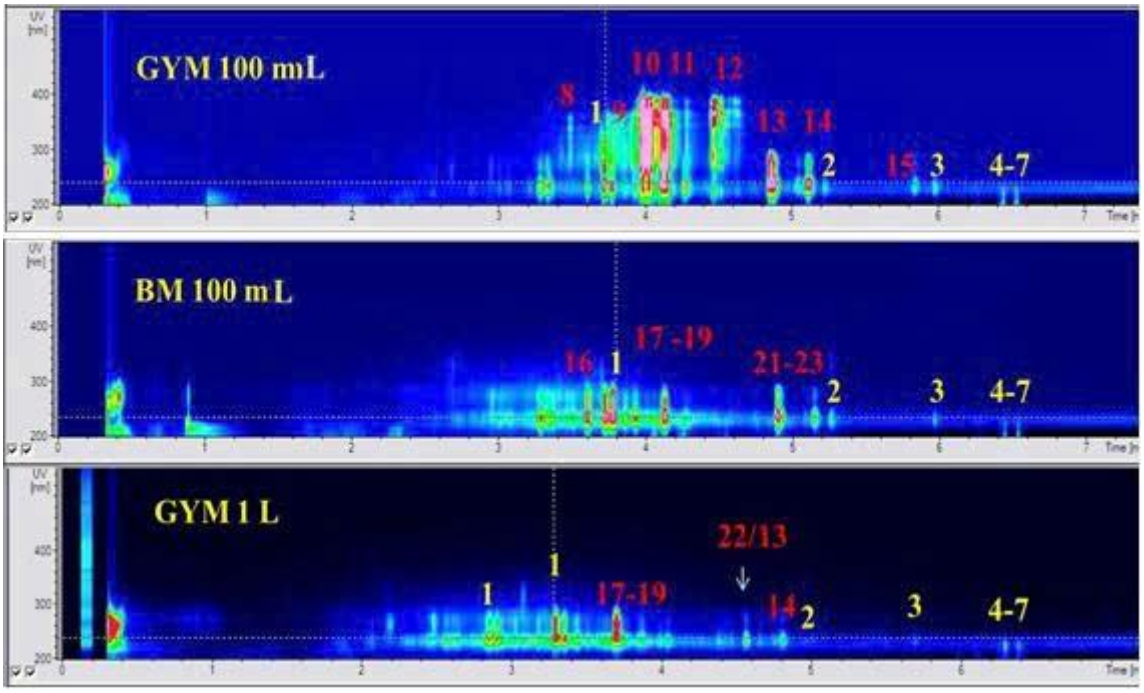

Figure 3. UV spectrums of C-1-10 in GYM and BM (100 mL) and 1 L cultures in GYM.

Bioactivities of the isolated compounds. All four purified compounds showed inhibition of *S. epidermidis* and also MRSA (Table 4). The compound 5 with molecular weight 1007 showed rather weak inhibition of Gram-negative *E. coli* and was inactive to phosphodiesterase 4, while the other compounds were inactive to *E. coli* and active against phosphodiesterase 4. However, all four pure compounds were inactive to other Gram-negative bacteria and fungi and exhibited no cytotoxic activity, and no inhibition to glycogen synthase kinase, and protein-tyrosine-phosphatase.

Table 4

Bioactive activities of pure compounds isolated from C-1-10

Compounds	*Antibacterial*						*Antifungal*			*Cytotoxic*		*Enzyme inhibition*			
	Bacillus subtilis	*Staphylococcus epidermidis*	*Staphylococcus aureus MRSA*	*Escherichia coli*	*Klebsiella pneumoniae*	*Propionibacterium acnes*	*Candida albicans*	*Septoria tritici*	*Trichophyton rubrum*	NIH-3T3 (mouse fibroblasts)	Hep G2 (human liver cancer)	Acetylcholinesterase	Glycogen synthase kinase	Phosphodiesterase 4 B2	Protein-tyrosine-phosphatase
4 (*m/z* 993)	-	52	86	-	-	-	-	-	-	-	-	-	-	39	-
5 (*m/z* 1007)	23	96	98	22	-	-	-	-	-	-	-	-	-	-	-
6 (*m/z* 1021)	-	75	74	-	-	-	-	-	-	-	-	-	-	31	-
7 (*m/z* 1035)	23	53	63	-	-	-	-	-	-	-	-	-	-	41	-

The numbers indicate the percentage of inhibition, (-) indicate negative results.

