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Antimicrobial potential of methanolic extract of *Bacillus aquimaris* isolated from the marine waters of Burmanallah coast, South Andaman

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Abstract: Marine environments are natural endowments, known to be "treasured hideouts" of novel molecules of biologically dynamic chemical species of potent activity, lingered largely uncharted, prospecting vibrant applications in pharmaco-dynamics and drug kinetics. Bioprospecting of marine microbes has discerned many astonishing milestones in pharmacology, drug designing, and therapeutics. Marine *Bacillus* are known to produce structurally diverse versatile secondary complexes such as lipopeptides, polypeptides, macrolactones, polyketides and coumarins showcasing a wide array of biological bustles, ranging from antimicrobial, antialgal and anticancer in nature, heavy metal detoxification, carotenoids production to biocontrol agents and biopesticides. In this respect, species of *Bacillus aquimaris,* isolated from the coastal water of Burmanallah, South Andaman and phenotypically characterized by routine biochemical tests. The antibacterial activity of its methanolic extract was assessed by agar well diffusion assay confirming the presence of active metabolites exemplified by LC-MS peaks, thereby, warranting a 'multiplex of approach' for applicative advances and pharma-settings.

Keywords: Marine waters, *Bacillus aquimaris*, Methanolic extract, LC-MS, Secondary metabolites, Antibacterial activity

Introduction

Due to the over exploitation and limitations in discovery of novel natural products from terrestrial sources, natural product chemists have commenced an intense exploration of novel biological compounds from marine biological sources. The 'blue domain of Ocean' covers nearly two-thirds of planet earth's topology seizing tremendous degrees of biological and chemical multiplicities. The diversity of marine milieu, complexes varied assemblage of microbial species sustaining in the extreme environments of variability in pressure, salinity, and temperature, has exercised enormous pressure on the microbial evolution and selection to accomplish new adaptations and production of metabolites leading to the emergence of potential pharmaceutical candidates (Lindequist, 2016; Romano *et al*., 2017).

In early 1960s, researchers surveyed microbial variability and diversity as a prospective source of bioactive molecules with unique potentiality covering majority of microbial in origin, devoid of any evident role in growth and development, referred to as secondary metabolites (Yalla *et al*., 2018). Thus, marine microorganisms tender an underexplored profile of potential metabolites for commercial exploration (Hamdache *et al*., 2011). An enormous bacterial diversity is widely acclaimed as one of the major driving force designing the oceanic organic composition with the maintenance of physiological and genetic virtues that allows high degrees of adaptableness, metabolic diversification and dissemination into several environmental habitats and niches, assembling them as cosmopolitan entities (Boottanun *et al*., 2017).

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Micro-organisms are important players of nutrient bio-transformation, viz cycles of carbon, nitrogen and sulfur, shaping their heterogeneity in marine ecosystems than the soil system (Agrawal *et al*., 2017), as majority of the described phyla of bacterial origin are represented in oceans, in contrast to about half of the terrestrial members (Ray, 1988).

Bacillus species are Gram-positive rods found in manifolds, dwelling in diverse quarters of ecosystem, owing to their asset of endospore formation under harsh conditions like high temperature, irradiation and offensive chemicals (Errington, 2003). Besides sporulation, they are capable of producing secondary metabolite products, embellishing them with an additive arsenal in competition against other organisms (Sansinenea and Ortiz, 2011). Bacteriocin-type substances released by *Bacillus subtilis* were found to be inhibitory in nature and suppressed the growth of clinical pathogens like *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhi, Bacillus cereus* (Xie *et al*., 2009) while *Bacillus licheniformis* restricted the activities of spoilage bacteria (Guo *et al*., 2012). Ramli *et al*., (2012) reported significant reduction of biofilm formation in *Burkholderia pseudomallei* due to the presence of N-acyl homoserine lactone in the culture supernatant from *Bacillus* sp. Additionally, the endosporal form of *Bacillus* strain TKS1 inhibited and restricted the growth and incidence of citrus bacterial canker (Huang *et al*., 2012). Some species of *Bacillus* are renowned for the production of multitude of metabolites like *B. amyloliquefaciens* FZB42 producing lipo peptides [fengycin, surfactin, bacillomycin D, dipeptide bacilysin and polyketide (difficidin)] encoded by 8.5% of its genome, suppressing the growth of *Erwinia amylovora* (Chen *et al*., 2007; 2009). The present study is focused on *Bacillus aquimaris*, a marine bacterium isolated from coastal marine waters and its antibacterial activity against test pathogenic strains.

