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Antimicrobial polyketide furanoterpenoids from seaweed-associated heterotrophic bacterium *Bacillus subtilis* MTCC 10403

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ABSTRACT

Brown seaweed *Anthophycus longifolius* (Turner) Kützing (family *Sargassaceae*) associated heterotrophic bacterium *Bacillus subtilis* MTCC 10403 was found to be a potent isolate with broad range of antibacterial activity against important perceptive food pathogens *Vibrio parahaemolyticus, V. vulnificus,* and *Aeromonas hydrophila*. This bacterium was positive for polyketide synthetase gene (KC589397), and therefore, was selected to bioprospect specialized metabolites bearing polyketide backbone. Bioactivity-guided chromatographic fractionation of the ethyl acetate extract of the seaweed-associated bacterium segregated four homologous polyketide furanoterpenoids with potential antibacterial activities against clinically important pathogens. The minimum inhibitory concentration (MIC) assay showed that the referral antibiotics tetracycline and ampicillin were active at 25 μg/mL against the test pathogens, whereas the previously undescribed (4*E*)-methyl 13-((16-(furan-2-yl) ethyl)-octahydro-7-hydroxy-4-((*E*)-23-methylbut-21-enyl)-2*H*-chromen-6-yl)-4-methylpent-4-enoate (compound **1**) and methyl 3-(hexahydro-9-((*E*)-3-methylpent-1-enyl)-4*H*-furo[3,2-g]isochromen-6-yl) propanoate (compound **3**) displayed antibacterial activities against the test pathogens at a lesser concentration (MIC < 7 μg/mL). The title compounds were characterized by comprehensive nuclear magnetic resonance and mass spectroscopic experiments. Polyketide synthase catalyzed putative biosynthetic mechanism additionally corroborated the structural ascriptions of the hitherto undescribed furanoterpenoids from seaweed-associated bacterial symbiont. The electronic and hydrophobic parameters appeared to hold a conspicuous part in directing the antibacterial properties of the compounds. Seaweed-associated *B. subtilis* MTCC 10403 demonstrated to represent a potential source of antimicrobial polyketides for pharmaceutical applications.

1. Introduction

Polyketide classes of compounds are structurally divergent molecules with potential pharmacological properties, and were reported to occur in microorganisms and higher organisms (Winter et al., 2016). Varying sequences of molecular condensation, β-ketoreduction, dehydration, and chain elongation of the acyl-coenzyme A elementary units in the catalytic motif of polyketide synthetase gene (*pks*) result in the biosyntheses of convoluted polyketide compounds with unique carbon architecture (Hertweck, 2009). Marine natural products with polyketide frameworks are valuable sources of previously undescribed pharmacophore leads (Chakraborty et al., 2016). This group of compounds can potentially bind to the target enzymes required to exhibit the bioactivities, and were therefore, found potential applications in the discovery of more effective pharmacophores (Driggers et al., 2008; Kazlauskas et al., 1982; Stout et al., 2009). The specialized metabolites of microorganisms living in extraordinary state of conditions, such as symbiotic association with higher organisms, particularly of marine origin, have recently developed into a formidable origin of biomedically important pharmacological leads. Even though marine microbial flora is known to be an attractive producer of diverse bioactive natural products, they are less characterized in comparison to the terrestrial microbial population (Uzair et al., 2008). Recently, seaweed-associated symbiotic bacterial communities have gained the attention of researchers with regard to their ability to produce various bioactive compounds of polyketide origin with biotechnological and pharmaceutical interests (Chakraborty et al.,

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2016; Gomez et al., 2010). Seaweed-associated bacterial flora was reported as potential sources to isolate valuable antimicrobial metabolites to control deleterious human food pathogenic strains (Ben Ali et al., 2012; Goecke et al., 2010). The seaweed surfaces were found to be rich in symbiotic bacteria having potential antimicrobial properties, which provide the host with bacterial-mediated defense properties.

Seaweed-bacterial affiliations are interesting in ecological perspectives to comprehend the biosynthesis of the bioactive compounds within the bacterial cell as well as their biological and chemical effect on seaweed bacterial association (Goecke et al., 2010). Reports of microbial origin of eukaryotic bioactives make them an interesting research area (Kubanek et al., 2003; Quevrain et al., 2014). Heterotrophic symbiotic bacteria associated with eukaryotes were believed to be included in the synthesis of several lead molecules with significant biological activities (Kubanek et al., 2003). Increasing reports of seaweed-associated bacterial isolates with antimicrobial activity revealed their importance to isolate pharmacologically active microbial specialized metabolites (Kanagasabhapathy et al., 2008; Penesyan et al., 2009). Investigations of seaweed-bacterial affiliations linger behind those of other marine eukaryotes (Goecke et al., 2010). Among the reported seaweed-associated bioactive bacterial population, a major number falls under *Bacillus* sp (Chakraborty et al., 2016; Kanagasabhapathy et al., 2008). *Bacillus* strains and their metabolites were reported to find potential applications in food and pharmaceutical applications due to their inhibitory activities against the seafood pathogens and their GRAS (generally recognized as safe) status (Baruzzi et al., 2011).

