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Proteomic typing of invasive group B *Streptococcus* isolated from humans and fish (*Oreochromis* spp.; Pisces) in Malaysia

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Abstract

Streptococcus agalactiae, also called Group B *Streptococcus* (GBS), is a major cause of several infectious diseases in humans and fish. However, the likelihood of GBS transmission between different host species leading to a potential zoonotic problem is less well studied. Understanding the proteomics of GBS is crucial for elucidating its zoonotic potential and developing effective diagnostic and prevention strategies. This study utilized comparative proteomic analysis using LC–MS/MS technique to investigate the similarities and differences between GBS isolates from human and fish sources. The core proteome was found to be highly conserved, with 82.5% of the 1405 identified proteins shared between the two groups. Functional categorization using the Cluster of Orthologous Genes (COG) database revealed that the majority of the proteins were involved in various metabolic pathways, reflecting the diverse catabolic capabilities of GBS. Notably, specific proteins were exclusively expressed in human or fish isolates, suggesting distinct functional roles and adaptation strategies. These findings highlight the importance of comparative proteomic studies in understanding the zoonotic potential and pathogenicity of GBS. The insights gained from this research could inform future risk assessment, diagnostic development and prevention strategies targeting this opportunistic pathogen.

Keywords *Streptococcus agalactiae* · Group B *Streptococcus* · Invasive GBS infections · Proteomics

Introduction

Streptococcus agalactiae, (also known as Group B *Streptococcus* or GBS), is an opportunistic commensal Gram-positive diplococci bacterium that is present in 17–30% of healthy human adults worldwide without causing symptomatic disease(Shabayek and Spellerberg 2018). Nonetheless, GBS is primarily recognized for its role in severe

Significance This study provides valuable insight into the critical role of comparative proteomics in elucidating the complexities of GBS pathogenicity and zoonotic potential, paving the way for improved public health responses and therapeutic interventions. With a clearer understanding of its proteomic profile, researchers can identify novel targets for drug development, potentially leading to more effective treatments against this opportunistic pathogen.

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diseases, such as urinary tract infections, meningitis, and sepsis, particularly among vulnerable populations, such as pregnant women, the elderly, and immunocompromised individuals(Francois Watkins et al. 2019). Furthermore, GBS is an important pathogenic bacterium causing infections in cultured fish worldwide, including economically important cultured fish, such as tilapia (*Oreochromis* species), channel catfish (*Ictalurus punctatus*), and rainbow trout (*Oncorhynchus mykiss*)(Zamri-Saad et al. 2014). The disease is commonly affecting various tilapia-producing countries in Southeast Asia, including Malaysia, Indonesia, Vietnam, and Thailand, where mortality can be as high as 70%, resulting in significant economic losses of approximately 250 million USD annually and posing a threat to the development of the aquaculture industry's development in this region(Sentani et al. 2014; Kayansamruaj et al. 2019; Syuhada et al. 2020).

In 2015, Singapore experienced a rare outbreak of invasive diseases caused by GBS ST283. This outbreak was linked to the consumption of contaminated raw fish salad, known as yusheng, which typically includes freshwater fish species, such as Asian bighead carp and snakehead fish. The outbreak affected 238 people, resulting in severe infections, such as septic arthritis, meningitis, and septicemia, primarily among non-pregnant adults. This was the first documented case of invasive GBS associated with foodborne transmission(Chau et al. 2017). The outbreak was notable for its rapid spread and severity. This led to significant public health measures, including a public advisory to halt the sale of raw fish dishes made with these fish species and subsequent legislation banning the sale of raw freshwater fish as ready-to-eat food. Despite these measures, sporadic cases of ST283 infections continued to occur, indicating ongoing risks. The ST283 strain was found to be widespread in Southeast Asia, affecting not only Singapore but also other countries, such as Thailand, Vietnam, Laos and Malaysia. This strain is considered more virulent than other GBS strains, as it causes severe disease in otherwise healthy adults. The outbreak highlighted the importance of food safety and the need for continued surveillance to prevent future incidents(Chau et al. 2017).

Current studies have realized that the genome alone is not always enough to provide useful information, as genes can be translated into a myriad of proteins depending on various factors, including the organism's environment (Rodriguez Amado et al. 2017; Abril et al. 2020). Proteomic studies make it possible to identify and quantify sets of proteins expressed by microorganisms under specific culture conditions (Zhang et al. 2014). Proteomics can be used to compare the qualitative and quantitative proteome across strains. This allows for the interpretation of bacterial physiology and promotes knowledge of the genetic variation of each isolate(Tavares et al. 2019). Thus, a proteomic study of GBS strains that infect both humans and fish would allow for the analysis of protein variability within the strains belonging to the genotypes among humans and fish. The current study would increase scientific knowledge about the adaptation and pathogenesis of GBS infections in both humans and fish, and provide insight into the potential interspecies transmission between human and fish hosts. Additionally, this approach could identify antigenic proteins that can be used as targets for vaccine development.

The findings of this study will improve health protection in Malaysia through productive partnerships for the preparedness, planning, prevention, prompt detection, characterization, containment, and control of emerging diseases. Additionally, it aims to provide risk assessment data to support outbreak control and prevention measures. Comparative proteomics of GBS can help us better understand its zoonotic and virulent potential, thereby expanding our knowledge of genetics for future manipulation. The data will serve as a reference for comparative analysis with other studies worldwide on genetic properties and population. This shared knowledge will be useful for prospective risk assessment, diagnostic procedures, and prevention strategies. Therefore, this study aims to describe and compare the proteomic profiles of selected representative human and fish GBS isolates using the LC–MS/MS technique.

Materials and methods

Bacterial isolates

Selected representatives of five human GBS isolates of clinical origin (including 2 isolates ST283 and 3 new STs, namely ST1668, ST1669 and ST1670, labelled as HSA14, HSA35, HM13, HTJ9 and HSB7, respectively) and three fish GBS isolates (ST283) from cage-bred tilapia (*Oreochromis niloticus* × *Oreochromis mossambicus*) (labelled as F7, F31 and F49, respectively) available from our previous studies, were selected for proteomic profiling analysis(Azmai et al. 2010; Muthanna et al. 2023b). Only human GBS isolates were registered on PubMLST under the following IDs: 12,410, 12,411, 12,261, 12,262 and 12,263. The isolate selection was based on ST283 and new STs. Since all fish isolates in our collection were ST283, only three isolates were randomly selected. All were isolated from the invasive sites of the diseased hosts.

GBS was confirmed by colony morphology, β hemolysis on Columbia Agar with 5% sheep blood (Isolab Sdn. Bhd., Kuala Lumpur, Malaysia), pink colonies on CHROMAgar (Isolab Sdn. Bhd.) and GBS brilliance agar (Isolab Sdn. Bhd.). A Gram stain, catalase test, CAMP test, and latex agglutination test were performed (Oxoid, Basingstoke, UK) (Muthanna et al. 2023a). Furthermore, polymerase chain

reaction (PCR) was performed to further confirm the identification of GBS.

Proteomics profiling

Protein extraction

The procedure outlined for extracting whole bacterial lysates from GBS isolates involves several key steps, primarily focusing on bacterial culture, lysis, and purification, as described by Tavares et al. (2019). The GBS isolates were cultured overnight in 600 mL of Brain Heart Infusion (BHI) broth at 37 °C for 24 h. The bacteria were harvested by centrifugation at 16,100 × g for 20 min at 4 °C, which effectively pelleted the bacterial cells. The bacterial pellets were washed three times with 10 mL of 50 mM Tris–HCl (pH 7.5) to remove the residual broth component. After washing, the pellets were resuspended in 1 mL of lysis buffer, which contained 7 M urea, 2M thiourea, 4% (w/v) 3-[(3-Cholamidopropyl)dimethylammonio]−1-propanesulfonate (CHAPS), 12.5 mM Tris- HCl, and 1.5% (w/v) dithiothreitol (DTT). Additionally, 10 µL of a protease inhibitor mix was added to prevent protein degradation during lysis. The resuspended lysate was sonicated on ice using an ultrasonic cell disruptor for one minute to mechanically disrupt the cells and release their contents. Following sonication, the lysate was centrifuged at 21,900 × g for 40 min at 4 °C to separate soluble proteins from cellular debris. The supernatant was subjected to five cycles of centrifugation at 15,000 × g for 30 min at 20 °C using Vivaspin 500 centrifugal concentrators with a cutoff threshold of 3 kDa. This step concentrated the proteins and removed smaller molecules. Between each centrifugation cycle, the lysis buffer was exchanged for 50 mM ammonium bicarbonate (pH 8.5) to remove detergents from the samples. This comprehensive method ensures that high-quality bacterial lysates are obtained for further analysis or experimentation, maintaining protein integrity and minimizing degradation throughout the process.

Protein quantification

A Bradford protein assay kit (Sangon Biotech, Shanghai, China) was used to quantify the extracted proteins following to the manufacturer's instructions. Protein levels were determined by comparing the color response of protein assay standards prepared as a series of known dilutions of bovine serum albumin (BSA). The color change results were measured spectrophotometrically at 595 nm. The average 595 nm measurement was subtracted for the blank replicates from the 595 nm measurements of all other individual standards and sample replicates. A standard curve was generated by plotting the average blank-corrected 595 nm measurement for each BSA standard, and the standard curve was used to determine the protein concentration of each sample.

Protein digestion

A volume of 50 µL containing 1000 to 2000 µg/mL of protein extract was collected from each replicate and transferred to a 1.5 mL tube containing 10 µL of 50 mM ammonium bicarbonate. The proteins in the sample were denatured by adding of 25 µL of 0.2% (w/v) RapiGEST SF surfactant (Waters, Manchester, UK) and heating at 80 °C for 15 min. Thiol groups were reduced using 2.5 µL of 100 mM DTT (Sigma Aldrich, Saint Louis, USA) at 60 °C for 30 min and then alkylated using 2.5 µL of 300 mM iodoacetamide (Sigma Aldrich) and incubated at room temperature for 30 min in a dark chamber. The proteins in the sample were then enzymatically digested by adding 5 µg of sequencing-grade modified trypsin (Promega, Madison, USA) and incubated at 37 °C for 16 h. Digestion was stopped by the addition of 10 µL of 5% (v/v) trifluoroacetic acid (Sigma-Aldrich) and incubation at 37 °C for 90 min. The resulting peptide extracts were centrifuged at 21,900 × g for 30 min at 6 °C. The supernatants were collected, transferred to Waters Total Recovery vials (Waters), supplemented with 5 µL of 1 N ammonium hydroxide (Sigma Aldrich), and stored at − 70 °C until use.

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to separate the proteins based on their size. The polyacrylamide gel was prepared with a 12% concentration of acrylamide and bisacrylamide, following a previous study(Tuasikal et al. 2017). The extracted protein was diluted with gel loading dye containing 62.5 mM Tris-HCI (pH 6.8), 25% Glycerin, 2% SDS, 0.01% Bromophenol blue and DTT (Sangon Biotech, Shanghai, China) by adding 10 µL of the protein sample and 10µL of the loading dye to a 1.5 mL centrifuge tube. The mixture was vortexed well and boiled for 10 min at 95 °C in a water bath. After that, 10 µL of the sample was applied to the SDS-PAGE gel. The BLUeye Prestained Protein Ladder marker (Genedirex, Taiwan) was used as a reference for the molecular weight range of the isolated proteins. The electrophoresis tank was closed and run at 200 V for 60 min or until the dye front reached the bottom of the gel. The visualization was carried out using Coomassie Brilliant Blue R-250 dye (Vivantis).

Liquid chromatography–mass spectrometry (LC–MS-MS)

The LC–MS/MS analysis was performed at the Halal Products Research Institute, Universiti Putra Malaysia, based on a previous study with some modification(Amir et al.

2021). High-performance liquid chromatography (Agilent 1200 series) was used to separate the digested protein sample, which included an autosampler (G1367D), binary pump (G1312B), and column oven (G1316A) coupled to AB SCIEX 4000 QTrap MS (Singapore). The sample was injected into a Phenomenex Kinetex Core–shell C18 reversed-phase column (Torrance, CA; 100 × 2.1 mm, 2.6 µm, 100 Å) at a temperature of 45 °C with a flow rate of 300 µL/min for 25 min. Mobile phases A and B were 0.1% formic acid (FA) and 100% Acetonitrile (ACN), respectively. The column was equilibrated with a volume of 15 µL for 10 × column volume before sample injection. After 1 min, the concentration of mobile phase A was maintained at 97%, then gradually decreased to 3% after 19 min, and then maintained at 3% for the next 22 min. The concentration of mobile phase A was then brought back to 97% after 22.1 min and held at this value for 25 min. A standard chemical kit (AB SCIEX) containing polypropylene glycol at a concentration of 2 µM was used to calibrate MS Q1 and Q3 before conducting the MS analysis. At 400^{O}C, nebulizer voltage of 5200 V and pressure of 40 psi, Turbo Spray (ESI) ionization was used in positive mode. The curtain gas was maintained at 30 psi, while the pressures of source gases 1 and 2 were 40 and 30 psi, respectively. The declustering potential (DP) was 50 V. Data-dependent acquisition (DDA), consisting of enhanced MS (EMS), enhanced resolution (ER), and enhanced production (EPI), was used for data acquisition. For EMS, the mass range of the instrument was m/z 400–2000 at a scan speed of 4000 Da/s to achieve the highest sensitivity. To calculate the charge and exact mass of the peptides, the ER scan mode was used with a scan speed of 250 Da/s to achieve the best resolution. For tandem analysis MS, the EPI scan mode was used with a scan range of m/z 100–2000 divided into two segments (m/z 100–280 and m/z 275–2000) at a scan speed of 4000 Da/s. Collision-induced dissociation analysis (CID) was performed at a collision energy (CE) of 35 V and a CE spread (CES) of 15 V. The selection of ions for the tandem MS was limited to the 1–4 most intense peaks with charge states 2 +, 3 +, and 4 +. Instrument control, data acquisition, and data processing were performed using Analyst 1.5.1 software. For protein identification, peak lists were searched via MS/MS ion search using Trans-proteomic pipeline (TPP v6.0.0 OmegaBlock Build 202,108,111,440-exported, Windows_NT-x86_64).

The raw data from the LC–MS/MS was processed against the *S. agalactiae* UniProt/TrEMBL database which contained 13,528 reviewed and 1,290,635 unreviewed protein sequence entries, using TPP. The following constraints were used for the searches: semi-tryptic cleavage with up to two missed cleavage sites and tolerance windows of 1.2 Da for precursor ions and 0.6 Da for MS/MS fragment ions. The variable modifications that were allowed were as follows: (M*) methionine oxidation (+ 15.99 Da), (C*) carbamidomethylation of cysteine (Cys) (+ 57.02 Da), and acetylation of the N-terminus of the protein (+ 42.0106 Da).

Bioinformatics analysis

The interactive web-based tool was used to evaluate the number of proteins identified in each GBS strain through Venn diagrams (Heberle et al. 2015). The core proteome consisted of a subset of identified proteins in all evaluated strains. The accessory proteome consisted of a subset of identified proteins shared between at least two strains, and the unique proteome consisted of proteins that were identified exclusively in a particular strain. Predicted protein clustering (PPC) was performed to indicate the homologous genes between the strains using OrthoMCL software(Li et al. 2003) with default parameters. To predict orthologous groups based on functional category and subcellular localization, the sequences of identified proteins were analyzed using the Cluster of Orthologous Genes (COG) database(Galperin et al. 2015). The COG database search was performed using an in-house script (available at https://github.com/aquacen/blast_cog). Parameters of Gram-positive bacterium and similarities to host proteins of humans were set. Only proteins with adhesion probabilities ≥ 0.51 were included in the results.

Results

SDS-PAGE

The results of the protein analysis using the SDS-PAGE technique on the GBS strains isolated from humans and fish are shown in Fig. 1. All eight GBS isolates exhibited the presence of protein bands with molecular weights (MW) ranging between 20 and 225 kDa, without variation in the protein patterns. This suggests that the protein characterization of isolates from humans and fish shows no difference in properties based on MW, regardless of ST. Therefore, the subsequent analysis combined fish and human isolates.

Proteomic profiling

A total of 21,945 peptides with a normal distribution of 10 ppm error were identified. The total number of proteins identified in three biological replicates of the eight GBS strains was 1405 proteins. A total of 761 proteins were identified in the HSA14 (ST283) isolate, 710 proteins in the HSA35 (ST283) isolate, 668 proteins in the HTJ9 (ST1169) isolate, 611 proteins in the HM13 (ST1668) isolate, 750 proteins in the HSB7 (ST1670) isolate, 725 proteins in the F7 (ST283)

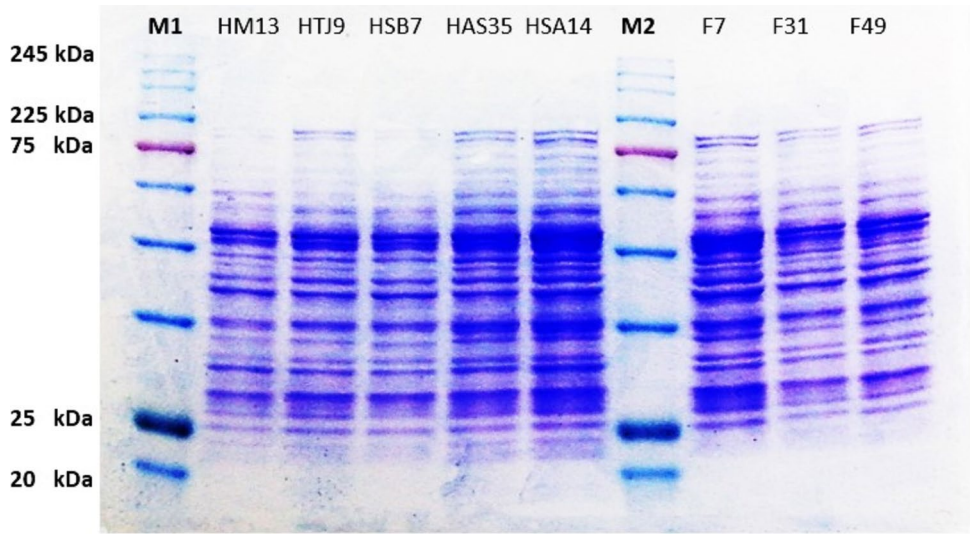

Fig. 1 GBS protein pattern in polyacrylamide 12% gel stained with coomassie brilliant blue. M = marker protein molecular weight: 25–245 kDa

isolate, 723 proteins in the F31 (ST283) isolate and 690 proteins in the F49 (ST283) isolate, with protein similarity ranging from 52.2% to 60.7% between the isolates (Fig. 2).

The Trans-Proteomic Pipeline software was used to merge proteins from human isolates and fish isolates to analyze their shared proteomes. A comparative analysis of the

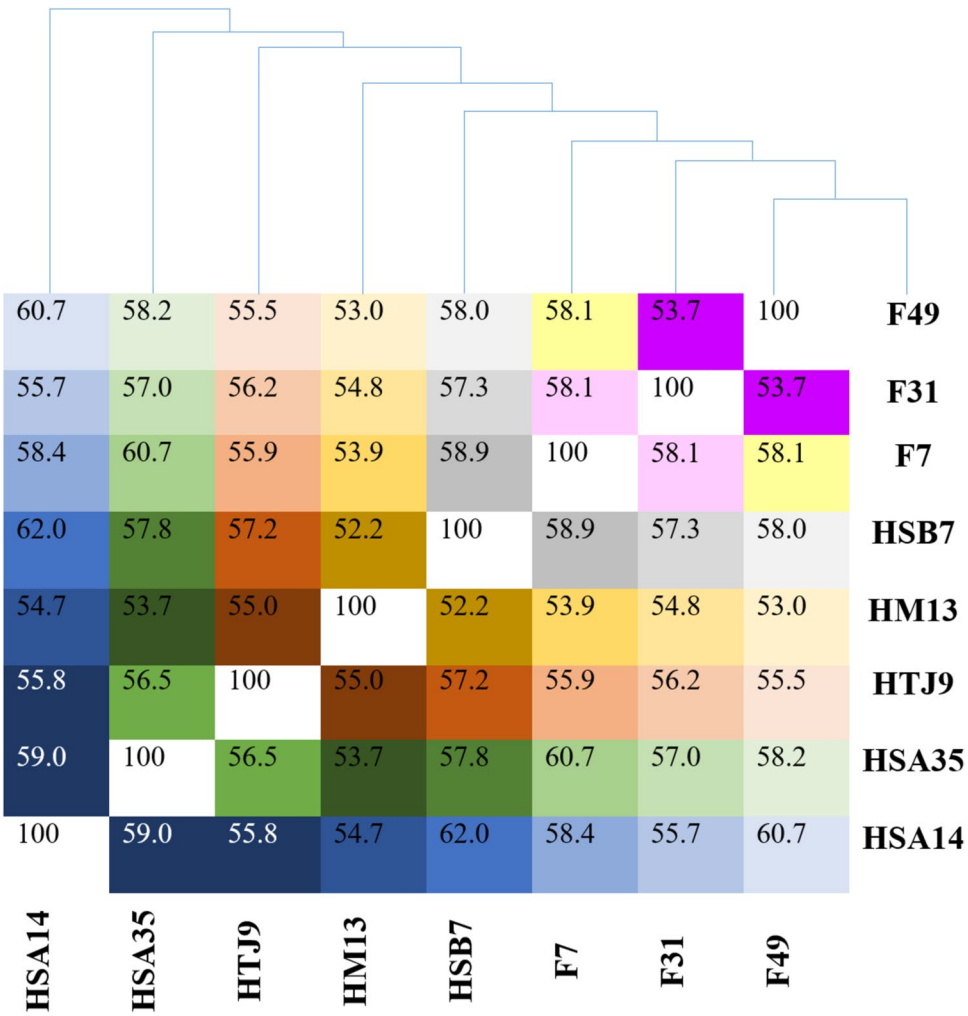

Fig. 2 Proteome similarity matrix of human and fish GBS isolates. The numbers inside the frames indicate the percentage of similarities among the strains

combined identified human and fish proteins was performed using Venn diagrams. A total of 1162 proteins were identified in the core proteome, 140 proteins were present in the human isolates, and 107 proteins were identified in the fish isolates (Fig. 3). The identification of 1162 common proteins in human and fish isolates shows that human and fish GBS strains are closely related in terms of protein content (82.5% similarity), even if the strains originate from different lineages of ST.

Among the top most abundant proteins identified in human and fish GBS strains, several key proteins, including Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Enolase, Elongation factor Tu (EF-Tu), Fructose-bisphosphate aldolase, Phosphocarrier protein HPr, and 50S ribosomal proteins were found. These proteins have been highlighted for their roles in metabolism, virulence, and cellular function.

Classification of human and fish GBS proteins based on COG

Approximately 92% of the proteins ($n = 1296$) from human and fish GBS isolates were classified into 20 functional categories using COG. The remaining 8% of identified proteins ($n = 113$) were classified as proteins with unknown functions. The most common categories included translation/ribosomal structure and biogenesis ($n = 145$), general function prediction ($n = 87$), amino acid transport and metabolism ($n = 84$), cell wall/membrane/shell envelope biogenesis ($n = 77$), transcription ($n = 74$), carbohydrate transport and metabolism ($n = 69$), replication, recombination and repair ($n = 64$), nucleotide transport and metabolism ($n = 60$), and coenzyme transport and metabolism ($n = 57$). Table 1 shows the proteins detected in the human and fish isolates based on their functional categories. The categories were divided into three main groups: cellular component, molecular function, and biological process. All data were obtained from the COG and UniProtKB databases. The characteristics of highly expressed proteins in human and fish GBS isolates are annotated in Tables 2 and 3, respectively. A comparative analysis was performed to evaluate the abundance of specific proteins in human and fish GBS isolates. A total of 79 abundance proteins were identified in human isolates compared to 77 in fish isolates. Proteins such as alpha-1,4-glucan phosphorylase, ATP synthase subunit beta, glutamine-fructose-6-phosphate aminotransferase, glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent, isoleucine tRNA ligase, maltose/maltodextrin -Transporter ABC, maltose/maltodextrin binding protein, sensor histidine kinase, and UvrABC system protein C were specifically present in human isolates. The proteins exclusively expressed in human GBS isolates are involved in various functions, including mobilizing storage polysaccharides ($n = 1$), binding proteins ($n = 1$), controlling glucose flux, processing of DNA lesions ($n = 2$), catalyzing reversible oxidative decarboxylation ($n = 1$), inhibiting protein synthesis ($n = 1$), transporting ligands ($n = 1$), and regulating various processes ($n = 1$). On the other hand, the proteins identified exclusively in fish GBS isolates, include DNA polymerase III PolC-type, DNA topoisomerase 4 subunit A, glycerol kinase, ribosomal protein S1, and streptococcal histidine triad family

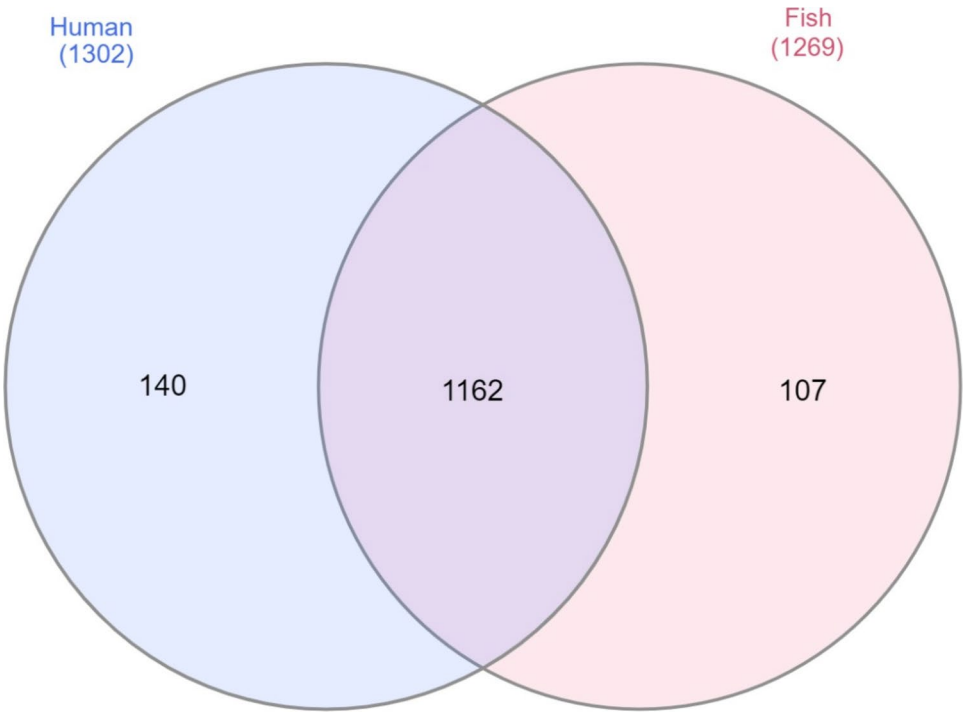

Fig. 3 Venn Diagram showing the number of unique and shared proteins in human and fish GBS isolates

Table 1 The main proteins identified and classified according to COG functional categories in human and fish GBS isolates

COG Category	Function	Proteins
Cellular component		
Cell cycle control, cell division, chromosome partitioning	Cell division	Cell division protein FtsA, FtsE, FtsH, FtsK, FtsW, FtsY, FtsZ, DivIVA
Cell wall/membrane/envelope biogenesis	Capsular polysaccharide biosynthesis	CpsA, CpsB, CpsC, CpsG, CpsH, NeuB, NeuC
	Peptidoglycan biosynthesis	MurM, MurT
	Group B antigen	Glucose-1-phosphate thymidylyltransferase, dTDP-glucose 4,6-dehydratase
	Multidrug resistance	Pbp1A penicillin-binding protein 1 A, Pbp2A penicillin-binding protein 2 A,DltD, Transcriptional regulatory protein DltR,Sensor protein DltS
	Immunoreactive antigen	PcsB, Sip
Cell motility	Transport	accessory Sec system protein Asp1, Asp2, Asp23, Competence protein CglA, CglB
Post-translational modification, protein turnover, and chaperones	Heat shock	GroL, GroS, GrpE, Chaperone protein DnaK, Chaperone protein DnaJ
	Oxidative stress resistance	Thioredoxin, Thioredoxin reductase, FeS assembly protein SufB, SufD
Signal transduction mechanisms	Two-component system	*CiaR regulatory protein*, Serine/Threonine Phosphatase Stp1, serine/threonine protein kinase
	Stress response	Universal stress protein
Intracellular trafficking, secretion, and vesicular transport	Protein translocation	Protein translocase subunit SecA 1, SecA, SecY and SecG
Defense mechanisms	Multidrug resistance	ABC-2 type transport system ATP-binding protein, Beta-lactamase
Molecular Function		
Translation, ribosomal structure and biogenesis	30S ribosomal proteins	RpsA,RpsB,RpsC, RpsD, RpsE, RpsF, RpsG, RpsH, RpsI, RpsJ, RpsK, RpsL, RpsM, RpsN, RpsO, RpsP, RpsR, RpsS, RpsT, RpsU
	50S ribosomal proteins	RplA, RplB, RplC, RplD, RplE, RplF, RplK, RplM, RplN, RplO, RplP, RplQ, RplR, RplS, RplT, RplU, RplV, RplW, RpmB, RpmC, RpmD, RpmG, RpmL
Transcription	RNA polymerase	RpoA, RpoB, RpoC, RpoE, RpoZ
	Transcriptional regulators	MutR, GntR, LysR, LacI, MarR, DeoR, TetR
Replication, recombination and repair	DNA replication	DnaA, DnaN, GyrA, GyrB
	DNA mismatch repair	MutL, MutS
Biological processes		
Energy production and conversion	Proton transporting	AtpA, AtpC, AtpD, AtpF, AtpG
	Pyruvate metabolism	pdhC, TPP-dependent acetoin dehydrogenase complex, Dihydrolipoyl dehydrogenas
Amino acid transport and metabolism	Arginine metabolism	ArcA, ArcB, ArcC, ArcD, ArgF, ArgG, ArgH, ArgR, ArgS
	Threonine metabolism	Hom, ThrB, ThrC
	Glycine metabolism	GlyA
	Glutamic acid metabolism	AlaT
	Glutamine metabolism	GlnA
	Serine metabolism	SdhA, SdhB, SerB, SerC
	Aspartic acid metabolism	AsnA
	Cysteine metabolism	Cysteine desulfurase
	Methionine metabolism	MetK, MetN
Nucleotide transport and metabolism	Purine biosynthesis	PurA, PurB, PurC, PurD, PurE, PurF, PurH, PurK, PurM, PurN, PurR
	Pyrimidine biosynthesis	PyrC, PyrD, PyrE, PyrG, PyrH, PyrR

Table 1 (continued)

COG Category	Function	Proteins
Carbohydrate transport and metabolism	Glycogen synthesis	PgmA
	Glycolytic pathway	Eno, Pga, Pgk, TpiA
	Pentose phosphate pathway	AroD
	PTS system	beta-glucoside transporter, family porter component HPr, sugar transporter, cellobiose transporter, fructose transporter, glucose transporter, lactose transporter, mannose transporter, galactitol and fructose transporter
Coenzyme transport and metabolism	Riboflavin biosynthesis	RibBA, RibD, RibE, RibH
Lipid transport and metabolism	Fatty acid biosynthesis	AccD, FabD, FabF, FabG, FabH, FabT, FabZ
Inorganic ion transport and metabolism	Transporter	Iron, Nickel, Ferrichrome, Manganese, Magnesium, Potassium, Phosphate, Heme
	Zinc metabolism	Zinc-binding protein
	Copper metabolism	CutC
Secondary metabolites biosynthesis, transport, and catabolism	Multidrug resistance	DltA, Bleomycin resistance protein
Poorly characterized		
Mobilome	Prophages	Phage repressor protein
	Transposons	Transposase
Function unknown	Stress response	Gls24, General stress protein

protein. These proteins are involved in the duplication of chromosomal DNA ($n = 1$), replication of DNA ($n = 1$), regulation of channel- and promoter-independent uptake of glycerol into the cell ($n = 1$), growth and translation of mRNA ($n = 1$), and surface exposure ($n = 1$).

Heat map analysis of protein expression

Heat map analysis was also performed to assess differences in protein expression between human and fish GBS isolates compared to the *S. agalactiae* UniProt/TrEMBL database. Proteins with lower peptide counts were excluded. Among these, 10 proteins were identified as highly expressed, and 40 proteins as less expressed in all human and fish GBS strains. Proteins that were found to be highly expressed, include glyceraldehyde-3-phosphate dehydrogenase, fructose bisce binding aldolase, EF-Tu, phosphocarrier protein HPr, 50S ribosomal protein L7/L12, DNA-binding protein HU, 16, triosephosphate isomerase, 50S ribosomal protein L5, phosphoglycerate kinase, and enolase, while proteins such as the chaperone protein DnaK, the Gls24 protein, putative, EIIAB-Man, 50S ribosomal protein L11, 50S ribosomal protein L22, 50S ribosomal protein L4, 50S ribosomal protein L20 Alkylhydroperoxide reductase C, 30S ribosomal protein S6, 50S ribosomal protein L2, trigger factor, aldehyde dehydrogenase family protein, 50S ribosomal protein L17, 50S ribosomal protein L23, carbamate kinase, ATP-dependent 6-phosphofructokinase, pyruvate kinase, DNA -Polymerase III PolC type, cysteine synthase, ribosomal protein S1, penicillin binding protein 2 A, 30S ribosomal protein S20, C5a peptidase, 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase, elongation factor Ts, Gls24 protein, putative, 50S ribosomal protein L10, 30S ribosomal protein S2, 30S ribosomal protein S10, ESAT-6-like protein, arginine deiminase, ATP synthase subunit beta, elongation factor G, protease, putative, ornithine carbamoyltransferase 2, catabolic, 30S ribosomal protein S8, 30S ribosomal protein S5, 50S ribosomal protein L31 type B, 60 kDa chaperonine and acetoin (diacetyl) reductase, were found to be less expressed.

Discussion

Our study utilized eight isolates of GBS, five from humans and three from fish, for a comprehensive LC–MS/MS proteomic analysis. Due to the limited number of representative isolates, the data were grouped based on their origin— either humans or fish. This analysis successfully identified 1,405 proteins expressed by the isolates, highlighting the proteomic diversity between human and fish strains of GBS. Research on the comparative proteome of GBS has utilized techniques such as SDS-PAGE coupled with mass spectrometry, revealing a limited number of proteins identified in earlier studies. Specifically, studies by Li et al. in 2014 and 2015(Li et al. 2014, 2015) reported the detection of only 65 proteins in their analyses of GBS proteomes. In another study utilizing nanoLC-UDMS, 1273 proteins were identified across three fish isolates and one human isolate of GBS(Tavares et al. 2019). Our study on the

Table 2 The characteristics of abundantly expressed proteins in human GBS isolates

Accession	Proteins	Total of peptides	pl/Mw (kDa)	Probability	% Coverage Weight	% Spectrum ids NSP
Q8DXS8|Q8DXS8	Glyceraldehyde-3-phosphate dehydrogenase	108	35.984	1.0000	65.8%	11.21%
P64081|ENO	Enolase	81	47.103	1.0000	53.6%	8.25%
Q8E270|Q8E270	Fructose-bisphosphate aldolase	76	31.499	1.0000	56.7%	7.84%
Q8E0H1|EFTU	Elongation factor Tu	76	43.983	1.0000	42.2%	7.53%
Q8E0B3|Q8E0B3	Phosphocarrier protein HPr	61	8.933	1.0000	88.5%	5.56%
Q8DZ21|RL7	50S ribosomal protein L7/L12	41	12.323	1.0000	61.2%	3.68%
Q8DXT0|PGK	Phosphoglycerate kinase	26	42.089	1.0000	25.6%	2.69%
Q8E2C2|RL5	50S ribosomal protein L5	22	19.803	1.0000	26.1%	2.23%
Q8E157|Q8E157	DNA-binding protein HU	17	9.584	1.0000	73.6%	1.66%
Q8E2C0|RS8	30S ribosomal protein S8	17	14.791	1.0000	37.1%	1.48%
Q8E2B7|RS5	30S ribosomal protein S5	17	17.081	1.0000	51.2%	1.71%
Q8E140|Q8E140	Acetoin(diacetyl) reductase	15	26.791	1.0000	40.9%	1.56%
Q8CX00|CH60	60 kDa chaperonin	11	57.297	1.0000	12.6%	1.08%
P66199|RL31B	50S ribosomal protein L31 type B	11	9.851	1.0000	36.0%	1.15%
P65606|OTCC2	Ornithine carbamoyltransferase 2, catabolic	11	38.096	1.0000	17.5%	1.11%
Q8DZR0|ESX2	ESAT-6-like protein SAG1039	11	10.684	1.0000	29.2%	1.09%
P0A3J3|DNAK	Chaperone protein DnaK	11	64.941	1.0000	23.6%	1.03%
Q8E0H0|TPIS	Triosephosphate isomerase	11	26.622	0.9951	6.3%	1.07%
P66345|RS10	30S ribosomal protein S10	9	11.607	1.0000	39.2%	0.92%
Q8E292|TIG	Trigger factor	9	47.010	1.0000	13.8%	0.80%
Q8DZ20|RL10	50S ribosomal protein L10	9	17.791	1.0000	45.2%	0.91%
Q8DZG4|Q8DZG4	Gls24 protein, putative	8	19.467	1.0000	29.4%	0.79%
Q8DXB7|Q8DXB7	PTS system, IIB component, putative	8	11.078	1.0000	62.4%	0.83%
Q8DXL8|RS2	30S ribosomal protein S2	7	28.598	1.0000	19.9%	0.62%
Q8DWQ2|ARCA	Arginine deiminase	7	46.526	1.0000	14.9%	0.71%
Q8DZG6|Q8DZG6	Gls24 protein, putative	7	19.251	1.0000	28.4%	0.69%
Q8E2B9|RL6	50S ribosomal protein L6	7	19.327	0.9951	9.0%	0.75%
P66180|RL29	50S ribosomal protein L29	7	7.958	0.9854	14.7%	0.72%
Q8E1J3|Q8E1J3	EIIAB-Man	6	36.116	1.0000	19.6%	0.62%

Table 2 (continued)

Accession	Proteins	Total of peptides	pl/Mw (kDa)	Probability	% Coverage Weight	% Spectrum ids NSP
Q8E0G9|GPMA	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	6	25.945	1.0000	20.0%	0.62%
P66094|RL1	50S ribosomal protein L1	6	24.279	0.9999	11.8%	0.63%
Q8DXS7|EFG	Elongation factor G	5	76.524	1.0000	9.4%	0.51%
Q8DZH7|Q8DZH7	Aldehyde dehydrogenase family protein	5	50.377	1.0000	12.4%	0.53%
Q8DYG1|RL11	50S ribosomal protein L11	5	14.878	1.0000	37.6%	0.43%
P64056|EFTS	Elongation factor Ts	5	37.594	1.0000	8.1%	0.53%
Q8DXW8|Q8DXW8	Formate acetyltransferase	4	86.742	1.0000	6.4%	0.42%
Q8E2C8|RL2	50S ribosomal protein L2	4	29.995	1.0000	31.0%	0.44%
Q8E2B0|RL17	50S ribosomal protein L17	4	14.514	1.0000	42.2%	0.39%
Q8E072|ATPB	ATP synthase subunit beta	4	50.913	1.0000	10.7%	0.42%
Q8E1M0|Q8E1M0	Cysteine synthase	3	32.627	1.0000	18.5%	0.28%
Q8DWP8|Q8DWP8	Carbamate kinase	3	33.987	1.0000	16.0%	0.30%
Q8E2D1|RL3	50S ribosomal protein L3	3	22.413	0.9956	7.2%	0.29%
Q8E2B6|RL30	50S ribosomal protein L30	3	6.310	0.9991	49.2%	0.24%
P66598|RS6	30S ribosomal protein S6	3	10.978	0.9997	35.8%	0.28%
P66443|RS16	30S ribosomal protein S16	3	10.277	1.0000	28.9%	0.25%
Q8DZZ2|RS20	30S ribosomal protein S20	3	8.897	1.0000	14.6%	0.19%
Q8E2B5|RL15	50S ribosomal protein L15	3	15.442	0.9816	8.2%	0.32%
Q8DYV3|RL21	50S ribosomal protein L21	3	11.195	0.9787	16.3%	0.32%
Q8E178|Q8E178	Cell division protein DivIVA, putative	3	29.578	0.9581	5.1%	0.30%
Q8E2C9|Q8E2C9	50S ribosomal protein L23	3	10.699	0.9999	12.2%	0.21%
Q8E1E1|Q8E1E1	C5a peptidase	3	13.5801	0.9996	4.1%	0.19%
Q8E0B1|Q8E0B1	Glyceraldehyde-3-phosphate dehydrogenase, NADP-dependent	3	51.188	0.9984	5.7%	0.20%
Q8E000|Q8E000	Pyruvate kinase	3	54.124	0.9970	5.8%	0.19%
Q8DYN7|Q8DYN7	Alpha-1,4 glucan phosphorylase	3	86.364	0.9951	5.7%	0.23%
Q8E057|Q8E057	Acetoin dehydrogenase, thymine PPi dependent, E1 component, beta subunit	3	35.784	0.9933	8.1%	0.17%
Q8E2C7|RL22	50S ribosomal protein L22	3	12.410	0.9920	25.4%	0.20%
Q8E2D0|RL4	50S ribosomal protein L4	3	22.101	1.0000	20.3%	0.46%

Table 2 (continued)

Accession	Proteins	Total of peptides	pl/Mw (kDa)	Probability	% Coverage Weight	% Spectrum ids NSP
Q8DZ90|UVRC	UvrABC system protein C	3	68.350	0.9911	3.5%	0.18%
Q8DYN4|Q8DYN4	Maltose/maltodextrin ABC transporter, maltose/maltodextrin-binding protein	3	45.190	0.9806	7.5%	0.22%
Q8DYY4|Q8DYY4	ABC transporter, ATP-binding/permease protein	3	64.820	0.7025	7.9%	0.12%
Q8DWP3|Q8DWP3	ABC transporter, permease protein, putative	3	64.820	0.6546	8.5%	0.10%
Q8E1Y6|RS9	30S ribosomal protein S9	3	14.258	0.9583	18.5%	0.15%
Q8DWT6|Q8DWT6	Sensor histidine kinase	3	49.960	0.9343	6.5%	0.16%
Q8E2E7|Q8E2E7	Acetyl xylan esterase, putative	3	37.203	0.8754	3.4%	0.20%
Q8E177|SYI	Isoleucine–tRNA ligase	3	10.4832	0.8117	1.0%	0.17%
P66111|RL20	50S ribosomal protein L20	3	13.644	0.7361	11.8%	0.19%
Q8DZZ7|GLMS	Glutamine–fructose-6-phosphate aminotransferase [isomerizing]	3	65.596	0.6688	4.6%	0.12%
Q8E1K9|Q8E1K9	Acyl carrier protein	3	8.292	0.9801	14.9%	0.11%
Q8E1Q5|Q8E1Q5	DD-transpeptidase	3	86.160	1.0000	8.4%	0.19%
Q8DXL7|Q8DXL7	Alkyl hydroperoxide reductase C	3	20.551	0.7501	7.5%	0.10%
Q8DWS6|RS4	30S ribosomal protein S4	3	23.053	0.9721	4.9%	0.11%
Q8E1B9|Q8E1B9	Conserved domain protein	3	11.078	0.8185	8.3%	0.10%
P64196|G6PI	Glucose-6-phosphate isomerase	3	49.555	0.9549	4.2%	0.11%
Q8DXM2|Q8DXM2	ATP-dependent Clp protease, ATP-binding subunit	3	90.525	0.8127	4.9%	0.12%
Q8DZY3|LDH	L-lactate dehydrogenase	3	35.397	0.8070	10.9%	0.11%
Q8E0A2|Q8E0A2	Coagulase domain-containing protein	3	85.775	0.7955	4.4%	0.10%
Q8DXY8|Q8DXY8	Drug resistance transporter, EmrB/QacA family	3	53.918	0.7448	10.3%	0.11%
Q8DX06|Q8DX06	C5a peptidase	3	172.188	0.9996	4.1%	0.19%
Q8DZF3|Q8DZF3	Membrane protein, putative	3	24.558	0.3959	19.0%	0.09%

pl isoelectric point, *Mw* molecular weight, *kDa* Kilodalton

Table 3 The characteristics of abundantly expressed proteins in fish GBS isolates

Accession	Proteins	Total of peptides	pl/Mw (kDa)	Probability	% Coverage Weight	% Spectrum ids NSP
Q8DXS8|Q8DXS8	Glyceraldehyde-3-phosphate dehydrogenase	157	35.984	1.0000	69.0%	14.57%
P64081|ENO	Enolase	83	47.103	1.0000	49.4%	7.39%
Q8E270|Q8E270	Fructose-bisphosphate aldolase	64	31.499	1.0000	52.9%	6.01%
Q8E0B3|Q8E0B3	Phosphocarrier protein HPr	54	8.933	1.0000	88.5%	3.88%
Q8E0H1|EFTU	Elongation factor Tu	48	43.983	1.0000	42.2%	4.47%
Q8DZ21|RL7	50S ribosomal protein L7/L12	43	12.323	1.0000	61.2%	3.66%
Q8E2C2|RL5	50S ribosomal protein L5	28	19.803	1.0000	26.1%	2.68%
Q8E157|Q8E157	DNA-binding protein HU	26	9.584	1.0000	68.1%	2.41%
Q8DXT0|PGK	Phosphoglycerate kinase	25	42.089	1.0000	23.9%	2.37%
Q8E2B7|RS5	30S ribosomal protein S5	22	17.081	1.0000	59.1%	1.87%
Q8E2C0|RS8	30S ribosomal protein S8	19	14.791	1.0000	46.2%	1.75%
Q8E0H0|TPIS	Triosephosphate isomerase	14	26.622	1.0000	23.4%	1.28%
Q8DXL8|RS2	30S ribosomal protein S2	14	28.598	1.0000	34.0%	1.25%
P0A3J3|DNAK	Chaperone protein DnaK	13	64.941	1.0000	27.1%	1.19%
P66199|RL31B	50S ribosomal protein L31 type B	12	9.851	1.0000	36.0%	1.07%
Q8E1J3|Q8E1J3	EIIAB-Man	11	36.116	1.0000	28.6%	0.99%
P65606|OTCC2	Ornithine carbamoyltransferase 2, catabolic	11	38.096	1.0000	23.4%	0.96%
Q8DZ20|RL10	50S ribosomal protein L10	11	17.791	1.0000	36.1%	1.04%
P66345|RS10	30S ribosomal protein S10	10	11.607	1.0000	39.2%	0.93%
P64056|EFTS	Elongation factor Ts	10	37.594	1.0000	8.1%	0.91%
Q8DZG4|Q8DZG4	Gls24 protein, putative	9	19.467	1.0000	29.4%	0.86%
Q8E2C9|Q8E2C9	50S ribosomal protein L23	9	10.699	0.9786	12.2%	0.83%
Q8DZG6|Q8DZG6	Gls24 protein, putative	8	19.251	1.0000	28.4%	0.76%
Q8DYG1|RL11	50S ribosomal protein L11	8	14.878	1.0000	45.4%	0.74%
Q8DXW8|Q8DXW8	Formate acetyltransferase	7	86.742	1.0000	8.1%	0.59%
Q8DZR0|ESX2	ESAT-6-like protein SAG1039	7	10.684	1.0000	29.2%	0.66%
Q8E2C8|RL2	50S ribosomal protein L2	6	29.995	1.0000	18.1%	0.48%
Q8DYV3|RL21	50S ribosomal protein L21	6	11.195	1.0000	26.9%	0.54%
Q8DWQ2|ARCA	Arginine deiminase	5	46.526	1.0000	9.8%	0.47%
P66094|RL1	50S ribosomal protein L1	5	24.279	1.0000	12.7%	0.48%
Q8E2D0|RL4	50S ribosomal protein L4	5	22.101	1.0000	20.3%	0.46%
Q8E2C7|RL22	50S ribosomal protein L22	5	12.410	1.0000	30.7%	0.48%
P66598|RS6	30S ribosomal protein S6	5	10.978	0.9996	49.5%	0.39%
Q8DZH7|Q8DZH7	Aldehyde dehydrogenase family protein	5	50.377	0.9974	11.4%	0.40%
Q8E2B9|RL6	50S ribosomal protein L6	5	19.327	0.9956	9.0%	0.48%
P66111|RL20	50S ribosomal protein L20	4	13.644	1.0000	29.4%	0.36%
Q8E140|Q8E140	Acetoin(diacetyl) reductase	4	26.791	1.0000	13.8%	0.38%
Q8DXB7|Q8DXB7	PTS system, IIB component, putative	4	11.078	1.0000	34.7%	0.30%
Q8DWP8|Q8DWP8	Carbamate kinase	4	33.987	1.0000	11.6%	0.35%
Q8DXS7|EFG	Elongation factor G	4	76.524	1.0000	6.6%	0.36%
P66443|RS16	30S ribosomal protein S16	3	10.277	1.0000	28.9%	0.25%
Q8E1M0|Q8E1M0	Cysteine synthase	3	32.627	1.0000	19.8%	0.29%
Q8E000|Q8E000	Pyruvate kinase	3	54.124	1.0000	9.4%	0.26%
Q8E292|TIG	Trigger factor	3	47.010	1.0000	6.8%	0.28%
Q8DXS6|RS7	30S ribosomal protein S7	3	17.685	1.0000	16.7%	0.27%
Q8CX00|CH60	60 kDa chaperonin	3	57.297	0.9999	9.1%	0.22%
Q8DZF1|Q8DZF1	Ribosomal protein S1	3	43.713	0.9997	11.2%	0.20%
P63984|DPO3	DNA polymerase III PolC-type	3	16.6015	0.9997	4.8%	0.18%

Table 3 (continued)

Accession	Proteins	Total of peptides	pl/Mw (kDa)	Probability	% Coverage Weight	% Spectrum ids NSP
Q8E2B6|RL30	50S ribosomal protein L30	3	6.310	0.9991	49.2%	0.24%
Q8E2D1|RL3	50S ribosomal protein L3	3	22.413	0.9956	7.2%	0.29%
Q8E2B0|RL17	50S ribosomal protein L17	3	14.514	0.9956	14.1%	0.28%
P66180|RL29	50S ribosomal protein L29	3	7.958	0.9791	14.7%	0.28%
Q8E1K9|Q8E1K9	Acyl carrier protein	3	8.292	0.9505	14.9%	0.28%
Q8DWZ3|Q8DWZ3	DD-transpeptidase	3	86.160	1.0000	8.4%	0.19%
Q8DZZ2|RS20	30S ribosomal protein S20	3	8.897	1.0000	14.6%	0.19%
Q8E2B5|RL15	50S ribosomal protein L15	3	15.442	0.9994	14.4%	0.15%
P65697|PFKA	ATP-dependent 6-phosphofructokinase	3	35.947	0.9988	15.0%	0.15%
Q8E1Y6|RS9	30S ribosomal protein S9	3	14.258	0.9908	9.2%	0.19%
Q8DXL7|Q8DXL7	Alkyl hydroperoxide reductase C	3	20.551	0.9907	14.0%	0.17%
Q8DZE8|Q8DZE8	DNA topoisomerase 4 subunit A	3	92.909	0.9905	3.9%	0.15%
Q8DWS6|RS4	30S ribosomal protein S4	3	23.053	0.9901	9.4%	0.17%
Q8E1B9|Q8E1B9	Conserved domain protein	3	11.078	0.9456	18.4%	0.14%
Q8E178|Q8E178	Cell division protein DivIVA, putative	3	29.578	0.9388	5.1%	0.16%
P64196|G6PI	Glucose-6-phosphate isomerase	3	49.555	0.8930	7.3%	0.13%
Q8DXM2|Q8DXM2	ATP-dependent Clp protease, ATP-binding subunit	3	90.525	0.8127	4.9%	0.12%
Q8DZY3|LDH	L-lactate dehydrogenase	3	35.397	0.8070	10.9%	0.11%
Q8E0A2|Q8E0A2	Coagulase domain-containing protein	3	85.775	0.7955	4.4%	0.10%
Q8DXY8|Q8DXY8	Drug resistance transporter, EmrB/QacA family	3	53.918	0.7448	15.0%	0.11%
Q8DX06|Q8DX06	C5a peptidase	3	172.188	0.7248	3.8%	0.11%
Q8DYY4|Q8DYY4	ABC transporter, ATP-binding/permease protein	3	64.820	0.7025	7.9%	0.12%
Q8E1T0|GLPK	Glycerol kinase	3	55.400	0.6978	11.8%	0.10%
Q8DWP3|Q8DWP3	ABC transporter, permease protein, putative	3	64.820	0.6546	8.5%	0.10%
Q8DZ81|Q8DZ81	Streptococcal histidine triad family protein	3	92.344	0.6479	5.5%	0.10%
Q8E215|Q8E215	Membrane protein, putative	3	25.631	0.6194	19.0%	0.09%
Q8E0G9|GPMA	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	3	25.945	0.9808	7.0%	0.10%
Q8E057|Q8E057	Acetoin dehydrogenase, thymine PPi dependent, E1 component, beta subunit	3	35.784	0.9718	3.6%	0.10%
Q8E2E7|Q8E2E7	Acetyl xylan esterase, putative	3	37.203	0.8754	3.4%	0.20%

pl isoelectric point, *MW* molecular weight, *kDa* Kilodalton

core proteome of human and fish isolates of GBS revealed the identification of 1162 proteins out of all detected proteins. The similarity of these proteins between the different isolates ranged from 52 to 61%. This indicates a significant degree of conservation in the protein profiles among the strains, reflecting their evolutionary relationships and potential for cross-host pathogenicity. A similar study was conducted on the core proteome of invasive fish and human GBS isolates revealing 1070 proteins in both species, with over 60% of these proteins matching between the isolates(Tavares et al. 2019). Our study revealed significant conservation of protein expression between human and fish isolates, with recent findings indicating that 82.5% of the total proteins identified were shared between these strains. This high degree of overlap suggests that the core proteome is conserved across species, which is crucial for understanding the pathogenicity and adaptability of GBS in different hosts, as reported in previous studies, with coverage of 73% to 92% of the panproteome reported in various studies(Zhang et al. 2014; Jhingan et al. 2016; Pragya et al. 2017; Silva et al. 2017).

Environmental factors may significantly influence protein expression in GBS through various mechanisms. These factors include conditions like oxygen levels, pH, temperature, osmotic strength, and nutrient availability(Dobrut & Brzychczy-Włoch 2022). Temperature changes and nutrient scarcity can cause errors in protein synthesis, such as stop codon recoding, allowing cells to adapt and produce diverse protein isoforms to survive stress. GBS, in particular, exhibits metabolic flexibility by utilizing various carbon sources for energy, which is essential for thriving in different environments. This is facilitated by systems like the PTS and ABC transporters. Proteins involved in sugar transport

and various metabolic pathways, such as glycogen synthesis and glycolysis, were identified in GBS strains, highlighting their diverse catabolic potential. Key enzymes, including triosephosphate isomerase and phosphoglycerate kinase, were notably abundant in human and fish GBS isolates, emphasizing their importance in carbohydrate metabolism(Neijssel 1997).

Streptococcus species, like *S. pneumoniae*, *S. sanguinis*, and *S. agalactiae*, rely on pyruvate as an alternative energy source when glucose or lactose is scarce, which is vital for their survival, especially in the human body. The core proteins involved in pyruvate metabolism in human and fish GBS isolates include pyruvate dehydrogenase and other related enzymes. These enzymes convert pyruvate into acetyl-CoA, a key component for fatty acid synthesis and acetate production. GBS strains also break down pyruvate to generate energy-rich compounds such as formate, acetate, acetoin, lactate, and ethanol(Yamamoto et al. 2006; Fuchs et al. 2012; Fan et al. 2025). In addition, the enzymes acetate kinase and L-lactate dehydrogenase have also been identified in the core proteome, which increase the formation of acetate and lactate to produce more energy and enable bacterial survival in aerobic and low-oxygen environments(Pragya et al. 2017). Several amino acids have been identified as essential for the growth of GBS strains under both aerobic and anaerobic conditions. Research indicates that a uniform requirement for specific amino acids exists across various GBS strains, which is crucial for their growth and metabolism(Fan et al. 2025). Our study identifies proteins involved in the metabolism of essential amino acids (glycine, serine, glutamine, aspartic acid, threonine, alanine, and asparagine) in human and fish GBS isolates. GBS is auxotrophic, meaning it relies on external sources of certain amino acids, necessitating specialized transport proteins and peptidases(Glaser et al. 2002; Rajagopal 2009). Our results further demonstrates that GBS strains adapt to aquatic environments by expressing distinct sets of proteins involved in amino acid uptake and metabolism, including ABC transporters, which facilitate nutrient acquisition under these conditions(Wichgers Schreur et al. 2011). Additionally, our study identifies proteins involved in metal ion homeostasis, such as copper (Cu) and zinc (Zn), which help GBS resist host defense mechanisms. These proteins, including metalloregulatory proteins, manage metal ion balance, which is crucial for bacterial survival under stress. The TrkA protein, responsible for potassium uptake, plays a key role in osmoregulation by maintaining internal pH and membrane potential(Di Palo et al. 2013; Gründling 2013). We also identifies key enzymes involved in fatty acid metabolism, including carboxylase, malonyl-CoA acyl carrier protein transacylase, 3-oxoacyl-[acyl carrier protein] synthase, 3-oxoacyl-[acyl carrier protein] reductase, and acyl carrier protein (ACP). The ACP is particularly important in regulating fatty acid transcription and helps *Streptococcus* species adapt to low-pH environments. This adaptability is crucial for adjusting the composition of membrane fatty acids, which supports cellular integrity under acidic conditions(Lu and Rock 2006).

As high water temperatures are usually associated with outbreaks of GBS, water temperature has been identified as a risk factor for the development of GBS infection in fish(Mian et al. 2009). Bacteria respond to heat stress by expressing various proteins associated with thermal adaptation, such as chaperonins, heat shock proteins, and cold shock proteins(Yura and Mori 1993). These proteins help bacteria maintain cellular homeostasis and survive under stressful conditions. Our research highlights the presence of several proteins linked to thermal shock response, such as the chaperone protein DnaK and multiple chaperonins. These proteins play a crucial role in preventing the inactivation of other proteins and facilitating the breakdown of damaged ones(Yura and Mori 1993). Furthermore, our analysis reveals that GBS isolates are capable of adapting to environmental stress by boosting their transport mechanisms, increasing their energy production efficiency, and modifying the composition of their membrane lipids. Collectively, these observations underscore GBS's robust defense strategies against environmental challenges through the synthesis of a wide array of protective proteins(Nair et al. 2003).

For GBS to successfully move from the environment into a host, it often needs to undergo adaptive changes. One important mechanism that helps these bacteria detect and respond to environmental signals is the two-component signal transduction system. This system involves proteins that work together to sense changes and trigger appropriate responses, enabling the pathogen to adjust and survive in the new host environment(Faralla et al. 2014). Our study highlights key proteins, like the serine/threonine phosphatase Stp1 and the ciaR regulatory protein, involved in signal transduction that help GBS survive and spread in host tissues. These proteins are found in both human and fish GBS isolates, contribute to bacterial survival in serum and are linked to diseases like septicemia, meningitis, and encephalitis, though their exact role in disease development remains unclear(Doran et al. 2005; Santi et al. 2007). Our study also identifies several virulence factors in GBS, including proteins involved in adhesion, invasion, and immune evasion, some of which have been well-studied in human isolates. Notably, the BibA protein, which is essential for brain tissue invasion and blood survival, is also found in fish GBS strains, suggesting a similar pathogenic role in both humans and fish(Rajagopal 2009). Our study suggests that these proteins may play a key role in the development of GBS associated with bacterial meningitis and septicemia. However, additional research is required to definitively establish this connection.

Elongation factor Tu is another protein found in our study to be involved in bacterial adhesion. This protein has been shown to mediate the binding of bacteria to mucin, fibrinogen, and fibronectin(Granato et al. 2004; Schaumburg et al. 2004; Balasubramanian et al. 2008). Consistent with previous research(Li et al. 2014), our study identified Elongation Factor Tu as the most abundant protein detected, highlighting its prominent role and high expression level in GBS. Additionally, the integrative and conjugative protein AbiGII, which is linked to metal resistance and virulence in GBS(Dy et al. 2014), was found in both human and fish GBS isolates. Our study also highlighted the identification of EsxA, a virulence factor that facilitates bacterial dissemination and colonization in *Streptococcus* species, particularly in mice(Lai et al. 2017), and can induce antibodies in humans(Zhou et al. 2013). Other virulence proteins discovered in GBS isolates from humans and fish in our study included gluconate-5-dehydrogenase, which catalyzes the reversible oxireduction of D-gluconate, and glycosyltransferase, an enzyme responsible for the synthesis of important structural molecules such as glycoproteins and glycolipids, playing roles in bacterial evasion, immune recognition, and biofilm formation(Zhang et al. 2009). The pathogenicity of GBS in humans and fish is also enhanced by other proteins identified through proteomic analysis in our study, including those involved in nucleotide metabolism and oxidative stress. Previous research has shown that purine and pyrimidine metabolism are critical for bacterial survival and dissemination in human serum(Samant et al. 2008). This aligns with the understanding that multiple virulence factors, including surface proteins and enzymes, contribute to GBS pathogenesis by supporting bacterial adherence, immune evasion, and survival within the host environment(Campeau et al. 2021). GBS, on the other hand, showed dynamic metabolic adaptation when genes involved in purine and pyrimidine metabolism showed a significant change in transcription in response to incubation with human blood(Mereghetti et al. 2008). In addition, proteomic analysis in our study revealed the expression of proteins previously described in GBS strains, such as superoxide dismutase, SufB, SufC, SufD, thioredoxin, and NADH oxidase(Glaser et al. 2002; Godoy et al. 2013). Superoxide dismutase, one of these proteins, is also involved in virulence and contributes to making GBS dangerous by enabling bacterial survival in macrophages and maintaining a high bacterial load in the blood(Poyart et al. 2001).

Our study revealed that although the protein numbers of the human and fish GBS strains were comparable, there were differences in proteome expression. It was found that there was a correlation between fish GBS isolates and the expression levels of several proteins. Less protein was expressed in fish GBS isolates than in human GBS isolates. These results suggest that fish pathogenic GBS isolates have a lower potential for catabolism than human GBS isolates. Previous studies using genomic techniques hypothesized that adapting the bacterium to aquatic hosts might be responsible for the lower catabolic capacity of fish strains(Liu et al. 2013; Rosinski-Chupin et al. 2013).

A limitation of our study is that only two GBS ST283 isolates were obtained from human sources, whereas all fish GBS isolates belonged to ST283. Therefore, GBS ST283 strains should be monitored sequentially throughout Malaysia in hospital settings, and additional representative isolates derived from fish need to be characterized. Although the fish and human GBS isolates were isolated at different time points (human; 2019–2020, fish; 2008–2018), the occurrence of ST lineages may persist over time, so the current study may prompt more systematic studies with isolates from both settings. Furthermore, in our study, proteomic analysis using the LC–MS/MS technique was conducted on seven selected GBS isolates from four different STs. To gain deeper insights, further proteomic analyses involving a larger number of isolates from diverse STs, employing the more sensitive nano-ultra-performance liquid chromatography (nano-UPLC), are highly recommended. Additionally, GBS has been identified as a pathogen affecting various fish species beyond tilapia, including both farmed and freshwater fish(Geng et al. 2012; Soto et al. 2015). Tilapia was selected as the most commercially significant species affected by GBS in Southeast Asia for ST283 detection in fish farms. However, it is important to consider including other fish species in comparative analyses to fully understand the host range and impact of GBS infections.

Conclusion

The core proteome of GBS isolates from humans and fish exhibits a high degree of conservation, indicating that many proteins are shared across different strains. This suggests a fundamental similarity in the biological processes that govern GBS survival and pathogenicity in diverse hosts. Our study identified stress-related proteins, such as heat shock proteins, that are crucial for GBS survival under environmental stressors. These proteins are significantly expressed in both human and fish strains, reflecting their role in the organism's adaptability. Specific proteins identified in human strains, including virulence regulators like β-lactamase and biofilm regulatory proteins, highlight potential targets for novel antimicrobial therapies. The presence of these proteins underscores their roles in GBS pathogenicity, which is critical for understanding how these bacteria interact with their hosts. Our study also noted differences in metabolite profiles between human and animal strains. For instance, certain metabolites, such as L-aspartic acid, were more abundant in human strains, while others were uniquely expressed in animal strains. This variation can influence

metabolic pathways and contribute to the pathogenic capabilities of GBS. The findings advocate for further comparative studies involving a broader range of GBS isolates from both humans and fish. Such research could deepen our understanding of GBS pathogenicity, adaptability, metabolism, and the potential for interspecies transmission. This is particularly relevant given the zoonotic potential observed in certain strains.

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Authors Contributions ARM and MNMD conceived and designed the methodology and data analysis, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft. MHY, NS, NHZB and NDR performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft. MNMD, RH and SAN analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

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Data availability The datasets that are used and/or analyzed in this study can be made available upon reasonable request.

Declarations

Competing interest The authors unanimously declare that there are no conflicts of interest.

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