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Multiomics analyses reveal that NOD-like signaling pathway plays an important role against *Streptococcus agalactiae* in the spleen of tilapia

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ABSTRACT

Streptococcus aglactiae(GBS) infection in tilapia is a serious global disease that causes significant production loss. Here, we studied the role of GBS in the spleen and the spleen's response against the pathogen through dual RNA-seq and proteome technology. Animals were divided into three groups: control, virulent treated (HN016), and attenuated treated (YM001). Spleen samples were collected and analysis when a disease outbreak. Dual RNA-seq result showed the virulence factor genes of GBS, included CAMP factor, PGK, OCT, enolase, scpB, Sip, bca, were upregulation. downregulation of GapA, cylE, OCT, scpB, C5AP, rlmB, hly, FBP, in HN016 and YM001. But for proteomic, OCT and bca were downregulation, the others were upregulation. For host transcriptome KEGG analysis showed, the NOD-like receptor signaling pathway (NLRs) and TOLL-like receptor signaling pathway (TLRs) were upreguoation in HN016 infected fish than the control fish; But for proteome KEGG, only the NLRS was up, the TLRS was not change. Compared with YM001 infected fishes, for transcriptome, NLRs and TLRs in infected HN016 fishes were significance rise ($p < 0.01$); for proteome, the NLRs was up ($p < 0.05$), but TLRs was no change.Analysis of pathogen-host interaction showed that the peptidoglycan (PNG), CD2, LCK, and host's Zap70 were involved in the regulation of NLRs; PNG, LCK, and ZAP70 were involved in the regulation of TRLs. Conclusion: the virulent strain HN016 and attenuated strainYM001 differed in the quantity of virulence factors. In tilapia's innate immune system, NLRs was the main defense factors, but bacteria avoided the host defense through TLRs.

1. Introduction

Streptococcus agalactiae (Group B Streptococcus, GSB) is an important broad-spectrum pathogen [1]. It has been shown to cause a number of deadly disease in tilapia in the recent years.

Tilapia is one of the most economically farmed fish and serves as an important source of proteins worldwide. Unfortunately, *S. agalactiae* infection has been shown to cause some significant loss [2] and globally researchers have been making unremitting exploration to understand the mechanism of action of this pathogenic bacteria on tilapia as well as devising methods to prevent the disease. On one hand, we have discovered the pathogenic genes of *S. agalactiae* and their role in the pathogenesis of the disease [3,4] from our previous studies; and on the other hand, other groups have reported tilapia's defense mechanism against the pathogen [5]. The latter revealed that the important barriers of tilapia against *S. agalactiae* infection include mucosal immunity, humoral immunity, and cellular immunity [6]. With the development of scale sequencing technology, in addition, the disease resistance of tilapia against *S. agalactiae* pathogenesis was studied by sequencing. However, it is important to note that these studies are independent, either done on the pathogen [7], or the host [8]. *S. agalactiae* infection and tilapia resistance is a dynamic process and dependent on each other. Thus, in our study, we investigated to study this relationship between *S. agalactiae* infection and tilapia resistance, using a novel dual RNA-Seq method [9].

Dual RNA-Seq technique does not require the isolation of pathogen from the hosts [10]. RNA libraries were constructed directly from the same sample, and then reverse transcribed to obtain the cDNA. This unique dual sequencing technique allowed both bacterial and host RNA to be sequenced at the same time. The technology has shown great promise in explaining the host-pathogen relationship at the genetic level, the process of initiating infection by the bacteria, and thus has

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been applied in many research fields [11]. However, many groups have reported that the transcriptome and proteome were not the similar trend [12].

The difference between the immune system of fish and mammals is that the fish lacks bone marrow and lymph nodes [13]. Lymphocytes, monocytes, macrophages, neutrophils, and surface immunoglobulin positive cells were found in the spleen [14], making the thymic and splenic cellular organization appear very similar to that of the mammalian counterparts. The thymus serves as the primary hematopoietic and peripheral lymphoid organ for T lymphocytes in the fish [15], and it contains antibody producing cells, which is the major source of immunoglobulins [16]. It also functions as an endocrine tissue [17]. It is well-known that the spleen of bony fishes plays an important role in immunity. The gene expression of interleukin IL-1β, IL-1 receptor antagonist (Ra), IL-6, IL-8, IL-10, IL-11, IFN-γ, and IL-1 receptor I and II (IL-RI and IL-RII) were increased in the spleen during the primary infection with *S.agalactiae*, but not during the secondary infection [14]. The antimicrobial peptides (AMPs) of piiscidins were found in fish spleen [18]. Through spleen transcriptome of grass carp found that there were six immune-related genes [16]. These suggest that the spleen of fish plays an important role in resisting the invasion of pathogenic bacteria.

In our previous study, we were working on a vaccine derived from *S. agalactiae* [19], and found that YM001 was immunogenic and HN016 was highly toxic. From there, the question we wanted to solve was the relationship between YM001 or HN016 and spleen. In this study, we used the virulent HN016 and attenuated YM001 strains to infect tilapia. The purpose of this study was to reveal the differences between YM001 and HN016 with the host defense system using dual RNA-seq and TMT-proteome technology [20]. This would provide a new target for vaccine control against *S. agalactiae* infection and aid in the future prevention and subsequent treatment of the disease. Our study also has a reference significance, for other broad-spectrum pathogens.

2. Materials and methods

2.1. Bacterial strains and fishes

S. agalactiae namely HN016 was originally isolated from a moribund cultured tilapia(*Oreochromis niloticus*) with typical clinical and pathogenic characteristics of meningoencephalitis, in 2010 in China, and it belongs to *S. agalactiae* serotype Ia, multilocus sequence type seven (ST7). This field strain was gradually attenuated by 840 continuous passaging in TSB medium (Tryptic Soy Broth) to obtain the strain YM001. Virulence test revealed that YM001 have no virulence on tilapia, and exhibit good safety, immunogenicity, and stability [19]. The bacteria was cultured in a shaker in TSB medium at 37 °C for overnight. The bacterial concentration was detected by the plate count method and recorded as colony-forming unit (CFU). Non-infected Nile tilapia (*Oreochromis niloticus*) with average weight of 400 ± 25 g was provided by the National Tilapia Seed Farm (Nanning, Guangxi, China). Prior to experiment, the fished were acclimated in fiber-reinforced plastic tanks to adapt to the indoor circulating water system for two weeks. The experimental fishes were confirmed to be negative for the bacterial infection using bacteriological analysis from the brain and kidney sample.

After anesthesia by immersion into a bath of 10 mg/L benzocaine (Sigma, USA), a total of 60 fishes were equally divided into three groups, each group with two tanks (10 fishes/tank, with 2 replicates). The attenuated YM001 group was intraperitoneally injected (IP) with 0.2 ml concentrated YM001 (2.5×10^9 CFU/ml). The virulent HN016 group was injected with 0.2 ml concentrated HN016 (2.5×10^9 CFU/mL). The control group was injected with 0.2 ml of sterile TSB. The infected fishes were monitored and raised in fiber-reinforced plastic tanks at 33 ± 1 °C.

The health status of the experimental fish in each group was measured every 30 min. Preliminary studies under the same condition, showed that the fish with HN016 strain began to die 7 h after the challenge, and then all died within the next 20–40 min. In our study, when the first tilapia with strain HN016 was on the point of death, all the experimental fish in the three groups were anesthetized within 30 min, and the brains, liver, spleen, head and kidney, and intestines of 10 fishes in each group were collected randomly under sterile conditions. After they were frozen in liquid nitrogen, they were stored – 80 °C for subsequent tests. The same tissue from the same test group was mixed into a total sample, grinded into powder with liquid nitrogen and separated two parts for technical repetition. Meanwhile, the fish brain and kidney tissues of YM001 and HN016 challenge groups were isolated, cultured, and identified to verify the successful challenge. (Animal experimental ethical inspection of Guangxii University,NO:GXU2019-060). The tissues were also fixed for routine pathological analysis (4 samples/group) at the same time.