Discussion. Several database searches were conducted for compound 1 (*m/z* 686; UV_{max} 233, 271, 323). While multiple hits were obtained, none showed a perfect match in both biological origin and UV absorption characteristics. Excelside B (*m/z* 686; UV_{max} 230, 275, 283) was isolated from the seeds of *Fraxinus excelsior* and reported to inhibit adipocyte differentiation in 3T3-L1 cells (Bai et al., 2010). Similarly, mayoside C (*m/z* 686; UV_{max} 226, 276, 350) is a constituent of the bark of *Picramnia teapensis* (Rodŕiguez-Gamboa et al., 2000). Few reports describe a compound with these exact properties from microbial sources, indicating that compound 1 merits detailed structural characterization.

Prior to isolating fraction 1, UV spectral analysis of metabolites from 1 L cultures grown in GYM medium revealed several compounds with strong UV absorption between 2.5 and 3.5 min retention time (Figure 2 – blue line; Figure 3 – GYM 1 L). However, due to the limited quantities present, the masses of these compounds could not be fully identified (Figure 2). Compound 1 (*m/z* 686) was detected in this retention window and confirmed through extracted ion chromatogram (EIC) analysis at m/z 687 (green line, Figure 2), appearing at 2.9 and 3.3 min with similar UV maxima (Figures 2-4).

Interestingly, another compound with *m/z* 365/765, located adjacent to compound 1 in the chromatogram, displayed strong UV absorption (green line, Figure 2). This compound was only detected in fraction 1 after crude extract preparation, suggesting low abundance in the initial extract. Due to the small quantity of crude extract, compound 1 was prioritized for further separation. Semi-preparative HPLC within the retention range of 2.5-3.5 min revealed that compound 1 (*m/z* 686) existed as isomers with UV_{max} 230, 273 (MeOH). The *m/z* 365/765 compound, although still present in low amounts, exhibited strong UV absorption (UV_{max} 237, 268, 323) at 3.2 min (Figure 4).

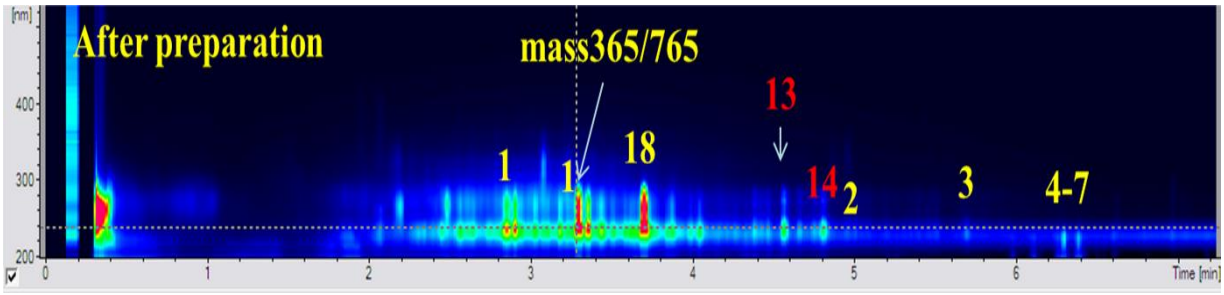

Figure 4. UV spectrums of metabolites of the strain C-1-10 after preparation.

The compound with *m/z* 364 and UV_{max} 234, 272, 312 might be an antibiotic LL Z1640-1 (UV_{max} 234, 272, 312; *m/z* 364) isolated from unidentified fungus and possessed antiprotozoal activity. Other hit was a zeaenol or antibiotic LL-Z1640-4 (UV_{max} 234, 271, 314; *m/z* 364) isolated from *Drechslera portulacae*. Antibiotic LL Z1640-1 and zeaenol isolated from Gorgonian derived fungus *Cochliobolus lunatus* showed antifouling properties (Shao et al., 2011). The molecular weight and UV maxima of compound 2

(UV_{max} 231, 275, 328; *m/z* 772) matched to those of 0-demethylpaulomycin A (UV_{max} 236, 274, 321; *m/z* 772) which was isolated from *S. paulus*. This compound was reported to be active against Gram-positive bacteria, especially *S. aureus* (Argoudelis et al., 1988b).

The compound 3 (*m/z* 502, UV_{max} 230) might be the cyclic hexapeptide bistratamide C (*m/z* 502, UV $_{max}$ 234 in CH_2Cl_2), which was isolated from the ascidian *L. bistratum* (Foster et al., 1992). The other hit was aleurodiscal (UV_{max} 237; *m/z* 502) isolated from *Aleurodiscus mirabilis.* This compound was reported as antifungal agent inhibition of *Fusarium oxysporum*, *Mucor miehei*, *Absidia glauca*, *Alternaria porri*, *Ascochyta pisi*, *Aspergillus ochraceus*, *Verticillium* sp. 153-83, and *Zygorrhynchus moelleri* with 2 µg $disc^{-1}$ by agar diffusion assay method. But no antibacterial activity was observed with *B. subtilis*, *E. coli*, and *S. aureus* with minimum inhibitory concentration (MIC) > 100 µg mL^{-1} (Lauer et al., 1989). As mentioned above for known surfactins which had similar molecular weight to compounds 4 (*m/z* 993) and 5 (*m/z* 1007) there was no UV information.

Searching of *m/z* and UV maxima revealed that micropeptin EI 992 (UV_{max} 224, 280; *m/z* 993) and hymenamide K (UV_{max} 221, 280; *m/z* 1007) were similar to compounds 4 (UV_{max} 226, 279; *m/z* 993) and 5 (UV_{max} 226, 280; *m/z* 1007) from this present study. Micropeptin EI 992 isolated from cyanobacterium was described as colourless oil, trypsin inhibitor (Ploutno et al., 2002). Hymenamide K isolated from a marine sponge *Hymeniacidon* sp. was a proline containing cyclic octapeptide and showed inhibition of protein tyrosine kinase at concentration 73 µg mL^{-1} (Tsuda et al., 1994). However, the compound 4 isolated from C-1-10 was white solid substance and showed antibacterial activity against Gram-positive bacteria and inhibited phosphodiesterase 4, whereas compound 5 inhibited Gram-positive bacteria and also Gram-negative bacteria and showed no inhibition of any tested enzymes. As mentioned above, compounds 6 (*m/z* 1021) and 7 (*m/z* 1035) were regarded as bacillopeptin A and B. However, these compounds isolated in this present study were antimicrobial substances. They showed antimicrobial activity against Gram-positive bacteria (Table 4), while bacillopeptin A and B isolated from *B. subtilis* FR-2 did not show any activity against bacterial and fungal tests including *B. subtilis*. *E. coli*, and *C. albicans* (Kajimura et al., 1995). The compound 8 with *m/z* 1049 was produced by *Bacillus* sp. strain H-6 (Pham et al., 2016). It might be a bacillopeptin C (UV_{max} 229, 278; *m/z* 1049), an antifugal cyclic peptide against *C. albicans* (Kajimura et al., 1995). However, this compound was not found in the crude extract of the strain C-1-10 in 100 mL cultures and also in 1 L cultures. The crude extract of the strain C-1-10 showed inhibition of four test microorganisms. Four purified compounds showed no inhitibion of *C. albicans*. Thus, the compound which responded to *C. albicans* inhibition was not identified.