Materials and Methods

Seawater sample were collected from the coast of Burmanallah (11°34'22.26"N, 92°44'22.51"E), South Andaman in sterile polyethylene bottle and transported to the laboratory under sterile conditions. 1 ml of the sample was transferred aseptically to a sterile conical flask containing 99 ml of filtered sterile seawater and incubated at 37°C for 3-6 h. From this, serial dilutions up to 10^{-6} were prepared and 0.1 ml was plated onto the successive Zobell Marine agar plates by spread-plate technique following incubation at 35°C for 24 hours. After incubation period, single and discrete isolated colonies were re-streaked, selected and single colony purity and morphology was observed under microscope. The pure isolates were maintained as slants, stabs and 10% glycerol cultures for further analysis.

Phenotypic characterization

Various biochemical tests were undertaken to determine the identity of isolates based on the phenotypic characters as described by Bergey's manual of Systematic Bacteriology (2009). Gram staining was carried out by standard method as proposed by Chapin (2007). A total of 32 biochemical tests were performed along with growth pattern at varying salt concentration (0%, 3%, 6%, 8%, 10% NaCl) and temperature (4°C, 20°C, 35°C, 40°C, 50°C). The results were interpreted by using Identax Bacterial Identifier software Version 1.2 with an identification score above 95% (Flores *et al*., 2009).

Screening and identification of bacteria with bactericidal activity

All pure isolates with different morphologies were primarily screened for their bioactivity by cross streaking method (Lemos *et al*., 1985) against various pathogens (*Aeromonas hydrophila* (IDH1585), *Shigella dysenteriae* type 5 (NK2440), Enteropathogenic *Escherichia coli* serotype (0115) and *Vibrio cholera* (0139). The test strains were streaked perpendicular across the pathogens in Muller Hinton agar medium (MHA) and incubated at 37°C for 24h.

Preparation of bacterial culture crude extract

Potent strains were inoculated in 250 ml sterile Zobell marine broth followed by incubation for 3-5 days at 27°C on an orbital shaker. After incubation period, the culture was centrifuged at 11000 rpm for 10 min followed by ensuing supernatant extorted by equivalent volumes of ethyl acetate and stirred overnight on a

magnetic stirrer. The extract was further concentrated using vacuum rotary evaporator (Buchi, Essen Germany) at 40-45°C and the final content was dissolved in methanol figuring to a final concentration of 50 mg/ml (Yalla *et al*., 2018).

Liquid chromatography-Mass spectrometry

LC–MS analysis was performed to detect the presence of organic compounds present in the methanolic extract of *B. aquimaris* using Agilent technologies fitted with column coupled to MS-6120 Quadrupole mass spectrometer with ESI ion source. The data analysis (Data acquisition and mass spectrometric evaluation) were carried out by Data Analysis software (QualBrowser; Thermo Electron, San Jose, CA). A column of Agilent Eclipse plus C-18 (4.6 × 250 mm) was used with mobile phase of ammonium acetate (10 mM) and 15:85 ratio of water to methanol for chromatographic separations with the flow rate maintained at 0.4 ml/min with injection volume of 10 µl.

Microbial cultures

Four human bacterial pathogens *Aeromonas hydrophila* (IDH1585), *Shigella dysenteriae* type 5 (NK2440), Enteropathogenic *Escherichia coli* serotype (0115) and *Vibrio cholera* (0139), maintained in our laboratory, were tested. All isolates were maintained periodically on Mueller Hinton agar (MHA) plates and stored as slants, stabs and 10% glycerol cultures.

Antimicrobial assay

Antimicrobial activity was assessed by well diffusion method as followed by Cherian *et al*., (2018). All pathogenic strains (cell density 2×10^7 CFU/ml) were plated on Mueller Hinton agar (MHA) plates and uniform sized wells were punctured onto the agar surface by gel borer. Variable concentrations of *B. aquimaris* methanolic extract (25, 50, 100, 200 µl) were added to the wells with Gentamycin disc (15 mg/ml) and methanol (200 µl) taken as positive and negative control, respectively. Plates were incubated at 37°C for 24 h and the diameters of inhibitory zones (mm) were measured. All the experiments were performed in triplicates.