2.2. RNA-sequencing

2.2.1. RNA extraction and sequencing

The method followed involved extraction of total RNA from the spleen [21]. The RNA quality was evaluated by test concentration (Nanodrop 2000) and agarose gel electrophoresis (AGE). Thereafter, the transcriptome library was constructed (Truseq RNA sample prep Kit, Illumina). Enrichment of RNA, fragment interruption, addition of adapters, size selection, PCR amplification, and RNA sequencing were performed by the staff of MajorBio (Shanghai, China). mRNA purification was then performed using the Ribo-zaro Magneti Gold kit (EpiCentre). The cDNA library was constructed using Truseq RNA sample prep Kit(Illumina) and quality controlled using QuantiFluor® dsDNA System (Promega). At last, a 10 ng cDNA library was used for cluster generation in cBot by TruSeq PE Cluster Kit (Illumina, USA) before sequencing using Illumina Hiseq™2500.

2.2.2. Gene sequence alignment and analysis

All the raw data with adaptors and low-quality reads was first filtered by SeqPrep (https://github.com/jstjohn/SeqPrep)and Sickle (https://github.com/najoshi/sickle). Contigs were assembled from the clean data via Hiast2 software (http://ccb.jhu.edu/software/hisat2/index.shtml). The following analysis were performed: 1. Assessment of ribosomal RNA contamination rate by Rfam (http://rfam.xfam.org/) [22]; 2. sequencing saturation analysis and genetic coverage analysis by RSeQC (http://rseqc.sourceforge.net/) [23,24]; 3. expression analysis by RSEM (http://deweylab.github.io/RSEM) [25]; 4. expression difference analysis by edger (http://www.bioconductor.org/packages/2.12/bioc/html/edgeR.html) [26]; 5. Gene Ontology (GO) classification statistics of differentially expressed genes by blasting the GO database and GO enrichment analysis of differential genes by Goatools [27–29]; 6. Enrichment analysis of differential gene KEGG (Kyoto encyclopedia of genes and genomes) pathway by KOBAS(http://kobas.cbi.pku.edu.cn/home.do); and lastly visualization of KEGG pathway of differentially expressed genes from the KEGG database (http://www.genome.jp/kegg/) [30,31].

2.3. TMT labeling, RPLC fractionation, and LC-MS/MS analysis

2.3.1. Protein extraction and enzymolysis

The spleen tissue was homogenized using liquid nitrogen. The protein lysate was added in a ratio of 1:15 (1% SDS, 8 M urea, and protein inhibitor) on the ice bath. 30 mines later, ultrasound treatment was performed on the ice for 2 min. Thereafter, the protein samples were centrifuged for 20 min at 4 °C at 12,000 g and the supernatant was obtained. The protein was quantified using the BCA method according to the manufacturer's instructions and SDS PAGE.

Total protein (100 μg) taken from each sample was mixed with 100 μL of the lysate. TCEP (10 mM), was added and then it was stored at

Table 1
Overview of transcriptome.

Sample	Reads	Base(bp)	Error%	Q20%	Q30%	GC%
CK_S1	50423836	7551400908	0.0228	98.98	96.34	48.61
CK_S2	53246314	7968317394	0.0228	99	96.42	48.98
HN016_1	15975432	2132830717	0.0117	98.6	95.87	37.93
HN016_2	16243082	2175788809	0.0115	98.69	96.11	37.68
HN016_S1	82020386	10633444478	0.0122	98.33	95.22	44.1
HN016_S2	82185434	10742714834	0.0122	98.36	95.18	44.16
YM001_1	17963378	2420122538	0.0118	98.54	95.73	38.29
YM001_2	18279062	2440975571	0.0118	98.58	95.69	38.35
YM001_S1	89778986	11843636025	0.0125	98.24	94.86	47.7
YM001_S2	84347910	11010868452	0.0123	98.33	95.09	47.11

NOTE:CK_S was control; HN016_n was HN016 GBS strain *in vitro*; HN016_s was HN016 infected in fish; YM001_n was YM001 strain *in vitro*; YM001_s was YM001 infected in fish.The following was the same.

37 °C 60 min later, iodoacetamide (40 mM) was added and stored away from light at room temperature for 40 min. To it each sample, cold acetone (v: v = 6:1) was added and stored at −20 °C for 4 h. Thereafter, the samples were centrifuged at 10,000 g for 20 min, and the supernatant was discarded. The pellet was collected and resoluted with 100 Mm TEAB and trypsin (1:50 by weight) was added at 37 °C stay overnight.

Tandem mass tags (TMT) labeling was done following the protocol as mentioned in literature [32]. TMT (Thermofisher, cat: 90111) reagent was taken out from −20 °C, and kept at room temperature; acetonitrile was added, quickly centrifuged, followed by the addition of the peptide prepared above (1 μg/tube) and kept at room temperature. 2 h later, hydroxylamine was added. The labeling was finished after 15 min, and the labelled samples were dried using a vacuum concentrator. The peptide was dissolved and one-dimensional separation with RPLC was performed (Waters). The sample was collected and dried by vacuum concentrator again. Lastly, MS-buffer was added to dissolve the sample and tested by LC-MS (EASY-nLC 1200, Q-Exactive, thermo, USA). Each sample was divided two parts and tested independently according to the above method.

Data analysis: All the data was analyzed using ProteomeDiscoverer software 2.1. Only proteins with FDR < 0.01 were considered as significantly differential proteins [33]. Following analysis such as the GO annotation and KEGG pathway was performed in a manner similar to the transcriptome [16].

2.4. Integration of the transcriptome and proteome data

The virulence factor genes and proteins expressed in HN016 and YM001 were compared. The differences in transcription and protein expression of common disease-resistant genes in tilapia was analyzed. The interactions between bacterial proteins and host proteins were conducted through the string database (https://string-db.org/) [33].

3. Result

3.1. The total transcriptome and proteome landscape in S. agalactiea-infected tipalia

Transcriptome sequencing data is shown in Table 1. For *S. agalactiea* infection, differential transcriptional analysis revealed that 1045 genes were differentially expressed after infection of HN016, 538 genes were differentially expressed after infection of YM001, and 895 genes were differentially expressed when comparing HN016 and YM001 strains. For the tilapia there were 12,338 differentially expressed genes in tilapia infected with the HN016 and 7705 differentially expressed genes in the YM001 infected fish. Total 7248 differentially expressed genes in tilapia infected with HN016 and YM001 strains were found, as shown in Table 2.

A total of 6456 proteins expressed were identified as belonging to the proteome of spleen, and 640 proteins were found to be expressed in *S. agalatiea*. Analysis of differentially expressed proteins showed that 327 proteins were upregulated and 1482 proteins were downregulated after tilapia was HN016 infected. In the YM001 infected spleen, 207 proteins were upregulated and 925 were downregulated. Comparing HN016 and YM001 infected spleen, there were 671 proteins differentially expressed, among which 299 were upregulated and 372 were downregulated. For *S. agalactiae*, 547 proteins were differentially expressed in the HN016 and YM001 strains, among which 522 were upregulated and 25 were downregulated.

The profiles of differentially expressed genes and proteins are shown in Fig. 1 and Fig. 2.

3.2. Differences in the gene and protein expression through Gene Ontology (GO) analysis

GO analysis was used to further understand the functional differences in the differentially expressed genes and proteins (Fig. 3). GO analysis is mainly divided into three categories: biological process (BP), molecular function (MF), and cellular component (CC). The transcription ratio of bacteria *in vivo* varies greatly as compared to that observed in *in vitro*. Upon comparing HN016 vs. YM001, IMP metabolic process (BP), ligase activity (MF), and protein-N(PI)-phosphohistidine-sugar phosphotransferase complex (CC) etc. revealed significant differences ($p < 0.001$). However, the protein differences are much less than the gene expression differences. The protein GO analysis, thedifferences were mainly organelle (CC),protein-containing complex (CC), cell part (CC), cellular component organization or biogenesis (BP), cellular process (BP), structural molecule activity (MF), catalytic activity (MF), and binding (MF).

When tilapia was infected with foreign substances, gene transcription was very active, and gene expression was very significant in both infected and control groups. However, upon analyzing the differential expression of proteins, it was found that the expression of proteins was much lesser as compared to the gene expression.

3.3. KEGG results of transcriptome and proteome

For bacterial strain HN016 and YM001, KEGG analysis showed that there was significant upregulation in the transcriptome of the genes belonging to fatty acid metabolism, fatty acid biosynthesis, and the same changes were also observed in the proteome analysis. In particular, the ribosome pathway was highly upregulated($p < 0.01$). The down-regulated proteins were found to be involved in arginine biosynthesis and glycerophospholipid metabolism. The results are shown in Table 3.