The genus *Bacillus* sp. is a well-known source of cyclic peptides so far. They had similar molecular weight to some compounds isolated in this study. However, UV information was not found. For examples, surfactins 1 (*m/z* 1035), and 2 (*m/z* 1021) produced by *B. subtilis natto* showed moderate anti HIV activity (Itokawa et al., 1994). Bacillomycin L (*m/z* 1021, normal-C_{14}) isolated from *B. subtilis* showed weak antifungal activity against *Ophiostoma ulmi* and *Cryphonectria parasitica*, while bacillomycin $L_{1,2}$ (*m/z* 1035, anteiso C_{15} and iso C_{15}) and bacillomycin $L_{3,4}$ (*m/z* 1049, iso C_{16} and n-C_{16}) showed stronger activity than those of bacillomycin L (Eshita et al., 1995). Halobacillin which was produced by a marine *Bacillus* sp. was an acylpeptide of the iturin class with *m/z* 1035. Halobacillin showed moderate human cancer cell cytotoxicity, no antifungal, and no antibiotic activities (Trischman et al., 1994). The compounds 4 (*m/z* 993), 5 (*m/z* 1007), 6 (*m/z* 1021), and 7 (*m/z* 1035) were the major compounds produced by strain C-1-10. They were successfully purified by UV-HPLC separation and showed different antimicrobial activities to known cyclic peptides from Dictionary of Natural Products (DNPs). This study suggested that compound 4 (*m/z* 993), 5 (*m/z* 1007), 6 (*m/z* 1021) and 7 (*m/z* 1035) might be relative cyclic peptides. Further detailed nuclear magnetic resonance analysis is required to confirm the name and structure of each compound. The search by molecular weight and UV maxima yielded 5 hits for compound 8 (*m/z* 758 UV_{max} 227, 284, 305, 320, 337, 355). Three hits were original sources from

microorganisms. These were 0-demethylpaulomycin B (*m/z* 758, UV_{max} 237, 276, 320) isolated from *S. paulus* (Argoudelis et al., 1988b), antibiotic disorazole A1 (*m/z* 758, UV max 251, 265sh, 272, 298, 306, 321sh) which was produced by a myxobacterium *Sorangium cellulosum* strain So ce 12 (Jansen et al., 1994), and unguisin A (*m/z* 758, UV_{max} 219, 274, 281, 290) isolated from marine fungus *Emericella unguis* (Malmstrøm, 1999). Disorazole A1 showed strong inhibition of many fungi but no inhibition of yeasts (*Sacharomyces cerevisiae*, *C. albicans*, *Debaryomyces hansenii*) and bacteria including of *B. subtilis*, *S. aureus*, *Micrococcus luteus*, *Streptococcus faecalis*, *E. coli* and *Salmonella typhimurium* (Irschik et al., 1995; Hopkins & Wipf, 2009). Unguisin A showed moderate inhibition of *S. aureus* and *Vibrio parahaemolyticus* (Malmstrøm, 1999). Other hits had the same molecular weight but different in UV maxima and biological sources. The best hit for compound 9 (*m/z* 742, UV_{max} 232, 270, 306, 322, 338, 357, 387) were bacillaene B (*m/z* 742, UV_{maxima} 292, 328, 343, 361, 382) which was isolated from *B. amyloliquefaciens* strain FZB 42 (Moldenhauer et al., 2010) and licoagrone (*m/z* 742, UV_{max} 230, 278, 324, 396) isolated from hairy root cultures of plant *Glycyrrhiza glabra* (Asada et al., 1999). The most similar hit to compound 10 (*m/z* 744, UV_{max} 226, 286, 324, 341, 362, 384) was paulomycin D (*m/z* 744, UV_{max} 236, 275, 321) which was isolated from *S. paulus.* This compound showed activity against the Gram-positive bacteria *S. aureus*, *S. epidermidis*, *S. faecalis* and *S. pneumoniae* (Argoudelis et al., 1988a). Searching by molecular weight and UV maxima revealed that unguisin B (*m/z* 726, UV_{max} 220, 274, 280, 290) which was isolated from the marine fungus *E. unguis* (Malmstrøm, 1999) and bambusicolaside IV (*m/z* 726, UV_{max} 232, 298, 344, 356, 456) were most similar to compound 11 with *m/z* 726 and UV maxima 235, 273, 322, 343, 350, 362, 384 (Zhang et al., 1997). The best hit for compound 12 (*m/z* 580, UV_{max} 223, 285, 327, 345, 349, 363, 383) was bacillaene with molecular weight 580 and UV maxima 292, 328, 343, 361, 382. Bacillaene was isolated from *B. subtilis*, and is active against a broad spectrum of bacteria inhibiting prokaryotic protein synthesis and showed strong inhibition of *E. coli* (Patel et al., 1995). The compounds 13 and 14 with identical *m/z* 462 and UV_{maxima} 235, 272, 286 but different retention time (4.8 and 5.1 min) were found in GYM medium (100 mL and 1 L cultures). The two compounds might be considered as isomer compounds. No hits were found for these compounds from DNPs by searching of *m/z* and UV maxima and biological sources as well. The most similar compound was aranotin (*m/z* 462, UV_{max} 222, 270) which was isolated from *Arachniotus aureus*, and showed antiviral property (Nagarajan et al., 1968). Other possible hits to this compound with molecular weight 462 were mostly isolated from plants. For example, bletilol C (*m/z* 462 UV_{max} 234, 274, 285, 302) was found in the tubers of *Bletilla striata* (Yamaki et al., 1993) and phyllodulcon 8-glucoside *m/z* 462, UV_{max} 233, 285, 295 was isolated from leaves of the plant *Hydrangea macrophylla* var. *thunbergii* and supported for the polyphenol biosynthesis in host plant (Suzuki et al., 1977). Chemical analysis of metabolite of crude extract in 1 L cultures showed that peak 13 including of two compounds with *m/z* 462 and 745, compound 14 (*m/z* 462), and compound 22 (*m/z* 780) were found at 4.8 to 5.1 min with similar UV maxima (Table 3, Figures 2 and 3). Mass spectrum showed that the isomers (*m/z* 462) existed in rather low amounts, and the compound 22 and compound with *m/z* 745 were found in a bit higher amount. These compounds existed in this retention time showed good UV signals when the crude extract was applied in standard HPLC (Figure 4). As mentioned above, the isomers were regarded as interesting compounds and purified with semi preparation. The UV spectrum of fractions 2 and 3 after preparation showed that the main compound in those fractions were compound with *m/z* 745 and compound 22, respectively. The isomers *m/z* 462 which were found in crude extract also presented after preparation of those fractions but in very low amounts, and shared UV maxima with compound *m/z* 745. The compound 22 was found in rather high amounts but in very weak UV signals. As a result, all compounds which were found in fractions 2 and 3 were not purified, because of either their existed in very low amounts or revealed in very weak UV signals. There was no clear hit for compound 15 (*m/z* 446, UV_{max} 233, 272, 286). The most similar compound was pseudopterosin E (*m/z* 446, UV_{max} 226, 275, 285) isolated from *Pseudopterogorgia elisabethae*. This diterpene glycoside showed very low acute toxicity in mice LC_{50} > 300

mg kg^{-1} and was an anti-inflammatory agent (Roussis et al., 1990). Other pseudopterosins possessed identical molecular weight 446 and UV maxima 213, 232, 276, 285 for pseudopterosin P, UV maxima 228, 277, 284 for pseudopterosin T, and UV maxima 277, 284 for pseudopterosin K. Two other hits were derivatives of eruberin A, and pneumatopterin C. A derivate eruberin was abacopterin which was constituent compound of *Abacopteris penangiana* with *m/z* 446 and UV maxima 232, 276 (Zhao et al., 2007). Pneumatopterin C was isolated from *Pneumatopteris pennigera* with *m/z* 446 and UV maxima 230, 280 (Tanaka et al., 1997). The compound 16 (*m/z* 586, UV_{max} 235, 270, 309) was found in screening BM medium in very low amounts but very good UV signals. Searching by molecular weight, UV maxima and biological sources revealed that this compound is not known as product of *Bacillus* so far. Among some substances with similar molecular weight but different UV maxima compared to compound 16, the most similar compound was integracin B/C (*m/z* 585, UV_{max} 225, 265, 302) isolated from from fungus *Cytonaema* sp. (Singh et al., 2002). The compound 18 (*m/z* 366, UV_{max} 235, 268, 311) might be a paecilomycin E (*m/z* 366, UV_{max} 233, 271, 311) or paecilomycin F (*m/z* 366, UV_{max} 253, 271, 314). Both were isolated from *Paecilomyces* sp. SC 0924 and a marine-derivate *C. lunatus* (Xu et al., 2010). Compounds 17, 19 and 20 were considered as bacillomycin D1, bacillomycin D2, and bacillomycin D4/5, respectively (Peypoux et al., 1984). Recently, the compounds a_1, a_2 and a_3 which were bacillomycin D-like lipopeptides of 14, 15 and 16 carbon fatty acid long chains showed strong inhibition of *C. albicans*. Especially, compound a_3 with molecular weight 1058 and very similar bacillomycin D structure had strongest antifungal activity (Tabbene et al., 2011). The compounds 17, 19, and 20 were considered as known peptides. All were found in 100 mL cultures in BM medium, and in 1 L cultures in GYM medium but with low amounts and weak UV signals. As a result, those compounds were not able to purify. The compound 21 (*m/z* 766, UV_{max} 231, 275, 287) had similar molecular weight with the antibiotic SB 22484 (*m/z* 766), but different UV maxima (SB 22484, UV_{max} 232, 327). This antibiotic SB 22484 was isolated from *Streptomyces* sp. (Selva et al., 1990). The most similar hit to compound 22 (*m/z* 780, UV_{max} 235, 273, 287) was kirrothricin isolated from *Streptomyces cinnamonensis* (Thein-Schranner et al., 1982). The most similar hit to compound 23 (*m/z* 787, UV_{max} 232, 274, 287) was the antibiotic BE 32030 also called BE 32030B (*m/z* 787, UV_{max} 242, 248, sh 258, 305). This substance was isolated from *Nocardia* sp. A 32030 and showed inhibition the growth of P388 murine leukemia, DLD-1 human colon cancer, PC-13 human lung cancer and MKN-45 human stomach cancer cell lines (Tsukamoto et al., 1997). The compound 24 (*m/z* 779) was found only in 100 mL cultures in BM medium and its UV maxima could not be determining, therefore this compound was not considered for further analysis. Analysis data from the literature revealed that compound 2 with *m/z* 772 possessed the UV maxima matched to the UV maxima of the compound 0-demethylpaulomycin with *m/z* 772 which was isolated from *S. paulus*. In addition, *S. paulus* also produced paulomycin D (*m/z* 744), paulomycin D was found as closed hit for the compound 10 from this study. Unguisin A (*m/z* 758) and unguisin B (*m/z* 726) which were both isolated from marine derived fungus *E. unguis*, those compound were the closed hits for the compound 8 (*m/z* 758) and the compound 11 (*m/z* 726) in this study. Those unguisins were cyclic heptapeptides contained gamma aminobutyric acid (GABA) in the ring, the synthetic cyclic peptide containing GABA has been tested as antitumor agent (Malmstrøm, 1999). Bacillaene B (*m/z* 742) and bacillaene A (*m/z* 580) isolated from *Bacillus* genus were closed hits for the compound 9 (*m/z* 742) and the compound 12 (*m/z* 580) respectively. All close hits for compounds which were produced by the strain C-1-10 were bioactive substances. It was interesting to find that best hits for compounds 2 and 10, compounds 8 and 11, and compounds 9 and 12 were antibiotic substances which were produced by the same sources (*S. paulus*: producer of hit compounds for compounds 2 and 10, *E. unguis*: producer of hit compounds for compounds 8 and 11, *Bacillus* sp.: producer of hit compounds for compounds 9 and 12. Actual, further exploitation should be carried out analysis of the metabolites of the strain C-1-10 for identification of all its metabolites and for determination of biological activities as well.