Results and Discussion

The persistent clamor for exclusive biological compounds with promising activity towards multi drug-resistant (MDR) pathogens and their survival-management stratagems to existing medical remedial strategies has heightened the delve towards microbial world (Cherian *et al*., 2018; Cherian *et al*., 2019). The microbial entities of bacteria and fungi are accounted to be potential sources of structurally assorted metabolites with imminent activities, glaring them as excellent aspirants of therapeutics (Mondol *et al*., 2013; Agrawal *et al*., 2017). Among cosmopolitan populations of bacteria, isolates of marine *Bacillus*, belonging to heterogeneous groups (both in phylogenetics and phenogenetics), are ubiquitous in the marine surroundings sustaining under adverse environmental conditions of salinity, pH, temperature and pressure (Rampelotto, 2010; Mondol *et al*., 2013). Their fastidious growth rate, nutritional and space competence with other species are some of the rationale at the rear of production of potent secondary compounds to ward off their competitors and evade micropredation (Sayem *et al*., 2011), diverging from their terrestrial counterparts in accordance with the prototype of metabolic successions by producing unique metabolites, manifested from their genomic revisions (Feling *et al*., 2003; Blunt *et al*., 2015).

In total, 15 isolates were isolated from the sea water and maintained on Zobell marine agar medium. The morphologies pertaining to their size, shape and color were analyzed along with the screening results by cross streaking method depicting one of the isolates, based on various biochemical tests (Table 1) and Identax result interpretations, found to be *Bacillus aquimaris* with 98% identification score. A diverse range of varied structural compounds were reported by LC-MS chromatogram of the methanolic extract of *B. aquimaris* (Fig. 1, Table 2). A total of 11 major peaks of the 25 peaks were detected, each corresponding to the type of organic compounds present in the bacterial extract. The major peak at m/z value of 138.0 corresponds to compound 4-(Pyrrolidinyl) but-2-en-4-ol followed by Butanoic acid, pentyl ester at m/z value of 156.0, Ethyl 3-hydroxy-4,4-dimethyl

pentanoate at 177.1, Ethyl (trans)-4-(benzyloxy)-8-ethoxy-5-oxaspiro[2.5] oct-6-ene-6-carboxylate at 302.0. The compounds of (5-Bromo-2-hydroxy-phenyl)-(1-phenyl-1H-pyrazol-4-yl) ketone and 2-{2-bromo-5H-indolo[2,3-b]quinoxalin-5yl}-1-(2,4-dichlorophenyl) ethan-1-one were detected at m/z values of 320.0 and 444.8, respectively. The minor peaks of other specific compounds were also present indicating a complex of diverse organic compounds present in the extract which may wield combinatorial bactericidal effects, thus, augmenting a strategic and tactical progression towards applicative pharmaco-dynamics (Yalla *et al*., 2018; Cherian *et al*., 2018). Furthermore, the methanolic extract of *Bacillus aquimaris* demonstrated antibacterial activity with variable inhibition zones against tested pathogens (Fig. 2 & 3). A moderate inhibition zone of 17.10mm was observed against *Vibrio cholerae* followed by an inhibition zone of 16.66mm against *Aeromonas hydrophila* at 200µl extract. The 200µl extract (50mg/ml concentration) showed maximum inhibition zone of 23.33mm against *E. coli* and the minimum inhibition zone of 12.0mm against *Shigella dysenteriae*. Inhibitory zones were not observed in the case of negative control pure methanol.

Production of antimicrobials provides an additive advantage to the producer strains as the former serves as one of the defense pathways in sustaining and protecting their natural niches from the intrusion of invasive microbial species. Apart from ecological character, the biological molecules of potent activity can also be harassed for the benefits of human life. Barsby *et al.,* (2001) reported the activity of a linear peptide molecule, Bogorol A isolated from *Bacillus laterosporus*, against MRSA (Methicillin resistant *Staphylococcus aureus*) and VRE (Vancomycin resistant Enterococci). The cyclic peptide molecules of YM- 266183 and YM-266184 isolated from *Bacillus cereus* were found to be effective against pathogenic staphylococci and enterococci (Nagai *et al*., 2003; Suzumura *et al*., 2003). Similarly, Desjardine *et al.,* (2007) isolated a lipopeptide, Tauramamide from *Brevibacillus laterosporus* effective against *Enterococcus* species at the concentration of 0.1µg/ml. Zhang *et al.,* (2004) isolated three cytotoxic cyclopeptides (Mixirin A–C) of class iturin from marine *Bacillus* sp. obtained from Arctic pole mud and illustrated their inhibitory activity against human colon tumor cells (HCT-116). The compounds of cyclodepsi peptide in origin, Bacillistatin 1-2 secreted by *Bacillus silvestris* were found to be anti-tumor in nature against an array of tumor inducing factors like P388 (murine lymphocytic leukemia); MCF-7 (breast); BXPC-3 (pancreas); SF-268 (CNS); KM20L2 (colon); NCI- H460 (lung); DU- 145 (prostate) (Pettit *et al*., 2009). Li *et al.,* (2011) summarized the anti-cancer activity of compound Turnagainolide A–B (cyclic peptide in nature) against PI3K pathway. The compounds of class Glycolipopeptides, namely Ieodoglucomide A-B (239) isolated from marine *Bacillus licheniformis* (from Ieodo Reef sediments, South Korea) showed moderate in-vitro antimicrobial activity along with cytotoxicity against stomach cancer and lung cancer cell lines (Tareq *et al*., 2012).

Table 1. Table showing results of biochemical tests

Morphology	Result
Gram staining	+
Motility	+
Colour	Light pale yellowish
Biochemical tests	
Catalase	+
Nitrate	-
Citrate	--
Urease	-
Indole	-
H_2S	+
Methyl-Red	-
Voges-Proskauer	-
Esculin Hydrolysis	+
Growth at 0% NaCl	+
Growth at 3% NaCl	+
Growth at 6% NaCl	+
Growth at 8% NaCl	+
Growth at 10% NaCl	+
Growth at 15% NaCl	--
Growth at 4°C	-
Growth at 20°C	-
Growth at 35°C	+
Growth at 40°C	-
Growth at 50°C	-
Sugar fermentation:	
Sucrose	+
Dextrose	-
Lactose	-
Fructose	+
Sorbitol	-
Mannitol	-
Inositol	+
Mannose	+
Xylose	+
Arabinose	+
Species identified with % identity	***Bacillus aquimaris*, 98%**

Table 2. Table showing observed m/z values corresponding to each compound by LC-MS technique

S.No.	Observed m/z values	Compound	Molecular formula
1.	54.2	Unknown	-
2.	117.0	Carbamic acid, dimethyl-, ethyl ester	$C_5H_{11}NO_2$
3.	120.0	Unknown	-
4.	135.0	Unknown	-
5.	138.0	4-(Pyrrolidinyl) but-2-en-4-ol	$C_8H_{15}NO$
6.	152.0	1-Propanamine, N-nitro-N-propyl-	$C_6H_{14}N_2O_2$
7.	156.0	Butanoic acid, pentyl ester	$C_9H_{18}O_2$
8.	177.1	Ethyl 3-hydroxy-4,4-dimethylpentanoate	$C_9H_{18}O_3$
9.	302.0	Ethyl (trans)-4-(benzyloxy)-8-ethoxy-5-oxaspiro[2.5] oct-6-ene-6-carboxylate	$C_{19}H_{24}O_5$
10.	320.0	(5-Bromo-2-hydroxy-phenyl)-(1-phenyl-1H-pyrazol-4-yl) ketone	$C_{16}H_{11}BrN_2O_2$
11.	444.8	2-{2-bromo-5H-indolo[2,3-b]quinoxalin-5 yl}-1-(2,4-dichlorophenyl) ethan-1-one	$C_{22}H_{12}BrCl_2N_3O$