For tilapia fish infected with HN016 (Table 4)as compared to

Table 2
The difference of transcriptome.

S.agalatiea	HN016_vs_HN016_S	YM001_S_vs_HN016_S	YM001_vs_YM001_S
Difference gen express number	1045	538	895
Tilapia	CK_S_vs_HN016_S	YM001_S_vs_HN016_S	CK_S_vs_YM001_S
Difference gen express number	12338	7248	7705

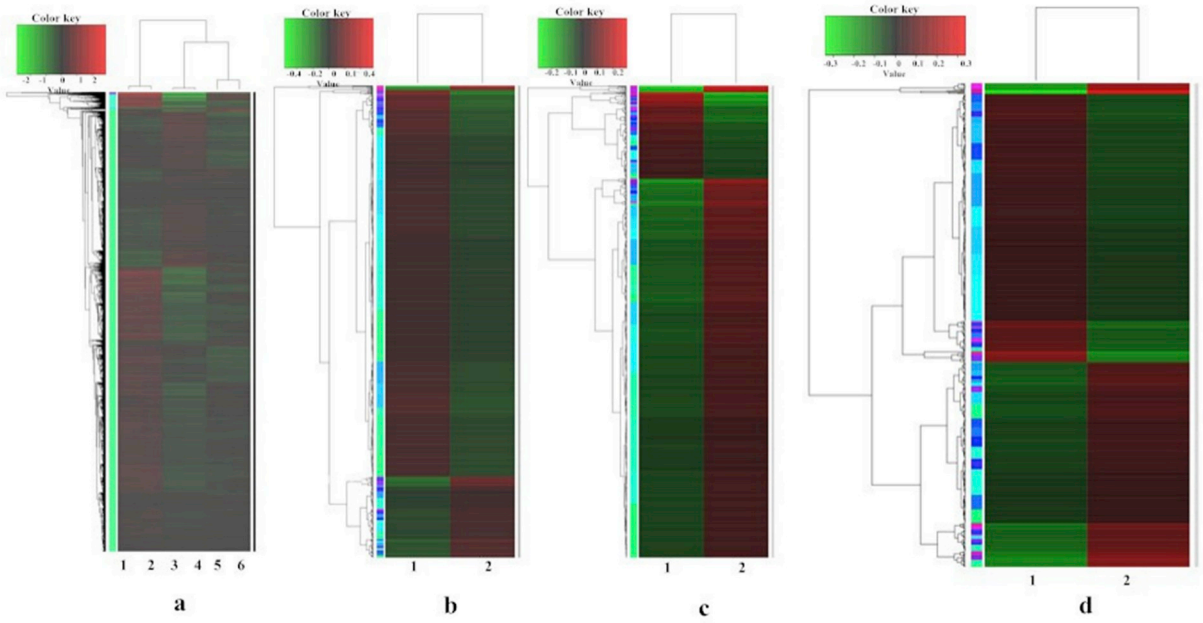

Fig. 1. Heatmaps showing Tilapia Transcriptome(a) and proteome(b-d) a: Transcriptome; 1 and 2 were similar to the one treated with HN016; 3 and 4 were control group; 5 and 6 were treated with YM001. b to d: proteome; b:1 Control group; 2. HN016 treated; c:1 HN016 treated; 2.YM001 treated; d:1.Control group; 2.YM001 treated.Two repeats separately.Red was upregulation, green was downregulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

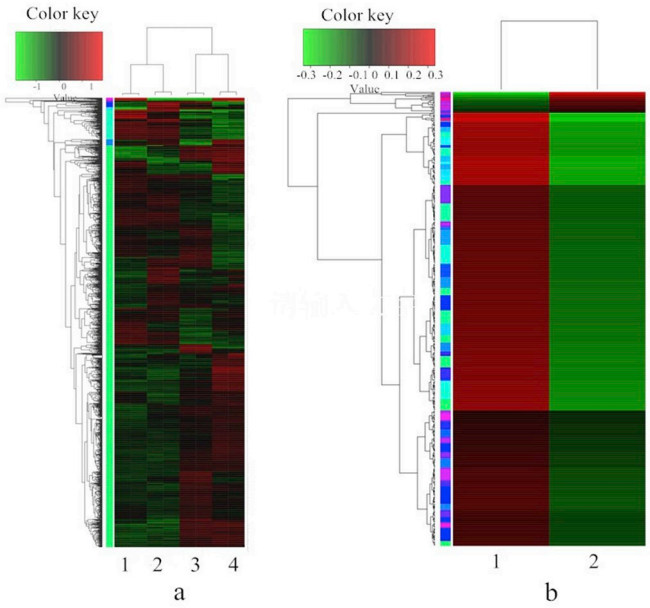

Fig. 2. Heatmaps showing the GBS transcriptome (a) and proteome (b) a: Transcriptome: 1. HN016 treated group; 2.YM001 treated group; 3.YM001 *in vitro*; 4.HN016 *in vitro*. b: Proteome of GBS in fish:1.HN016; 2.YM001. Red was upregulation; Green was downregulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

YM001(Table 5), the KEGG analysis revealed that the nod-like receptor signaling pathway after infection with HN016 was significantly upregulated ($p < 0.01$) in both transcriptome and proteome analysis. On the contrary, the genes of toll-like receptor signaling pathway were significantly upregulated ($p < 0.01$) after *S. agalatiae* infection, whereas the proteins were significantly downregulated, while there was no significant difference ($p > 0.05$) between the HN016 and YM001 infected fish(Table 6).

3.4. Comparison of the virulence factor genes and protein changes in S. agalactiae

The gene transcript expression and protein expression of HN016 and YM001 of *S. agalactiae* were analyzed. Virulence factor genes of HN016, including cylE, PGK, OCT, enolase, and C5AP were upregulated after invasion of the host. But genes of GapA, CAMP factor, Sip, rlmB, hly, bac, and FBP were downregulated. For YM001 live in the host, genes of CAMP factor, scpB, C5AP, bca, and FBP were upregulated; inverse of the gene of GapA, cylE, PGK, enolase, Sip, rlmB, and hly were downregulated. HN016 compared to YM001 revealed that the upregulated genes were CAMP factor, PGK, OCT, enolase, scpB, Sip, and bca; whereas the downregulated genes were GapA, cylE, OCT, scpB, C5AP, rlmB, hly, and FBP. In detail on the protein analysis, in addition to OCT and bca protein were lowered in HN016, the rest of the protein were significantly raised ($p < 0.01$). The results are shown in Table 7.

3.5. Comparison of disease-resistant genes and proteins in tilapia

Tilapia were infected with *S. agalactiae* and the splenic genes of CD59, CD2BP2, LCK, ZAP70, TLR1, TLR2, TLR3, TLR8, and TLR13 were downregulated. Genes of SAS, PTGS2, IL-1 β, TNF-α, CD2, MCP - 8, gelectin-8, NOD1 and NOD2, TLR7, and TLR9 were upregulated. The expression of COX2, IL-1 β, TNF-α, CD2, LCK, galectin-8, NOD1, TLR5, and TLR9 genes in spleen infected with the HN016 were significantly upregulated as compared to that of the YM001 infected spleens ($P < 0.01$). Interestingly, the CD59 gene was significantly downregulated ($p < 0.01$).

For proteome, the upregulated proteins are discussed in Table 5. TLR5 was significantly upregulated in virulent infection group as compared to the blank control group; but the proteins of COX2, CD2, CD2BP2, McP-8, LCK, zap-70, NOD1, TLR1, and TLR2 were downregulated. Compared to the blank control group, IL-1 β and TLR5 were significantly upregulated in the YM001 infected group. However LCK, ZAP-70, TLR1, TLR2, and TLR3 were significantly downregulated ($p < 0.01$). CD59, TLR1, TLR2, and TLR5 were significantly upregulated in the HN016 infected group as compared to theYM001 infected group (Table 8).

