TITLE: COMPARATIVE PROTEOMIC ANALYSIS OF *Streptococcus agalactiae* ISOLATES REVEALS DIFFERENCES BETWEEN HUMAN AND FISH STRAINS

AUTHORS: Tavares, G.C.[1], Barony, G.M.[1], Pereira, F.L. [1], Rezende, C.P. [1], Leal, C.A.G. [1], Figueiredo, H.C.P.[1]

INSTITUTION: [1] AQUACEN – National Reference Laboratory of Aquatic Animal Diseases, Ministry of Agriculture, Livestock and Food Supply (Veterinary School, Federal University of Minas Gerais, Belo Horizonte, MG 30123-970, Brazil).

ABSTRACT:

Streptococcus agalactiae has caused outbreaks of septicaemia and meningitis in human newborns and meningoencephalitis in fish. The aims of this study were to evaluate the protein expression among the main genotypes of *Streptococcus agalactiae* isolated from diseased fish in Brazil using a label free shotgun LC-UDMS^E approach and to compare the differential expression of proteins identified between fish and human strains. Six strains were used: 5 fish strains belonging to sequence typing ST-260, ST-927 or non-typeable (NT) lineage and 1 human strain (NEM316). Biological triplicates of each bacteria were cultured for protein isolation and analyzed by LC-MS using nanoAcquity UPLC 2D system coupled to a mass spectrometer. Raw data were processed with the software Progenesis QIP. *In silico* analysis were performed to predict subcellular localization, orthologous group by functional category and pathogenicity. In total, 1070 protein clusters were identified. Of these, 989 protein clusters were identified in all fish strains (core proteome), 62 proteins in 2, 3 or 4 strains simultaneously (accessory proteome), 1, 2, 2, 4 and 5 proteins were exclusively expressed in SA16 (NT), SA20 (NT), SA53 (ST-260), SA81 (NT) and SA95 (ST-927) respectively. Therefore, 1065 protein clusters corresponded to pan-proteome of fish strains. SurfG+ identified 885 proteins cytoplasmics, 99 surface-exposed, 57 membrane and 29 secreted. Translation/ribosomal structure, general function prediction only and amino acid metabolism were most common in functional classification of COG. Proteins involved in stress response, regulation of gene expression, metabolism and virulence (especially adhesion, invasion and immune evasion) were identified in core proteome. Proteins present in all putative pathogenicity island predicted by GIPSy tool were also identified. Evaluating the protein expression among different host, 5 and 26 proteins exclusively expressed in NEM316 and fish strains, respectively, were identified. Furthermore, 216 and 269 proteins from fish strains were up- and down-regulated, respectively, in comparison to human isolate. Our study showed that core proteome of fish strains was conserved, demonstrating a high similarity of expressed proteins, main between NT strains. Regardless the similarity in protein content, the global protein expression of NEM316 was different from fish strains and suggests distinct adaptations to mammal and fish host at regulatory level.

Keywords: Streptococcosis, fish, human, genotype, proteome

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