Short Communication

Cytotoxic potential of Nephthea sp.-derived actinomycetes supported by metabolomics analysis

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Abstract
Soft corals and associated microorganisms are known to produce leads for anticancer drugs. Keeping this in mind, Nephthea sp.; a Red Sea soft coral was investigated for the first time using the OSMAC approach. Two isolates, Streptomyces sp. UR63 and Micrococcus sp. UR67 were identified. Their extracts revealed the presence of alkaloids, macrolides, quinones, fatty acids and terpenoids. Further comparison through a set of multivariate data analyses revealed their unique chemical profiles. The extracts displayed inhibitory potencies against HepG-2, Caco-2 and MCF-7 tumor cell lines with IC50 values ranging from 11.4 to 38.7 μg/mL when compared with the positive control, doxorubicin. The study not only highlights the cytotoxic potential of soft coral-associated actinomycetes but also shows the advantage of using the OSMAC approach in this regard.

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1. Introduction

Marine environments are one of the most diverse ecosystems with a large assortment of different life forms (Huang et al. 2021). Various organisms occupying these environments have been appreciated as a prime source of valuable natural products with diverse structural features and therapeutic applications (Nie et al. 2018; Shaaban et al. 2021). Among them, soft corals belonging to the genus *Nepthea* have attracted considerable interest as a rich biochemical warehouse of several bioactive metabolites, e.g., steroids, terpenoids, quinones, nitrogenous compounds and fatty acids that demonstrated a wide spectrum of biological properties, e.g. anti-inflammatory, anticancer, antimicrobial, antidiabetic and antifouling activities (Abdelhafez et al. 2019, 2020; Abdelhafez, Fahim, El Masri et al. 2021; Abdelhafez, Fahim, Mustafa et al. 2021). Coral-associated microbial aggregates are also another promising source of chemically varied compounds with antiviral, antibacterial, antiproliferative and antifouling activities, among many others (Hou et al. 2019; Sang et al. 2019; Sharma et al. 2019; Qin et al. 2021). In this regard, research studies on different marine-derived *Streptomyces* species have reported the presence of a diverse pool of secondary metabolites of agricultural and medical value, e.g. antibacterial, antifungal and antitumor effects (Cao et al. 2019; Yang et al. 2020; Luo et al. 2021; Peng et al. 2021). Likewise, marine bacteria of the genus *Micrococcus* have also provided a number of chemical entities with cytotoxic and antibacterial potential (Kumari et al. 2020). On the other hand, the one strain many compounds (OSMAC) approach has emerged as a privileged strategy for expanding drug discovery from microbial sources (Gamaleldin et al. 2020). This technique has been effectively applied to obtain a wide array of medicinally important microbial metabolites, allowing the opportunity to produce new chemical entities from a single microbial strain by culturing in different media under different culture conditions (Meng et al. 2017; Romano et al. 2018).
Cancer is ranked among the major life-threatening pathologies worldwide, with an increasing number of cases reported annually as a result of environmental changes and life-style modernisation (Pu et al. Forthcoming). Recent epidemiological studies have also indicated that lung and breast cancers are the most frequently diagnosed types and the leading triggers of cancer-related deaths in men and women, respectively (Johnson et al. 2021). To date, as potent marine supplies of anticancer leads, a large variety of natural metabolites with praiseworthy potential against different cancer cells has been reported from various *Nephthea* species (Abdelhafez et al. 2019); however, the chemical and biological traits of their associated bacteria has not been deliberated yet. Inspired by this, and as part of our ongoing research interest in the genus *Nephthea* (Abdelhafez et al. 2019, 2020; Abdelhafez, Fahim, El Masri et al. 2021; Abdelhafez, Fahim, Mustafa et al. 2021), the current study describes the isolation and identification of two actinomycetes associated with the Red Sea soft coral *Nephthea* sp. for the first time, namely *Streptomyces* sp. UR63 and *Micrococcus* sp. UR67. The isolated bacterial strains were cultured using the OSMAC approach and their chemical profiles were explored by LC–MS-based metabolomics and compared by means of multivariate data analyses. The cytotoxic potential of the obtained bacterial extracts against a number of human cancer cells was also considered.