Fig. 3. GO analysis of transcriptome and proteome Tilapia infected HN016 VS YM001 GO analysis of transcriptome and proteome. Bars in red refer to the transcriptome measurements, whereas the green ones, to the proteome measurements. Transcripts were grouped under three main categories: biological processes, cellular components and molecular function, indicated in the bottom of the figure. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.6. The result of manifestations and histopathologic change

As shown in the figure (Fig. 4), the strong strain showed typical clinical symptoms and death, while the weak strain showed no clinical symptoms and died. Pathologically, the spleen of the virulent strain HN016 had structural damage whereas the attenuated strain YM001 and control did not.

4. Discussion

4.1. The differences between HN016 and YM001 strains

Most studies have focussed on the effect of virulent bacteria on host, or to study the differences *in vitro*. In this study, we have used virulent and attenuated strain for infection. The analysis revealed that the

Table 3
Comparison of the KEGG pathway of GBS.

GBS transcriptome	GBS protome up regulation	GBS proteome down regulation
GBS YM001_S VS HN016_S	HN016_S VS YM001_S UP	HN016_S VS YM001_S down
Phosphotransferase system (PTS)(*) ABC transporters(*) Alanine, aspartate and glutamate metabolism(*) Pyruvate metabolism(*) Propanoate metabolism(*) Fatty acid metabolism(**) Fatty acid biosynthesis(**)	Aminoacyl-tRNA biosynthesis(**); Ribosome (***); Mnino sugar and nucleotide sugar metabolism (*); Cysteine and methionine metabolism(*); Fatty acid biosynthesis (***); Fatty acid metabolism(***); Folate biosynthesis (*);; Glycerolipid metabolism(***) Propanoate metabolism (*)	Arginine biosynthesis (***); Glycerophospholipid metabolism (***); Plant-pathogen interation(*); PPAR signaling pathway(*).

Note: *,P < 0.05; **,P < 0.01; ***,P < 0.001.

Table 4
HN016_S VS CK_S the KEGG pathway.

HN016_S VS CK_S	HN016_S VS CK_S up	HN016_S VS CK_S down
NOD-like receptor signaling pathway(**)	Neuroactive ligand-receptor interaction (***)	Axon guidance(***)
Toll-like receptor signaling pathway(**)	Leishmaniasis (***)	Adrenergic signaling in cardiomyocytes(***)
TNF signaling pathway(**)	*Staphylococcus aureus* infection (***)	Chemokine signaling pathway(***)
FoxO signaling pathway(**)	Systemic lupus erythematosus (***)	Complement and coagulation cascades(**)
Apoptosis(*)	Arachidonic acid metabolism (***)	Natural killer cell mediated cytotoxicity(***)
Endocytosis(*)	Starch and sucrose metabolism (***)	Neurotrophin signaling pathway(***)
Autophagy-animal(**)	Complement and coagulation cascades (***)	T cell receptor signaling pathway(***)
Propanoate metabolism(*)	Protein digestion and absorption (***)	Long-term potentiation(**)
Insulin signaling pathway(*)	NOD-like receptor signaling pathway(*)	Leukocyte transendothelial migration(***)
Adipocytokine signaling pathway(*)	Glutathione metabolism(*)	Oxytocin signaling pathway(***)
Neurotrophin signaling pathway(*)	Pancreatic secretion(**)	Melanogenesis(**)
		Platelet activation(**)
		PPAR signaling pathway(***)

Note: *,P < 0.05; **,P < 0.01; ***,P < 0.001.

Table 5
YM001_S VS CK_S the KEGG pathway.

YM001_S vs CK_s	YM001_S vs CK_s:up	YM001_S vs CK_s:down
Cytokine-cytokine receptor interaction(**);	Neuroactive ligand-receptor interaction(*)	Cell adhesion molecules (CAMs) (***)
Ribosome(***);	Antigen processing and presentation(*)	Cytokine-cytokine receptor interaction(**)
Hematopoietic cell lineage(***);	Cholesterol metabolism(**)	Primary immunodeficiency(***)
Intestinal immune network for IgA production(**);	Complement and coagulation cascades(***)	Natural killer cell mediated cytotoxicity(***)
Trptophan metabolism(*);	Fat digestion and absorption(***)	T cell receptor signaling pathway(***)
Fatty acid biosynthesis(*);	Hematopoietic cell lineage(**)	Th17 cell differentiation(***)
Circadian rhythm-fly(*)	Pancreatic secretion(***)	Th1 and Th2 cell differentiation(***)
	Plant-pathogen interaction(*)	Thermogenesis(*)
	Protein digestion and absorption(***)	Toll-like receptor signaling pathway(*)
	Relaxin signaling pathway(*)	
	Vitamin digestion and absorption(**)	

Note: *,P < 0.05; **,P < 0.01; ***,P < 0.001.

virulence factor of the two strains of bacteria could not be detected *in vivo*. But the extent of expression varies greatly. The virulence factor quantitative protein of HN016 was higher than that of YM001. Although, virulence factor is an important criteria of the pathogenic bacteria, this study revealed that the amount of virulence factor was the most important factor. Virulence factors existed in both the strains, but the expression levels were different. The results varied greatly in host -one lethal, one tolerated. Hence, the amount of virulence factor may be the cause of death. Meanwhile, according to the GO analysis and KEGG analysis of differentially expressed genes, it was found that the differences between the HM016 strain and the YM001 strain was mainly reflected in metabolism, i.e. the strong strain propagated quickly *in vivo*, as compared to the weak strain. Wang [52] conducted a complete genetic analysis of the HN016 and YM001 and found that YM001 was missing a gene that codes for metabolism. Genes involved in phosphotransferase system (PTS), ABC transporters, fatty acid metabolism and biosynthesis were expressed higher in HN016 as compared to YM001.

Virulence was also found to be related to temperature. Clinical incidence mainly occurred in the high temperature season. A study by Tavares [53] showed that in *in vitro* culture, high temperature (32 ˚C) as compared to low temperature (22 ˚C) was the main difference between the metabolism and virulence factors. Studies by Laurent [54] showed that virulence factors, specifically hemolysis and exocrine proteins,

Table 6
HN016_S VS YM001_S the KEGG pathway.

HN016_S_vs_YM001_S	HN016_S_vs_YM001_S.up	HN016_S_vs_YM001_S.down
Apoptosis(***);	Biofilm formation - *Escherichia coli*(**)	Lysosome(***)
Cytokine-cytokine receptor interaction(**);	Insulin resistance(*)	Ribosome(***)
TNF signaling pathway(**);	Biosynthesis of antibiotics(**)	Biosynthesis of antibiotics(***)
NF-kappa B signaling pathway(**);	Starch and sucrose metabolism(***)	Oxidative phosphorylation(***)
Osteoclast differentiation(**);	Complement and coagulation cascades(**)	Purine metabolism(**)
Complement and coagulation cascades(***);	Fat digestion and absorption(**)	Pyruvate metabolism(*)
Antigen processing and presentation(***);	Glucagon signaling pathway(**)	Mineral absorption(***)
Hematopoietic cell lineage(***);	Inflammatory mediator regulation of TRP change Insulin signaling pathway(*)	Platelet activation(***)
NOD-like receptor signaling pathway(***);	NOD-like receptor signaling pathway(*)	Parathyroid hormone synthesis, secretion and action(*)
Intestinal immune network for IgA production (**);	Osteoclast differentiation(*)	Protein digestion and absorption(***)
T cell receptor signaling pathway(**);	Serotonergic synapse(*)	Relaxin signaling pathway(***)
Toll-like receptor signaling pathway(**);	Thermogenesis(*)	
RIG-I-like receptor signaling pathway(**).	Vitamin digestion and absorption(*)	

Note: *,P < 0.05; **,P < 0.01; ***,P < 0.001.

Table 7
Comparision of pathogenic factors.

gene	Seq_ ID	HN016_vs_HN016_S	YM001_vs_YM001_S	YM001_S_vs_HN016_S	description
Gap A [4]	SAHN016_RS08650	Down,no	Down,yes	Down,no	GAPDH, gapA
cylE [34]	SAHN016_RS03635	Up,yes	Down,no	Down,yes	cylE
CAMP factor [35]	SAHN016_RS09635	Down,no	Up,yes	Up,no	cfa
PGK [36]	SAHN016_RS08640	Up,no	Down,no	Up,no	PGK, pgk
ornithine carbamoyltransferase	SAHN016_RS10235	Up,yes	Down,no	Down,no	OTC, argF, argI
	SAHN016_RS10040	Down,no	Down,no	Up,no	OTC, argF, argI
enolase	SAHN016_RS03305	Up,no	Down,yes	Up,no	ENO, eno
scpB [37]	SAHN016_RS07745	Down,yes	Up,no	Down,no	scpB
C5AP	SAHN016_RS02480	Up,yes	Up,yes	Down,no	C5AP, scpA, scpB
Surface Immunogenic Protein [38]	SAHN016_RS00320	Down,yes	Down,no	Up,no	SIG
rlmB [39]	SAHN016_RS01340	Down,yes	Down,no	Down,no	rlmB
hly [40]	SAHN016_RS06415	Down',yes	Down,yes	Down,yes	hlyIII
bca [3]	SAHN016_RS01265	Down,yes	Up,no	Up,yes	PTS-Tre-EIIB, treB
fibrinogen-binding protein [41]	SAHN016_RS05385	Down,no	Up,yes	Down,yes	fibrinogen-binding protein

protein	Accession	YM001_S	HN016_S	FC(HN016_S/YM001_S)	log2FC(HN016_S/YM001_S)	Pvalue(HN016_S/YM001_S)	FDR	significant	regulate
GapA	AKT96771.1	2.21	4.1675	1.885746606	0.91513583	0.0001167	0.00146447	yes	up
	AKT95953.1	1.7605	3.4245	1.945186027	0.959908133	0.000853579	0.002576841	yes	up
CAMP factor	AKT96240.1	0.9475	1.46	1.540897098	0.623770521	0.018211394	0.023357299	yes	up
	AKT95735.1	1.5325	2.486	1.622185971	0.697939222	0.035123988	0.040797372	yes	up
PGK	AKT96769.1	2.7845	3.97	1.425749686	0.511720715	0.000196504	0.001533689	yes	up
OCT	AKT97077.1	3.27	1.528	0.467278287	−1.097646092	0.000282293	0.001571024	yes	down
enolase	AKT95740.1	2.2745	4.445	1.954275665	0.966633984	1.40623E-05	0.000999988	yes	up
C5AP	AKT96719.1	1.565	2.5605	1.636102236	0.710262902	1.53878E-05	0.000984821	yes	up
SIP	AKT95223.1	1.1	2.391	2.173636364	1.120110606	0.000453887	0.001936584	yes	up
rlmB	AKT95394.1	1.015	1.289	1.269950739	0.344772536	0.015470719	0.020206653	yes	up
hly	AKT96417.1	1.3165	1.928	1.464489176	0.55039753	0.008084854	0.0119499	yes	up
bca	AKT95537.1	1.461	3.0085	2.059206023	1.042088179	5.43852E-05	0.001338713	yes	up
	AKT95535.1	1.4195	2.592	1.825995069	0.868682869	0.000484279	0.001986787	yes	up
	AKT96647.1	2.61	1.794	0.687356322	−0.540869917	0.117157096	0.124759637	no	down
fibrinogen-binding protein	AKT95687.1	1.266	2.716	2.145339652	1.101206075	0.000138853	0.001433325	yes	up
	AKT96271.1	1.093	1.7115	1.565873742	0.646967891	0.022918681	0.028261957	yes	up

Note: Up was upregulation; down was downregulation; yes was $p < 0.001$; NO was $p > 0.05$.

were upregulated at 40 °C versus 30 °C. Hence, overall we can conclude that the different amount of virulence factor was the reason for the different pathogenicity of the two strains.

4.2. Tilapia against GBS through the NOD pathway and pathogens escape through the toll receptor pathway

In our study, the transcription and expression of disease-resistant genes were studied through infection, which closely reflects the body's defense system. The CD59 gene was downregulated when the virulent strain was infected, but in protein was upregulation. CD59 belongs to the mucosal immune protein and plays a crucial role in the regulation of complement activation. In healthy tilapia, it is reported that CD59 transcripts in all the tissues, and there was a clear time-dependent expression pattern in the skin, brain, head kidney, thymus, and spleen. The CD59 protein may possess both binding activities to PGN and LTA and inhibiting activity of *S. agalactiae*.

In general, the transcription level of disease-resistant genes in tilapia was significantly improved with HN016 infection as compared to the YM001 infection. But changes in the protein levels were mostly insignificant. The expression of TLR1,TLR5, and TLR13 was significantly higher than that of the attenuated group. Wang [55] studied the main role of TLR1 in liposaccharide immune response in large yellow crocean and found that TLR1 is mainly distributed in spleen, head, kidney, blood, liver, heart, gill, intestine, brain, and muscle. TLR5 was an acute-phase protein with integral flagellin-recognition activity [56]. TLR13 was not present in humans, but found in mice and was antibacterial when infected GBS [57]. TLR13 exists in the teleost fish [58]and shows high homology between TLR5 and TLR13 [59]. Many unannotated transcripts and proteins were also found. These provide clues to our further study. TRL13, for example, needs further study in tilapia.

Finally, these differential proteins were found in the KEGG pathway, and the main difference was in NOD and toll-like receptor pathways. HN016 infected group, the NOD like receptor pathway were upregulated significantly, but the TLR pathway was downregulated significantly.

4.3. NOD like receptor signaling pathway was assumed in tilapia

NOD-like receptor signaling pathway is one of the most important natural immune systems. Belong to specific families of pattern recognition receptors that responsible for detecting various pathogens and generating innate immune responses [60]. The intracellular NOD-like receptor (NLR) family contains more than 20 members in mammals and plays a pivotal role in the recognition of intracellular ligands. NOD1 and NOD2, the two prototypic NLRs [61], sense the cytosolic presence of the bacterial peptidoglycan fragments that escape from the endosomal compartments, driving the activation of NF-{kappa}B and MAPK, cytokine production, and apoptosis. On the other hand, a different set of NLRs induces caspase-1 activation through the assembly of multiprotein complexes called inflammasomes. The activated of caspase-1 regulates maturation of the pro-inflammatory cytokines IL-1B, IL-18 and drives pyroptosis.

Peptidoglycan (PGN) is an important component of the cell wall of gram-positive bacteria. It is an important effector that activates NOD receptor pathway. In our study, we found that the PGN related gene transcriptome and proteome were increased in infection groups, especially the transcriptional protein products were significantly increased in HN016 VS YM001 (Refer Table 9). Nucleotide-binding oligomerization domain-containing protein 1 (NOD1,gene:CARD4) was an important receptor in cells, it was significantly higher HN016 VS

Table 8
Host anti-disease gene.

gene	Seq _ ID	CK_S_vs_HN016_S	CK_S_vs_YM001_S	YM001_S_vs_HN016_S	description
acetylneuraminate synthase	gene7243	up,yes	up,no	up,no	NANS, SAS
	gene7245	down,no	up,yes	down,no	NANS, SAS
	gene7244	down,no	down,no	nochange	NANS, SAS
	gene7238	nochange	up,yes	up,no	NANS, SAS
	gene7241	nochange	nochange	nochange	NANS, SAS
	gene7242	nochange	nochange	nochange	NANS, SAS
cyclooxygenase-2	gene24116	up,yes	up,yes	up,yes	PTGS2, COX2
IL-1 β [42]	gene19793	up,yes	up,yes	up,yes	IL-1 beta
TNF-α [43]	gene31808	up,yes	up,no	up,yes	TNFA
	gene16338	up,yes	up,no	up,yes	TNFA
	gene3395	up,yes	down,no	up,yes	–
	gene27684	up,yes	up,no	up,no	ADAM17, TACE
	gene11667	up,yes	down,no	up,no	LITAF
	gene22126	up,no	up,no	up,no	ADAM17, TACE
	gene6452	nochange	nochange	nochange	LITAF
	gene7352	nochange	nochange	nochange	LITAF
	gene3856	nochange	nochange	up,no	LITAF
CD59 [44]	gene31908	down,yes	down,no	down,yes	Chromosome 3 SCAF14978, whole genome shotgun sequence
	gene7865	down,yes	down,yes	up,yes	CD59
	gene31024	down,no	down,yes	nochange	CD59
	gene26461	nochange	nochange	nochange	CD59
	gene40557	nochange	nochange	nochange	CD59
CD2 [45]	gene9760	up,yes	up,no	up,yes	CD2
	gene4058	down,no	down,yes	up,yes	–
	gene32249	up,no	down,no	up,no	CD2
	gene32252	up,no	down,no	up,yes	CD2
	gene32259	up,no	down,yes	up,yes	CD2
CD2BP2 [46]	gene4058	down,no	down,yes	up,no	CD2BP2, PPP1R59
MCP-8 [47]	gene33327	up,yes	nochange	up,no	GZMB
	gene33314	up,no	down,no	up,no	PRSS
	gene33614	nochange	nochange	nochange	PRSS
Lymphocyte-specific protein tyrosine kinase [48]	gene28409	down,no	down,yes	up,yes	LCK
ZAP-70 [49]	gene24104	down,yes	down,yes	up,yes	ZAP70
galectin-8 [50]	gene22119	up,no	up,no	up,no	LGALS8
	gene28458	up,no	down,yes	up,yes	LGALS8
	gene22120	up,no	down,no	up,no	LGALS8
NOD1 [51]	gene32862	up,yes	up,yes	up,yes	NOD1, CARD4
	gene32975	up,yes	up,yes	up,yes	NOD1, CARD4
	gene38042	up,yes	up,yes	up,yes	NOD1, CARD4
	gene32969	up,yes	up,yes	up,yes	NOD1, CARD4
	gene32850	up,yes	up,yes	up,yes	NOD1, CARD4
	gene32979	up,yes	up,no	up,yes	NOD1, CARD4
	gene14773	down,no	down,no	up,no	NOD1, CARD4
NOD2 [51]	gene1061	up,yes	up,no	up,no	NOD2, CARD15
Toll-like receptor	gene24056	Down,no	Up,no	Down,no	TLR1-like
	gene2126	Down.no	Up,no	Down,no	TLR1-like
	gene6586	down,yes	down,yes	down,no	TLR2
	gene6589	down,yes	down,yes	up,no	TLR2
	gene6587	down,yes	down,yes	down,no	TLR2
	gene6583	down,yes	dwon,no	down,yes	TLR2
	gene36128	down,no	down,no	up,no	TLR2
	gene6607	down,yes	down,no	down,no	TLR3
	gene21793	up,yes	up,yes	up,yes	TLR5
	gene21080	up,no	up,no	up,no	TLR5
	gene23301	up,no	up,no	up,no	TLR7
	gene23302	down,no	down,yes	up,no	TLR8
	gene23303	down,no	down,no	up,no	TLR8
	gene29377	up,yes	up,no	up,yes	TLR9
	gene35766	down,no	down,no	down,no	TLR13
	gene35765	down,yes	down,yes	up,no	TLR13
	gene12374	down,no	down,no	up,no	TLR13
	gene12373	down,yes	down,yes	nochange	TLR13
	gene12372	down,no	down,no	up,no	TLR13
	gene35763	down,no	down,yes	up,no	TLR13
	gene15805	down,no	down,no	down,no	TLR13
	gene30230	down,no	down,no	up,no	TLR13
	gene30234	nochange	nochange	nochange	TLR13
	gene35762	nochange	nochange	nochange	TLR13
	gene2125	down,no	down,no	down,no	_
	gene24056	down,no	up,no	down,no	_

(*continued on next page*)

Table 8 (*continued*)

protein	Accession	HN016 VS CK	YM001 VS CK	YM001 VS HN016
SANS	XP_003449999.1	Down,yes	no change,yes	no change,yes
	XP_003449998.1	no change,no	no change,no	no change,no
	XP_019211799.1	no change,no	no change,yes	no change,yes
	XP_005455950.2	Up,yes	Up,yes	no change,no
	XP_019210948.1	Down,yes	Down,yes	no change,yes
COX2	XP_005451550.1	Down,yes	no change,yes	no change,no
IL-1 β	XP_005474584.1	no change,no	UP,yes	Down,yes
TNF-α	XP_019211750.2	no change,yes	no change,no	no change,no
	XP_003450313.1	no change,yes	no change,no	no change,yes
	XP_003458234.1	no change,no	no change,yes	no change,yes
	XP_019204243.1	no change,yes	no change,no	no change,no
	XP_003440071.1	Down,yes	no change,no	Down,yes
	XP_025753460.1	no change,no	no change,no	no change,no
CD59	NP_001298266.1	no change,yes	no change,no	no change,no
	XP_005457336.1	no change,yes	no change,yes	no change,yes
	XP_003458330.1	Up,yes	no change,no	Up,yes
CD2	XP_005469032.1	Down,no	no change,yes	no change,yes
	XP_005460718.1	Down,no	no change,no	no change,yes
CD2BP2	XP_005469032.1	Down,yes	no change,yes	no change,yes
MCP-8	XP_025759172.1	Down,yes	no change,yes	no change,yes
lck	NP_001296334.1	Down,yes	Down,yes	no change,no
ZAP-70	XP_003445176.1	Down,yes	Down,yes	no change,no
galectin-8	XP_003446682.2	no change,no	no change,no	no change,no
	XP_013123916.1	no change,yes	no change,no	no change,no
NOD1	XP_025761637.1	Down,yes	no change,no	no change,yes
TLR1	XP_013126527.2	Down,no	Down,yes	no change,no
TLR1	XP_005460413.1	no change,yes	no change,yes	Up,yes
TLR2	XP_019215654.1	Down,yes	Down,yes	no change,no
TLR3	XP_019215641.1	no change,no	Down,yes	no change,no
TLR5	XP_019201018.2	Up,yes	Up,yes	Up,yes
TLR13	XP_025766318.1	no change,no	no change,yes	Up,yes
TLR13	XP_019218842.1	Down,no	no change,no	no change,es

Note:Up was upreguoation; Down was downregulation; yes was $p < 0.001$; No was $p > 0.05$.

YM001($P < 0.05$).The same to the NOD2 (gene: CARD15).

Nicotinamide phosphoribosyltransferase (visfatin gene: NAMPT) is important part in NOD pathway in tilapia. When the host infected the *S. agalactiae*, it was up-regulated ($P < 0.01$), HN016 caused signature rise than YM001 treated($P < 0.01$). But for the protein express HN016 treated were signature ($p < 0.01$), YM001 treated fish were not change ($p > 0.05$).

Therefore, it was found from the analysis that in tilapia spleen, the cell wall peptidoglycan of *S. agalactiae* acted on NOD1 and NOD2 finally regulate the transcription and translation of the nuclei to produce effect product IL - 1β, IL - 6, IL - 8 and TNF a. Another pathway from Visfatin (nicotinamide phosphoribosyltransferase) to NLRP3 (NACHT, LRR and PYD domains - containing protein 3).In terms of protein transcriptome analysis, the latter seems to be more significant. Otherwise, the heat shock protein 90(hsp90) were also involved.The complete NLRs pathway was shown in Fig. 5. Detail transcription and protein showed in supply1.

4.4. Toll-like receptor signaling pathway was assumed in tilapia

The other innate immune responses part was toll-like receptor signaling pathway (TLRs).They were membrane-bound receptors

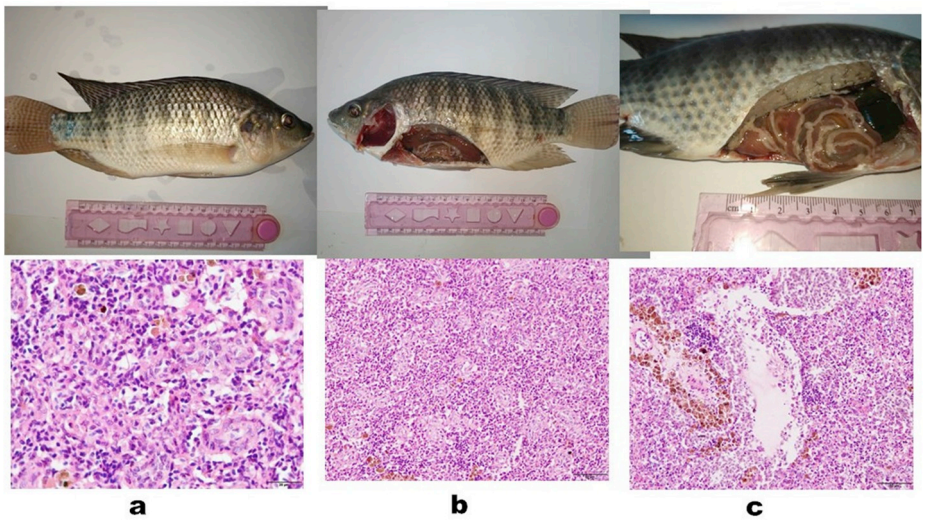

Fig. 4. Clinical manifestations and histopathologic changes of tilapia A:control group; b:attenuated(YM001) group; c:virulent(HN016) group.

Table 9
Peptidoglycan(PGN)transcriptome and proteome.

seq ID	YM001_S_count	HN016_S_count	logFC(HN016_S/YM001_S)	p-value	FDR	significant	regulate
SAHN016_RS06565	2215.5	2461.5	−2.25	2.49E-124	8.40E-123	yes	down
SAHN016_RS09720	1160	906.5	−2.76	1.28E-58	1.84E-57	yes	down
SAHN016_RS03030	285.5	2442.5	0.7	4.68E-11	1.66E-10	no	up
SAHN016_RS10150 protein	20.5	326	1.58	9.32E-09	2.90E-08	yes	up
AKT95209.1	0.964	1.783	0.887201651	0.000144581	0.001445805	yes	up
AKT95688.1	2.2965	4.239	0.884287183	0.026392078	0.03186968	yes	up

Note:Up was upregulation; Down was downregulation; yes was $p < 0.001$; no was $p > 0.05$.

identified as homologs of Toll in Drosophila. Mammalian TLRs are expressed on innate immune cells, such as macrophages and dendritic cells, and respond to the membrane components of Gram-positive or Gram-negative bacteria. Pathogen recognition by TLRs provokes rapid activation of innate immunity by inducing production of proinflammatory cytokines and upregulation of costimulatory molecules.

In terms of transcriptome, toll-like receptor pathway is up-regulated in all infected groups.Among them, the transcription of TLR1 and TLR2 receptors respond to bacterial peptidpolysaccharide (PGN) were significantly increased($P < 0.01$) [62], and the expression of TLR5 receptor was significantly increased respond to Flagellin [63]. Significantly increased TLR9 receptors respond to unmethyl CpG DNA. TR1 receptors, through PI3k-Akt signaling pathway and Myd88-dependedt pathway, eventually produce inflammatory cytokines, including TNF α, IL-1β, IL-6, IL-12. TLR2, TLR5, and TLR9 mediated produce were inflammatory cytokines, including TNF α, IL-1β, IL-6, IL-12.IL - 8 and MIP - 1 β. In addition, the high expression of TLR13 was detected in the study. It has been reported in grass carp [57], but the mechanism and product of TLR13 have not been reported in tilapia. On the contrary, in terms of protein analysis, the overall toll-like receptor pathway was decreased, in HN016 treated group fish was decline signature than the blank and YM001 treated group fish($p < 0.01$).

When the body was attacked by foreign substances, it correspondingly activated the body's resistance mechanism to resist foreign substances, so it initiates relevant immune genes for transcription. There were also modifications and splicing of mRNA between transcription and proteins. At the same time, the regulation effect of bacterial products and some virulence factors was reduced. This is the mechanism by which certain bacteria resist or evade the body's immune attack. Nod-like receptor and toll-like receptor signaling pathways(Fig. 6) are the

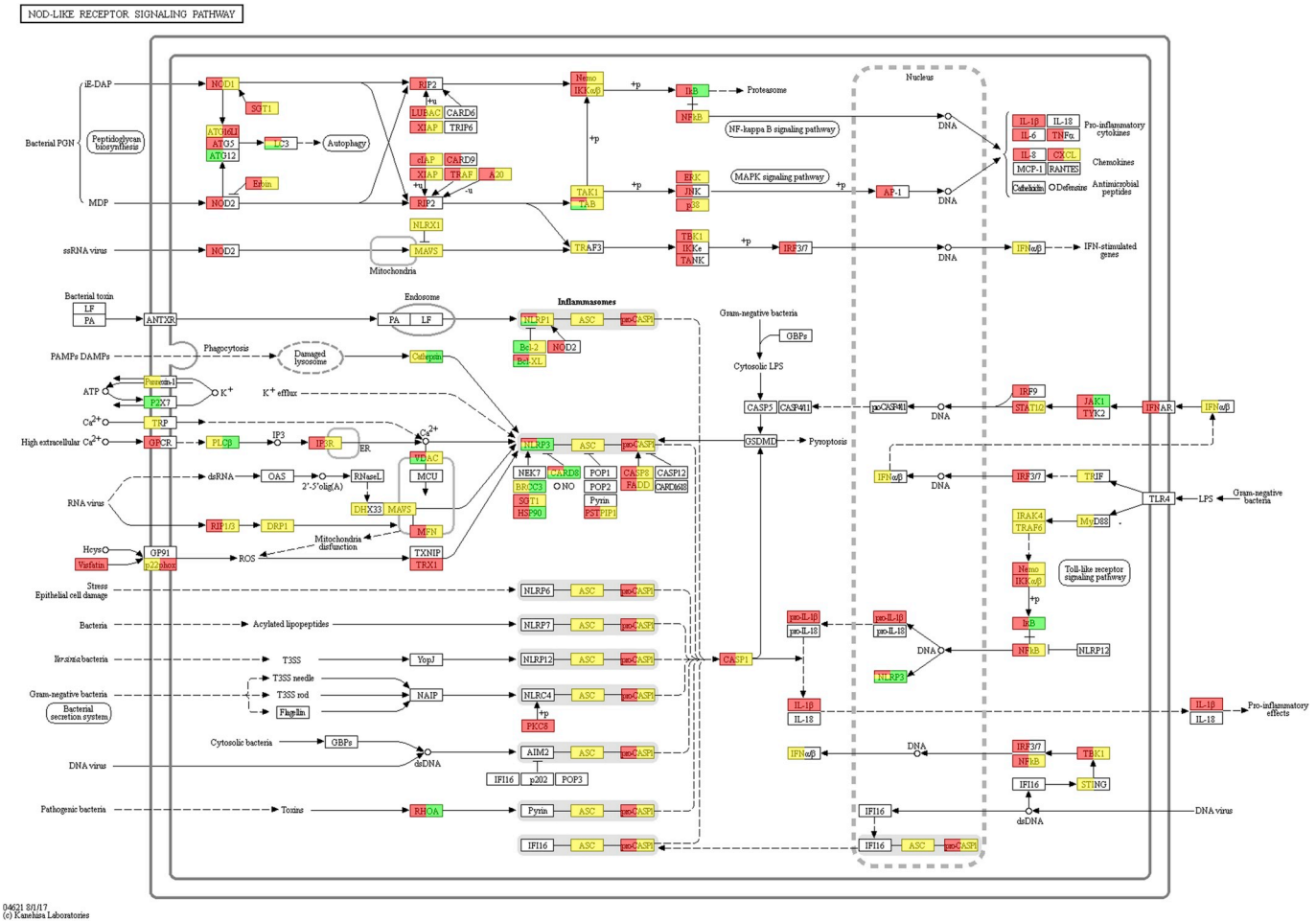

Fig. 5. NOD-like receptor pathway,(integrates transcriptome and proteome) Note:Red:RNA and protein up regulation; Yellow: RNA and protein no change; Green: RNA down, protein no change. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

