

Supplementary Material

Article

Identification of the Antibacterial Compound Produced by the Marine Epiphytic Bacterium *Pseudovibrio* sp. D323 and Related Sponge-Associated Bacteria

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Purification and Chemical Characterisation of TDA

To further purify the antibacterial compound, the spent culture medium of overnight liquid culture of *Pseudovibrio* sp. D323 was exhaustively extracted, concentrated under vacuum, yielding an orange-red residue. The residue was partitioned in acidified 60% aqueous methanol (0.05% trifluoroacetic acid, TFA). The remaining residue was dissolved in acetonitrile and purified by high-performance liquid chromatography on an Alliance 2695 module (Waters) with photodiode array detection on a Phenomenex Prodigy ODS3 (150 × 4.6 mm, 5 µm). The column was run with a linear gradient of 30–80% acidified methanol (0.05% TFA) over 15 min at 0.9 mL/min. In total, 6 peak fractions were collected, concentrated and tested in the TCL-BOA. A dominant bioactive peak fraction eluted at 6.957 min. This fraction was re-chromatographed on a LCQ Deca-XP LC-UV-MS system (Thermo Finnigan) using a Phenomenex Gemini C18 column (50 × 4.6 mm, 5 µm) and a linear gradient of acidified acetonitrile (0.05% TFA) ranging from 30 to 60% in 10 min. The peak representing the antibacterial compound **1** (Figure 4) was detected both, in positive electrospray ionization mode (ESI⁺) and atmospheric pressure chemical ionization mode (APCI⁺). Data were collected from *m/z* 100 to 220. The UV spectrum of **1** was in accordance with literature data for tropodithietic acid (TDA) [1]. The ESI⁺-mass spectrum showed a molecular ion [M + H]⁺ of 212.88 Da and a dominant fragment ion [M + H – H₂O]⁺ at 194.89 Da. The APCI⁺ mass spectrum showed a

dominant molecular ion $[M + H]^+$ of 212.94 Da and a dominant fragment ion $[M + H - CO_2]^+$ at 169.15 Da. Collision induced MS^2 of the molecular ion in ESI^+ did not result in further fragmentation. These data were in direct accordance with the LC-MS data of TDA reported by Bruhn *et al.* [2].

For NMR analysis **1** was dissolved in DMSO- d_6 (^{13}C) or benzene- d_6 (1H). Samples were analysed on a Bruker DPX 300 MHz system. The ^{13}C -NMR spectra in DMSO displayed eight carbon signals with one α , β -unsaturated enolic carbon, one carboxylic carbon, six aromatic carbons including two carbons connected with heteroatoms. The 1H -NMR spectrum in benzene displayed three adjacent proton signals in a A,B,C spin pattern. Measured shifts of proton and carbon signals of **1** were in accordance with the data previously obtained for TDA [1].

References

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