Measurement of G. aculeatus digestive enzymes (trypsin, amylase and intestinal alkaline phosphatase)

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Abstract

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Materials

0.01M Tris-HCl, pH 7 by Sigma Aldrich

Tricaine methanesulfonate MS222 by Sigma Aldrich

3 mM, N-benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA) by Sigma Aldrich

Alpha-Amylase reagent 984329 by Thermo Scientific

Alkaline phosphatase reagent TR11320 by Thermo Scientific

Protocol

Fish sacrifice and organ removal:

Step 1.

(a) After 24h starvation period, sacrifice fish after anesthesia with tricaine methanesulfonate MS222 (70 mg. L-1, SIGMA-ALDRICH, France).

(b) Remove the whole digestive tract on ice, and then clean it from exterior fat.

(c) Open longitudinally the digestive tract and rinse with cold Tris-HCl buffer (0.01 M, pH 7, SIGMA-ALDRICH, France).

(d) Store immediately the digestive tract in liquid nitrogen and then at -80 °C, until extraction step.

Digestive enzyme extraction:

Step 2.

(a) Grind digestive tracts with ceramic (3 mm Ø) and glass (1mm Ø) beads in cold Tris-HCl buffer (0.01 M, pH7), using PRECELLYS24® homogenizer (BERTIN TECHNOLOGIES, France), at 5,500 rpm 2x10 sec.

(b) Centrifuged at 15,000 x g for 30 min at 4°C.

(c) Recover the supernatant and store it at -80°C until analysis.

Digestive enzyme analysis:

Step 3.

a) Measurements of amylase and intestinal alkaline phosphatase (IAP) activity levels are performed according to Junge et al., (2001) and Panteghini and Bais (2008), respectively, using Thermo-Scientific Gallery ready-to-use reagents.

(b) Trypsin activity measurements are performed according to the Garcia-Carreño and Haard (1993) method, using N-benzoyl-DL-arginine 4-nitroanilide hydrochloride (BAPNA, 3 mM) as a substrate.

(c) All enzymatic assays are performed at 37 °C on the Gallery™ Automated Photometric Analyzer (Thermo Fisher Scientific Oy), by kinetic colorimetric assay at 405 nm.

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