Conclusions. The coral-derived *Bacillus* sp. strain C-1-10, isolated from *Alcyonium digitatum* in the Baltic Sea, demonstrated the capacity to produce multiple metabolites across two different culture media, including seven compounds common to both. Four purified cyclic peptides (compounds 4–7) showed inhibitory activity against *Staphylococcus epidermidis* and methicillin-resistant *Staphylococcus aureus* (MRSA). Other fractions, specifically fractions 1, 2, and 3, contained compound 1 (*m/z* 686), compound 18 (*m/z* 366), known cyclic peptides (*m/z* 1030 and 1044), an isomeric compound (*m/z* 462), and fraction 6 (a mixture of three known cyclic peptides, *m/z* 1007, 1021, and 1035). These components require further structural characterization and biological evaluation.

Strain C-1-10 represents a valuable candidate for the sustainable discovery of bioactive compounds from marine microorganisms. This aligns with the objectives of UN sustainable development goal 14 (Life Below Water), which encourages the conservation and sustainable use of oceans, seas, and marine resources. Harnessing bioactive metabolites from marine bacteria such as *Bacillus* spp. not only supports innovation in pharmaceuticals and biotechnology but also promotes the responsible exploitation of marine biodiversity, ensuring long-term benefits for both human health and marine ecosystem stewardship.

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Conflict of Interest. The authors declare that there is no conflict of interest.

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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