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Experimental procedures

1. Bacterial strains

Sphingomonas sp. strain ACM-3962, obtained from Australian Collection of Microorganisms, was cultured in a recommended peptone yeast extract medium (299) at 28°C for 48 hours. After two days of incubation, cells were centrifuged and washed in PBS buffer. *Escherichia coli* strains DH5 α and BL21(DE3), which were used for cloning, plasmid propagation and expression of recombinant proteins, were grown at 37°C in LB broth supplemented with ampicillin (100 μg mL^{-1}) where indicated.

2. Construction of recombinant plasmid

The sequence coding for MlrC was amplified by PCR using genomic DNA from *Sphingomonas* sp. strain ACM-3962 genomic DNA as the template and the primers mlrCF (5'-GTTCCATATGCTTGATCGTCGAACATTG-3') and mlrCRok (5'-GAAAGCGGCCGCGACA GGCTCGAATGGCCAC-3'). The amplified fragments were inserted into the pTZ57R/T cloning vector (Fermentas) and the sequence was verified. Subsequently, the fragments were excised using the *Nde*I and *Not*I restriction enzymes, and inserted into the expression vector pET21a (Novagen). The resulting construct, named pET21-mlrC, encodes MlrC with a polyhistidine tag at the C-terminal end of the protein.

3. Expression and purification of recombinant MlrC

The construct pET21-mlrC was transformed into *E. coli* BL21(DE3), and bacteria were plated on LB agar plates supplemented with ampicillin (100 μg mL^{-1}). Freshly transformed colonies were inoculated into LB medium supplemented with the same concentration of ampicillin, and grown at 37°C until an absorbance at 600 nm of 0.8 was reached. The temperature was then decreased to 30°C, recombinant expression was induced by the addition of isopropyl-beta-D-thiogalactopyranoside (IPTG) at a final concentration of 1 mM, and the cells were cultured for an additional 4 h. Subsequently, the bacteria were harvested by centrifugation (15,000 *g*, 20 min, 4°C). The pellet was suspended in lysis buffer (100 mM sodium phosphate, 10 mM Tris-HCl, 6 M guanidinium hydrochloride, pH 8.0), and the cells were disrupted by sonication. Recombinant MlrC was purified using NiTNA columns (Qiagen) under denaturing conditions, according to the protocol recommended by the supplier. The recombinant protein was refolded by dialysis against 50 mM sodium phosphate pH 8.0, 150 mM NaCl, and 10% glycerol.

4. MlrA activity assay

The linear MC variants, MC-LR and dmMC-RR, were produced by 24 h of incubation of MCs (100 μg mL–1) with recombinant MlrA as described by Dziga et al.[7]. The purified MlrC was used to check enzymatic activity towards these acyclic molecules. A total of 10 μL of different dilutions of enzyme preparations were added to 90 μL of MC solution. The enzyme and the linear MCs were suspended in PBS buffer (pH 7.0). The final concentration of the substrate was 1 μg mL^{-1} or higher if it was necessary to collect larger amounts of product. The incubation temperature was 20°C, and the reaction was stopped after 1 h by addition of 10 μL of 1% trifluoroacetic acid (TFA). Samples were cooled and analyzed using HPLC and/or MS.

5. HPLC and MS assays

All analyses involving HPLC, including primary identification of the products and determination of hydrolysis rates, were performed using a Waters HPLC system consisting of a 600E multisolvent-delivery system, a 717plus autosampler, a 996 photodiode array detector (PDA), Millenium^{32} SS software, and a Jetstream 2 plus column thermostat. The degradation products were quantified using a Purospher STAR RP-18 end-capped column (55 × 4 mm, 3 μm particles), as described by Meriluoto and Spoof (2005).[10] The mobile phase consisted of a gradient of 0.05% aqueous TFA (solvent A) and 0.05% TFA in acetonitrile (solvent B) with the following linear gradient program: 0 min 25% B, 5 min 70% B, 6 min 70% B, and 6.1 min 25% B.

MS analyses were performed using a Bruker Daltonics HCT Ultra Ion Trap MS with an electrospray (ESI) ion source operated in the positive electrospray ion mode. The drying gas temperature and flow rate were set at 350°C and 8 L min^{-1}, respectively. The Purospher STAR RP-18 end-capped column (55 × 4 mm, 3 μm particles) was kept at 40°C.

For LC-MS analysis, the mobile phase consisted of a gradient generated by mixing 0.1% aqueous formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) according to the following linear gradient program: 25% B to 70% B over 5 min, then to 90% B over 2 min, where it was held for 1 min.

6. Toxicity assay of Adda

The toxicity of the Adda obtained from HPLC separation was established by the PP1 inhibition assay described by An and Carmichael (1994).[11] The concentration of the MC-LR standard used to prepare the calibration curve ranged between 0.125 and 4.0 μg L^{-1}, whereas the concentration of Adda ranged between 2.5 μg L^{-1} and 200 μg mL^{-1}. This enabled calculation of the IC_{50} value for Adda.

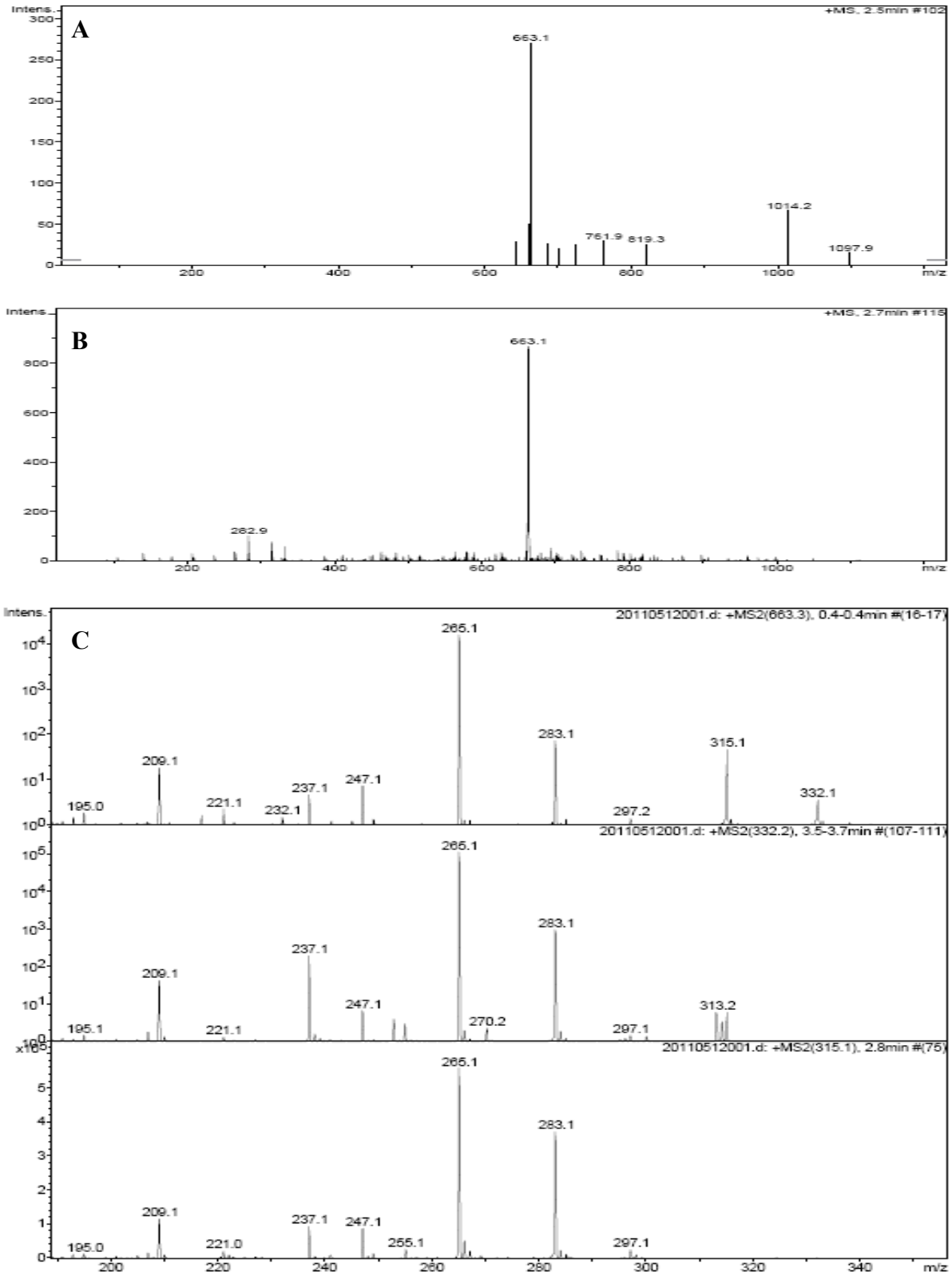

Figure 1S. MS spectra of the hydrolysis products of MlrC activity of linear MC-LR (A) and linear dmMC-RR (B), and fragmentation pattern of m/z 663.2 ion (C).