In the course of our ongoing exploration for previously undescribed pharmacophores with potential antibacterial activities against human food pathogenic bacteria from seaweed-associated bacteria collected off the Palk Bay of southeastern coast of Indian subcontinent, we have isolated and characterized four previously undescribed polyketide furanoterpenoids from the ethyl acetate extract of the brown seaweed *Anthophycus longifolius* (Turner) Kützing (family *Sargassaceae*) associated heterotrophic bacterium *Bacillus subtilis* MTCC 10403 (Fig. 1A–B). The previous studies carried out at our laboratory demonstrated that the seaweed associated Gram-positive bacteria belonging to the phylum Firmicutes (*Bacillus* spp) have been found their prominent place as a source of bioactive specialized metabolites of polyketide class of chemistry, connecting the hierarchy of actinobacteria and pseudomonads (Chakraborty et al., 2016; Thilakan et al., 2016). In view of this, the present study described the isolation, structural characterization, and antibacterial evaluation of the title compounds against important perceptive food pathogens *Vibrio parahaemolyticus, V. vulnificus, V. alginolyticus* and *Aeromonas hydrophila*.

Furanyl derivatives were considered as valuable intermediates to synthesize various bioactive lead molecules of clinical significance due to their comparatively lesser resonance energy (<20 kcal mol^{-1}) than other heterocycles, such as arene (36 kcal mol^{-1}), thiofuran (30 kcal mol^{-1}) and azole (20 kcal mol^{-1}) facilitating the thermodynamically favorable unambiguous transformation of furan containing compounds to non-aromatic derivatives (Wright, 2001). This group of heterocyclic compounds was accounted for to be connected through certain oxidative changes resulting in potential bioactivities (Roethle and Trauner, 2008). Furanosesterterpenes extracted from marine sponge *Psammocinia* sp was accounted for to be cytotoxic (Choi et al., 2005). There were reports of terpenoids belonging to tetraketide class of chemistry from fungal origin (Geris and Simpson, 2009). Previous literature reported that the *O*-heterocyclic compounds were endowed with potent antibacterial activities (Dodd et al., 1950). Furan bearing drugs, such as fumoxicillin, furazolidone, nifurquinazol were demonstrated as potential antibacterial agents (Banerjee et al., 2012). Furanoterpenoids were reported to occur in the corals and marine sponges (Faulkner, 1996). There were examples of furanated polyketides, such as plakorfuran A from the marine sponge *Plakortis simplex* (Liu et al., 2012), bicyclic furanolactones from *Plakortis* sp (Yong et al., 2011) and 5-hydroxy-3-methyl-4-(1-hydroxylethyl)-furan-2(5*H*)-one from the halotolerant fungus *Myrothecium* sp. GS-17 (Liu et al., 2013). Hyafurones A1–B were isolated from the myxobacterium *Hyalangium minutum* (Okanya et al., 2014). However, there were no reports of this group of compounds in the seaweed associated heterotrophic bacteria.

Bacteria associated with seaweed were found to be rich in

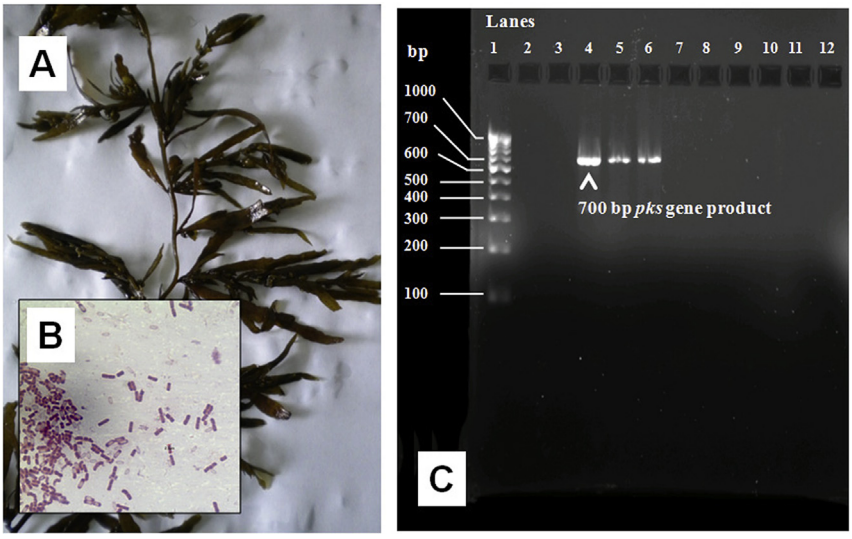

Fig. 1. (**A**) Thalli of red seaweed *A. longifolius*. (**B**) Gram staining photomicrograph of *B. subtilis* MTCC 10403 associated with *A. longifolius*. (**C**) Electrophoresis image of *PKS* gene profiles of the antagonistic isolates from seaweed on agarose gel. Lane 4 shows positive hit for *B. subtilis* MTCC 10403. Lane 1 shows the molecular marker used (Gene Ruler™ 100 bp DNA Ladder, Thermo Scientific, 100 bp-1000 bp). Lanes 2–3 show negative control (in duplicate). Lanes 5–6 show positive control (in duplicate). Lane 7 shows negative hit of the KS specific primer for *B. subtilis* MTCC 10407[B]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)