Yang *et al.,* (2002) reported anti-fungal and anti-human gastric tumor activities of the molecules of cyclic pepetides: Halolitoralin A (hexapeptide), Halolitoralin B and C (both tetrapeptides), isolated from *Halobacillus litoralis* YS3106 against *Candida albicans*, *Trichophyton rubrum* and BGC cell lines, respectively. The fermentation broth of *Bacillus mojavensis* B0621A isolated from *Pinctada martensii* (off Weizhou Islands, South China Sea) was found to be antifungal in nature (compound Mojavensin A, an iturinic lipopeptide) and inhibited the growth of HL-60 (Ma *et al*., 2012). Kalinovskaya *et al.,* (2013) isolated a glyceryl acid derived heptapeptide from marine species of *Paenibacillus profundus* Sl 79 found to be cytotoxic to SK-MEL-28 cell line along with growth inhibition of pathogenic species of *S. epidermis*, *S. aureus*, *Enterococcus faecium* and *B. subtilis*. Thus, the marine microbial entities (especially *Bacillus*) offer a plethora of potential secondary compounds displaying an expansive range of organic functions bestowing huge versatility in applicative modules of industrial and environmental interest, considering their mode and range of action against phytopathogens, foodborne flora and account of their safe use in food industry. Systematic approaches are need of the hour for the discovery and characterization of novel molecules.

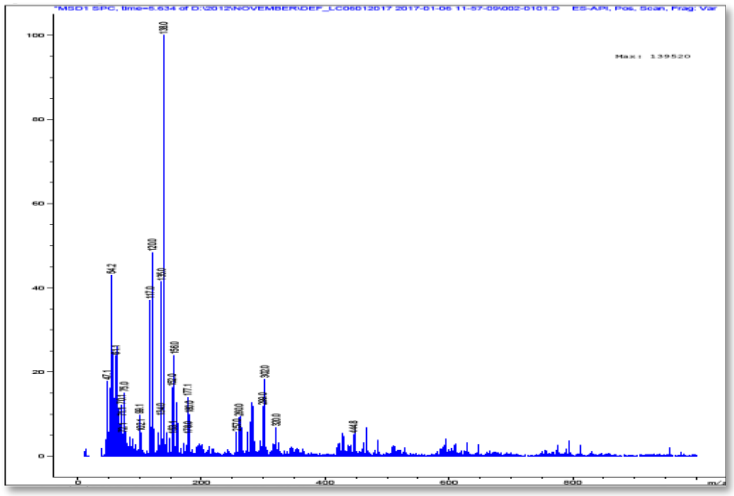

Figure 1. LC-MS chromatogram showing different peaks of compounds present in methanolic extract of *Bacillus aquimaris*.

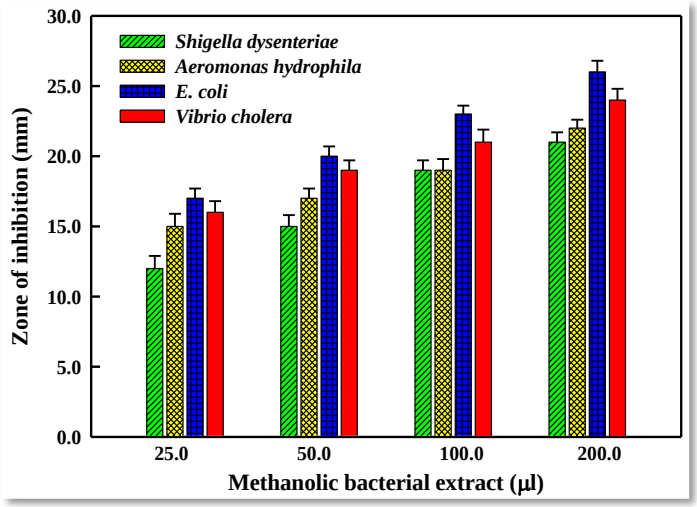

Figure 2. Comparative antibacterial activity of methanolic extract of *Bacillus aquimaris* in the concentration range (25-200 µl) against pathogenic strains**.**

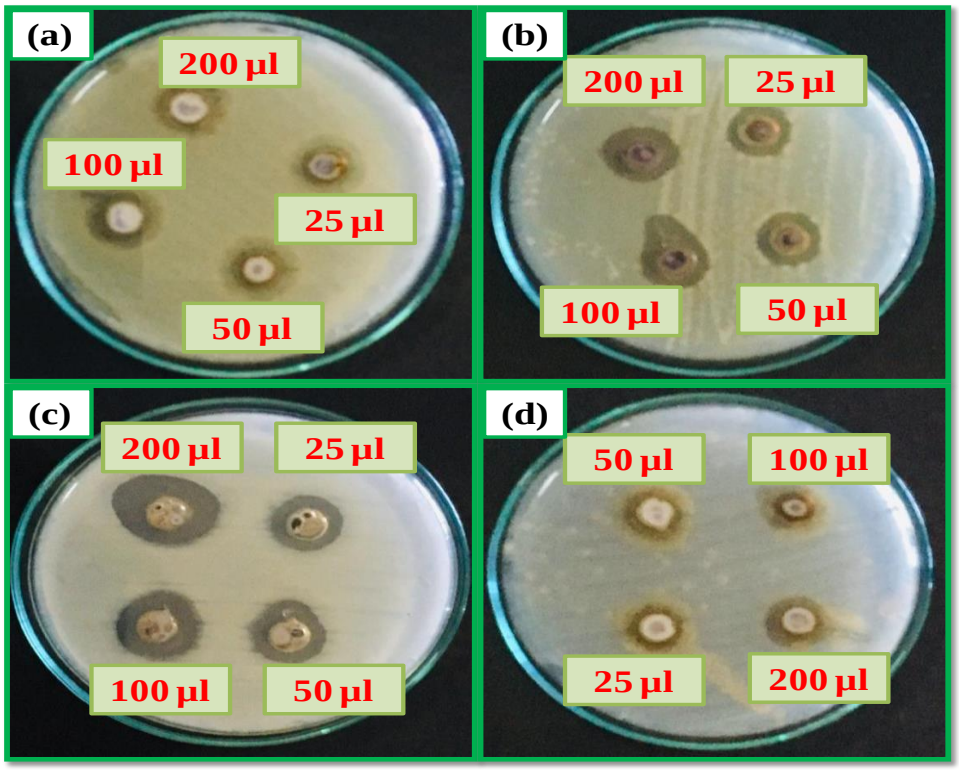

Figure 3. Assessment of antibacterial activity of methanolic extract of *Bacillus aquimaris* by well diffusion assay (a) *Aeromonas hydrophila*, (b) *Vibrio cholerae*, (c) *E. coli*, (d) *Shigella dysenteriae*.

Conclusion

The present study evidently exemplified the beneficial modules of marine bacteria *Bacillus aquimaris* (its methanolic extract) with emphasis of its antibacterial aspects on pathogenic strains clearly warranting further advanced inputs for its applicative strides in drug designing and pharmacology amendments. The comparative account of biosynthetic pathways, chemical nature of compounds and their activity coupled with implementations of genetic confirmation are warranted for 'safe and sound' usage in practical applications. Furthermore, the revisions on putative synergistic effects within these bio-active mixtures needs to be addressed as the concentration of compound(s) (purified or semi-purified) often remains vague or biologically immaterial.

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References

Agrawal, S, D Acharya, A Adholeya, CJ Barrow, and SK Deshmukh. "Nonribosomal peptides from marine microbes and their antimicrobial and anticancer potential." *Front. Pharmacol.* 8 (2017): 828. doi: 10.3389/fphar.2017.00828.

Barsby, T, MT Kelly, SM Gagné, and RJ Andersen. "Bogorol A produced in culture by a marine *Bacillus* sp. reveals a novel template for cationic peptide antibiotics." *Organ. Lett.* 3 (2001): 437–440. doi: 10.1021/ol006942q.

Bergey's manual of systematic bacteriology. Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman W. (Eds.) (2009) Volume 3.

Blunt, JW, BR Copp, RA Keyzers, M Munro, and MR Prinsep. "Marine natural products." *Nat. Prod. Rep.* 32 (2015): 116–211. doi: 10.1039/C4NP00144C.

Boottanun, P, C Potisap, JG Hurdle, and RW Sermswan. "Secondary metabolites from *Bacillus amyloliquefaciens* isolated from soil can kill *Burkholderia pseudomallei*." AMB Expr 7 (2017) :16, 1-11.doi: 10.1186/s13568-016-0302-0.

