Targeted analysis and profiling of prostaglandins produced by shrimp cyclooxygenase enzyme using ultra high-performance liquid chromatography-tandem mass spectroscopy

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Abstract: Cyclooxygenase (COX) enzyme converts arachidonic acid (ARA) into prostaglandin H₂, which is catalyzed to prostaglandin E₂ (PGE₂) and prostaglandin F₂α (PGF₂α) via downstream enzymes. Here, the enzymatic function of the Penaeus monodon COX (PmCOX) was studied due to its involvement in shrimp ovarian development. PmCOX was expressed in 293T cells and 10 µM ARA was supplemented as substrates. The cell culture supernatant was subsequently extracted with ethyl acetate. The extracted metabolites were separated on an Acclaim¹¹¹¹ C18 column (50 mm × 2.1 mm, 2 µm) using 0.01% (v/v) acetic acid in water/acetonitrile (70:30, v/v) as mobile phase at flow rate of 0.4 mL/min. Detection of all metabolite was monitored in negative ion mode using selected ion monitoring (SIM). The SIM scans were used for profiling and the MS2 scans were used for identification. PGB₁ was used as the internal standard. Detection of PGF₂α, PGE₂, PGB₁ and ARA employed ions with m/z of 353.2327, 351.2169, 335.2220 and 303.2220, respectively. PmCOX activity was verified based on the presence of PGF₂α and increasing amount of PGE₂ in the PmCOX expressing cells. The optimized extraction and UPLC-MS/MS analysis will be used to examine prostaglandin profiles in shrimp tissues in future studies.

Keywords: Prostaglandins; Arachidonic acid; Cyclooxygenase; Liquid-liquid extraction; Ultra high-performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS)