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Preliminary Report on First Database of Useful Protein Substances from Discus Fish Epidermal Mucus (*Symphysodon* spp.) Utilizing the Proteomics Platform

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Introduction

Many times, fish epidermal mucus has been reported as an essential component for fishes in the area such as mechanical protection, gas transportation, and osmoregulation (Shephard, 1994). Discus fish is a cichlid species demonstrating fry feeding behavior via the parental mucus secretion (Noakes and Barlow, 1972). This species also an important ornamental species with global trading status and is currently being produced locally. However, discus is also prone to diseases infection, with huge losses reported in discus farms due to mortality from diseases outbreak. Proteomics has emerged as an important tool to rapidly unlock the overall proteome or protein expression of a particular cell, tissue or organ system. Since a simple linear relationship between gene and final protein product do not exist due to process such as post-translational modifications, proteomics offers to look into the final product of gene for various physiological processes. Martin S.A.M *et. al* (2001) also has adopted proteomics methodology to analyze the proteome of rainbow trout (*Oncorhynchus mykiss*) liver proteins during a short period of starvation. Thus, it shows proteomics is commonly adopted for fisheries research in the post-genomic era.

Materials and Methods

Protein samples preparation

Fish mucus sampled from the dorsal portion of fish skin surface was centrifuged at 13,200rpm, 4°C for 20 minutes. The supernatant was desalted using Micro BioSpin Column 6 (BIORAD®) followed by freeze-drying. Dried mucus then re-dissolved with cold deionized distilled water prior to sample clean-up to reduce detergents and reductants which will affect the isoelectric (IEF) part later. Sample clean-up was done by using 2D Clean-Up Kit (BIORAD®). Pellet resulted from the clean-up step was resuspended with rehydration buffer (8M Urea, 50mM DTT, 4% CHAPS, 0.2% Carrier Ampholyte, 0.0002% Bromophenol Blue and deionized distilled water [18mΩ]) and RC DC Protein Assay (BIORAD®) was carried out to determine the amount protein to load onto the IPG strips.

2D-electrophoresis

Briefly, isoelectric focusing (IEF) was done by starting with passive in-gel sample rehydration for 16 hours. Protein samples were applied onto the pH3-6NL IPG strips. IEF was done in three steps which are 250V for 20 minutes (linear), 10,000V for 2.5 hours (linear) and 10,000V, 40,000 volt-hours for about 4 hours. After IEF, IPG strips were equilibrated with Urea, 20% SDS, 1.5M Tris-HCl (pH8.8), 50% Glycerol, ddH_2O and 2% DTT followed by second equilibration with the same buffer content except that 2.5% iodoacetamide substitute the 2% DTT. Equilibrated strips were applied on a SDS-PAGE large gel (12.5%) and the second dimensional electrophoresis was carried out at the constant ampere of 16mA/gel for the first 30 minutes and carry on until the end with 24mA/gel in a BIORAD® Protean II XL apparatus. Gels were stained with silver nitrate.

As for preparative gel, 455ug of protein sample for both parental and non parental mucus was loaded onto the pH3-6NL IPG strips. Gels were stained with Coomassie Brilliant Blue.