Chapin, KC. "Principles of stains and media, p. 182-191." In PR Murray, EJ Baron, JH Jorgensen, ML Landry, MA Pflaller (Eds.). Manual of Clinical Microbiology, 9th ed. (2007) ASM Press, Washington, DC.

Chen, XH, A Koumoutsi, R Scholz, A Eisenreich, K Schneider, I Heinemeyer, B Morgenstern, B Voss, WR Hess, O Reva, H Junge, B Voigt, PR Jungblut, J Vater, R Sussmuth, H Liesegang, A Strittmatter, G Gottschalk, R Borriss. "Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* Fzb42." *Nat Biotechnol* 25 (2007): 1007–1014.

Chen, XH, R Scholz, M Borriss, H Junge, G Mogel, S Kunz, R Borriss. "Difficidin and bacilysin produced by plant-associated *Bacillus amyloliquefaciens* are efficient in controlling fire blight disease." *J Biotechnol* 140 (2009): 38–44.

Cherian, T, K Ali, S Fatima, Q Saquib, SM Ansari, HA Alwathnani, AA Al-Khedhairy, M Al-Shaeri, J Musarrat. "*Myristica fragrans* bio-active ester functionalized ZnO nanoparticles exhibit antibacterial and antibiofilm activities in clinical isolates." *J. of Microbiol. Meth.* 166 (2019): 105716.

Cherian, T, W Jamal, SK Yalla, and R Mohanraju "One-pot green synthesis of biocompatible silver nanoparticles using leaf extract of *Piper nigrum*." *Int. J. of Pharm. and Biol. Sci.* 8 (2018): 1082-1088.

Desjardine, K, A Pereira, H Wright, T Matainaho, M Kelly, and RJ Andersen. "Tauramamide, a lipopeptide antibiotic produced in culture by *Brevibacillus laterosporus* isolated from a marine habitat: structure elucidation and synthesis." *J. Nat. Prod.* 70 (2007): 1850–1853. doi: 10.1021/np070209r.

Errington, J. (2003) "Regulation of endospore formation in *Bacillus subtilis*." *Nat Rev Microbiol* 1 (2003): 117–126.

Feling, RH, GO Buchanan, TJ Mincer, CA Kauffman, PR Jensen, W Fenical. "Salinosporamide A: A highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus *Salinospora*." Angew. Chem. Int. Ed. Engl. 20 (2003): 355–357.

Flores, O, LA Belanche, and AR Blanch. "New Multiplatform Computer Program for Numerical Identification of Microorganisms." *J. Clinic. Microbiol.* 47 (2009): 4133–4135.

Guo, Y, Z Yu, J Xie, R Zhang. "Identification of a New *Bacillus licheniformis* strain producing a bacteriocin-like substance." *J Microbiol* 50 (2012): 452–458.

Hamdache, A, A Lamarti, J Aleu, and IG Collado "Non-peptide metabolites from the Genus *Bacillus*." *J of natur. prod.* 74 (2011): 893–899. dx.doi.org/10.1021/np100853e.

Huang, T-P, DD-S Tzeng, ACL Wong, C-H Chen, K-M Lu, Y-H Lee, W-D Huang, B-F Hwang, K-C Tzeng. "DNA polymorphisms and biocontrol of *Bacillus* antagonistic to citrus bacterial canker with indication of the interference of phyllosphere biofilms." *PLoS ONE* 7 (2012) :e42124. doi:10.1371/journal.pone.0042124.

Kalinovskaya, NI, LA Romanenko, AI Kalinovsky, PS Dmitrenok, and SA Dyshlovoy. "A new antimicrobial and anticancer peptide producing by the marine deep sediment strain "*Paenibacillus profundus*" sp. nov.Sl 79." *Nat. Prod. Commun.* 8 (2013): 381–384.

Lemos, ML, AE Toranzo, LJ Barja. "Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds." *Microb Ecol.* 11 (1985): 149–163.

Li, D, G Carr, Y Zhang, DE Williams, A Amlani, H Bottriell *et al.*, (2011) "Turnagainolides A and B, cyclic depsipeptides produced in culture by a *Bacillus* sp.: isolation, structure elucidation, and synthesis." *J. Nat. Prod.* 74 (2011): 1093–1099. doi: 10.1021/np200033y.