2. Results and discussion

The fermentation of the two actinomycetes strains, *Streptomyces* sp. UR63 and *Micrococcus* UR67 was carried out using the solid approach on four different media (M1, ISP2, Malt and agar with natural seawater), followed by extraction with ethyl acetate to afford eight different extracts. Various metabolites produced by the two strains under different culture conditions were investigated by LC–MS-based metabolomics, which showed the presence of diverse chemical classes like macrolides, alkaloids, quinones, fatty acids and terpenoids (Supplementary Table S1 and Figures S1–S3). Different multivariate data analyses (MVDA) were then carried out using the raw MS data (Supplementary Figures S4–S8), of which the principal component analysis (PCA) was performed to highlight the differences and similarities of the resulting extracts (Supplementary Figure S4). The obtained outliers were for the extracts of *Micrococcus* sp. on ISP2 and agar with natural seawater media (O3 and O4, respectively), indicating their unique chemical profiles. Therefore, the PCA scores plot revealed the variation of these culture extracts that was further confirmed by their unique patterns in the heat map plot (Figure 1). Moreover, in the partial least squares-discriminant analysis (PLS-DA), the variable importance in projection (VIP) (Supplementary Figure S8) demonstrated the metabolites responsible for such variations among these extracts; these compounds were further identified through the comparison with the MarinLit, DNP and METLIN databases (Supplementary Table S2 and Figure S6). As a result, comparing different samples (O7–O10) of *Streptomyces* sp. UR63 indicated a high degree of similarity in the metabolic patterns of the samples O7, O9 and O10 (using agar and natural seawater, ISP2 and M1 media, respectively), whereas the extract O8 of *Streptomyces* sp. UR63 grown on Malt was the most different one. On the other hand, analysis of *Micrococcus* sp. UR67 extracts (O3–O6) showed the comparable metabolic profiles of
O5 and O6 (using M1 and Malt media, respectively), whereas those of O3 and O4 (using ISP2 and agar with natural seawater media, respectively) were markedly different. Taken together, these findings highlighted the OSMAC approach as a powerful tool for the discovery of new microbial secondary metabolites.

On the other hand, investigating the cytotoxic potential of the resulting bacterial extracts against a number of tumor cell lines using the MTT viability assay showed that the crude extract of *Streptomyces* sp. UR63 cultured on agar with natural seawater exhibited potent inhibitory activities against MCF-7, Caco-2 and HepG-2 cells, with IC$_{50}$ values of 11.4, 12.6 and 13.2 μg/mL, respectively, whereas the crude extract of *Micrococcus* sp. UR67 cultured on the ISP2 medium displayed moderate cytotoxic effects against the studied cell lines, showing IC$_{50}$ values of 27.3, 20.4 and 17.5 μg/mL, respectively. However, the cytotoxic potential of different extracts was lower than that of the positive control, doxorubicin in terms of IC$_{50}$ values (1.72, 2.1 and 1.3 μg/mL, respectively) (Supplementary Table S3).

3. Conclusion

Metabolic profiling of the crude extracts of *Streptomyces* sp. UR63 and *Micrococcus* sp. UR67 derived from the soft coral *Nephthea* sp. showed their richness in several classes of metabolites, e.g., fatty acids, alkaloids, quinones and terpenoids. Exploring the metabolic differences of these actinomycetes cultivated on different media revealed the evident potential of the applied OSMAC approach to induce the production of diverse microbial secondary metabolites. Besides, different extracts from both bacterial strains exhibited varying *in vitro* inhibitory potencies against HepG-2, Caco-2 and MCF-7 tumor cells, which also highlighted the importance of coral-associated actinomycetes as a prolific source of cytotoxic metabolites for the discovery of potential anticancer drug leads.
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