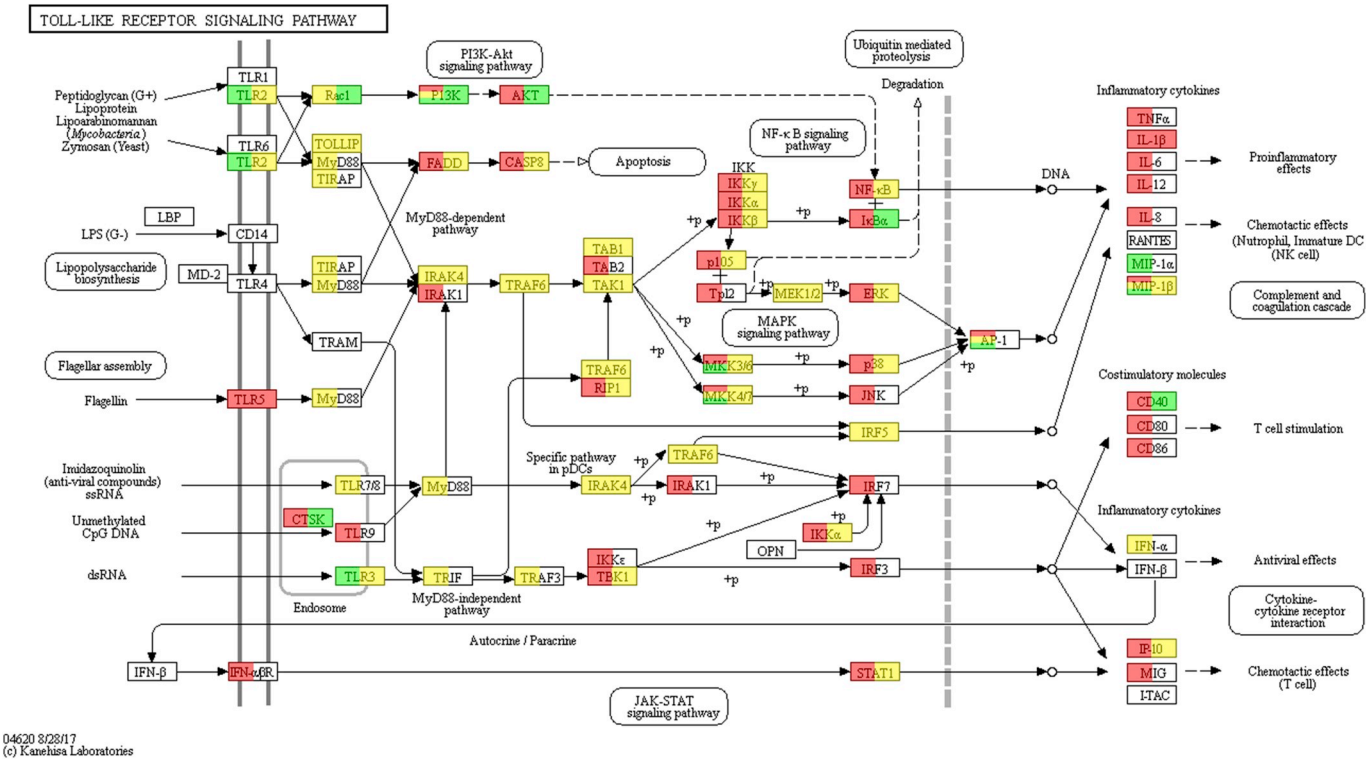

Fig. 6. Toll-like receptor pathway(integrates transcriptome and proteome) Note:Red:RNA and protein up regulation; Yellow: RNA and protein no change; Green: RNA down, protein no change. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

main signaling pathways of innate immune cells, which are the first line of defense against pathogens. Pathogens have evolved over a long period of selective evolution to develop antagonistic mechanisms against the NLR and TLR signaling pathways to facilitate their survival in the host [64]. By producing virulence factors or reducing the expression of PAMP that stimulates the activation of inflammatory corosomes, pathogens can achieve immune avoidance of NLR. Through the production of virulence factors, mitogen activated protein kinase cascade reaction is inhibited, NF-κB activation is inhibited, and TIR domain protein is produced, binding directly to TLR, thus interfering with downstream signal transduction. Ken et al. [65]. Found that toll-like receptor pathway mediated induction of NADPH oxidase complex and piscidins are important primary immune responses, but their researches mainly target transcriptome, no protein, and the biological effects of genes are ultimately reflected by proteins. The relationship between *S. agalactiae* and NLR, TRL is need studying deeply. Detail transcription and protein showed in supply2.

4.5. Relationship between bacterial virulence factors and NOD and TOLL signaling pathways

To study the relationship between bacterial virulence factors and signaling pathways, protein interactions were analyzed using the STRING database (https://string-db.org/). Through the various protein interaction, the relationship between bacterial virulence factors and the TOLL pathway was analyzed. Bacterial cell wall peptidoglycan (PGN)

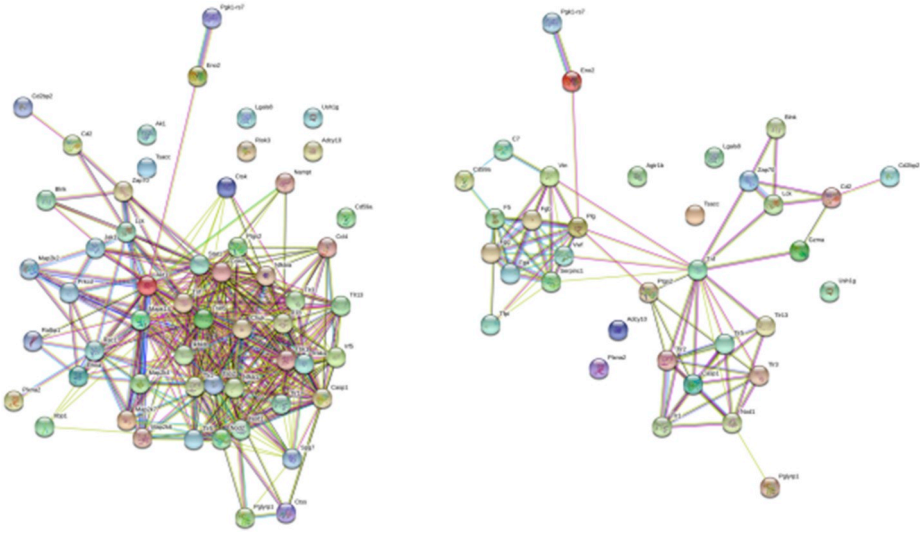

Fig. 7. NOD and TOLL pathway interaction with the GBS (Left:NLRS; Right:TLRS).

binds to the peptidoglycan receptor (Pglyrp1) on tilapia(Table 9), signals to NOD1, and interacts with TRLl1,TRL2, and TRL3 to produce tumor necrosis factor (TNF) and thus initiate toll-related reactions. On the other hand, the host's Zap70, Lck, and Gzma proteins that activate TNF, causes the TRL response. Thus, TNF plays an important role here.

PGN, Pglyrp1 and cathepsin (Ctss) interact, Phosphoglycerate protease (Pgk1) and Enolase (Eno2), CD2 antigen cytoplasmic tail-binding protein 2(Cd2bp2) and T-cell surface antigen CD2 (CD2), Tyrosine-protein kinase, Zap-70 (ZAP70), proto-oncogene tyrosine-protein kinase LCK (LCK) interacted with NOD. Hence, TRL5 and TRL13 are important for the reaction. As shown in Fig. 7.

In summary, with Dual RNA-seq and Protomics, we have gained a preliminary understanding of the interactions between the virulent and attenuated strains of *S. agalactiae* in the spleen of tilapia. TRL and NRL pathways are the main signaling pathways employed by bacteria to evade the primary immune response in the host. At the same time, we also see deficiencies about the mechanism of the deep. It is a dynamic process between pathogen and host, so it is possible to determine the expression of transcriptome and proteome landscape at different time points, so as to analyze the dynamic changes more accurately. The expression of transcriptome and proteome is inconsistent [66], and the mechanism is worth studying. For example, the regulatory mechanism of involvement of non-coding RNA in this context could be understood through the analysis of non-coding RNA [67].

5. Conclusion

Dual RNA-seq and Proteomics were used to study the interaction of tilapia infected with virulent and attenuated strain of *S. agalactiae* in the spleen of the host. On one hand, the differences in gene and protein expression of GBS in the body were suggested, laying a foundation for the search for novel gene targets and vaccines against *S. agalactiae*. On the other hand, it was revealed that in response to the invasion of *S. agalactiae*, the spleen used NOD signaling pathway to defend against pathogens, and the bacteria reduced the toll-receptor pathway in the host's defense strategy through certain factors, thus, evading the host's defense mechanism.

Declaration of competing interest

All authors are informed and have no conflict of interest.

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Appendix A. Supplementary data

ALL the data submitted to the **NCBI**(https://www.ncbi.nlm.nih.gov/), Tilipia transcriptome:SRR9672750(YM001 infected), SRR9672861(HN016 infected), SRR9673772(Control); GBS transcriptome:SRR9673797(HN016), SRR9673798(ym001); **Protein submitted to the ProteinXchagne(**http://proteomecentral.proteomexchange.org/cgi/GetDataset**) Project accession: PXD014616.**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.10.007.

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