Lindequist, U. "Marine-derived pharmaceuticals-challenges and opportunities." *Biomol. Ther*. 24 (2016): 561–571.

Ma, Z, N Wang, J Hu, and S Wang. "Isolation and characterization of a new iturinic lipopeptide, mojavensin A produced by a marine derived bacterium *Bacillus mojavensis* B0621A." *J. Antibiot.* 65 (2012): 317–322. doi: 10.1038/ja.2012.19.

Mondol, MAM, HJ Shin, and MT Islam. "Diversity of secondary metabolites from marine *Bacillus* species: chemistry and biological activity." Mar. Drugs 11 (2013): 2846-2872; doi:10.3390/md11082846.

Nagai, K, K Kamigiri, N Arao, K-I Suzumura, Y Kawano, M Yamaoka *et al.,* "YM-266183 and YM-266184, novel thiopeptide antibiotics produced by *Bacillus cereus* isolated from a marine sponge. I. Taxonomy, fermentation, isolation, physico-chemical properties and biological properties." *J. Antibiot.* 56 (2003): 123–128. doi: 10.7164/antibiotics.56.123.

Pettit, GR, JC Knight, DL Herald, RK Pettit, F Hogan, VJ Mukku *et al.,* "Antineoplastic Agents. 570. Isolation and structure elucidation of Bacillistatins 1 and 2 from a marine *Bacillus silvestris*." *J. Natl. Products* 72 (2009): 366–371. doi: 10.1021/np800603u.

Ramli, NS, C Eng Guan, S Nathan, J Vadivelu. "The effect of environmental conditions on biofilm formation of *Burkholderia pseudomallei* clinical isolates." PLoS ONE 7 (2012): 6.

Rampelotto, PH. "Resistance of microorganisms to extreme environmental conditions and its contribution to astrobiology." *Sustainability* 2 (2010): 1602–1623.

Ray, GC. "Ecological diversity in coastal zones and oceans." In Biodiversity. Ed. (1988) E. O. Wilson. National Academy Press, Washington, DC.

Romano, G, M Costantini, C Sansone, C Lauritano, N Ruocco, and A Ianora (2017) "Marine microorganisms as a promising and sustainable source of bioactive molecules." *Mar. Environ. Res.* 128 (2017): 58–69.

Sansinenea E, Ortiz A (2011) Secondary metabolites of soil *Bacillus* spp. Biotechnol Lett 33:1523–1538.

Suzumura, K-I, T Yokoi, M Funatsu, K Nagai, K Tanaka, H Zhang *et al.,* "YM-266183 and YM-266184, novel thiopeptide antibiotics produced by *Bacillus cereus* isolated from a marine sponge II. Structure elucidation." *J. Antibiot.* 56 (2003): 129–134. doi: 10.7164/antibiotics.56.129.

Tareq, FS, JH Kim, MA Lee, H-S Lee, Y-J Lee, JS Lee *et al.,* "Ieodoglucomides A and B from a marine-derived bacterium *Bacillus licheniformis*." *Org. Lett.* 14 (2012): 1464–1467. doi: 10.1021/ol300202z.

Xie, J, R Zhang, C Shang, Y Guo. "Isolation and characterization of a bacteriocin produced by an isolated *Bacillus subtilis* Lfb112 that exhibits antimicrobial activity against domestic animal pathogens." *Afr J Biotechnol* 8 (2009): 5611–5619.

Yalla, SK, T Cherian, and R Mohanraju. "Antimicrobial potential of secondary metabolites extracted from *Vibrio furnissii*, a luminescent bacterium associated with squid, *Uroteuthis duvauceli*." *Int. J. of Pharm. and Biol. Sci*. 8 .1 (2018): 530-534.

Yang, L, R-X Tan, Q Wang, W-Y Huang, and Y-X Yin. "Antifungal cyclopeptides from *Halobacillus litoralis* YS3106 of marine origin." *Tetrahedron Lett.* 43 (2002): 6545–6548. doi: 10.1016/S0040-4039(02)01458-2.

Zhang, HL, HM Hua, YH Pei, and XS Yao. (2004) "Three new cytotoxic cyclic acylpeptides from marine *Bacillus* sp." *Chem. Pharm. Bullet.* 52 (2004): 1029–1030. doi: 10.1248/cpb.52.1029.

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