Fish and Shellfish Immunology Reports 5 (2023) 100122

Contents lists available at ScienceDirect

Fish and Shellfish Immunology Reports

journal homepage: www.sciencedirect.com/journal/fish-and-shellfish-immunology-reports

Proteomic map of the differentially expressed proteins in the skin of *Ctenopharyngodon idella* against *Aeromonas hydrophila* infection

Shandana Ali [a,1], Farman Ullah Dawar [a,b,1,**], Waheed Ullah [c], Maizom Hassan [d], Kalim Ullah [a], Zhe Zhao [b,*]

[a] Laboratory of Fisheries and Aquaculture, Department of Zoology, Kohat University of Science and Technology Kohat, 26000, Khyber Pakhtunkhwa, Pakistan
[b] Jiangsu Province Engineering Research Center for Marine Bio-resources Sustainable Utilization, College of Oceanography, Hohai University, 1 Xikang Road, Nanjing, Jiangsu,210098, China
[c] Department of Microbiology, Kohat University of Science and Technology Kohat, 26000, Khyber Pakhtunkhwa, Pakistan
[d] Institute of System Biology (INBIOSIS), Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor, Malaysia

ARTICLE INFO

Keywords:
A. hydrophila
C. idella
DEPs
Immune response
Proteomic profile
Skin mucus

ABSTRACT

The skin mucus of fish is an important part of the innate immune system, which is poorly understood at the proteomic level. The study established a complete map of the proteins in the skin mucus of *Ctenopharangdon idella* (*C. idella*) and discussed the Differentially Expressed Proteins (DEPs) after *Aeromonas hydrophila* (*A. hydrophila*) infection. Using Label Free Liquid Chromatography-Mass Spectrometry (LC–MS/MS) analysis, a total of 126 proteins were identified as differentially expressed, 89 proteins of which were upregulated, and 37 proteins were downregulated. Functional annotations of DEPs showed that the upregulated proteins in the skin mucus of the treated group were mostly associated with complement system and cytoskeleton proteins, whereas downregulated proteins were associated with metabolism. The key upregulated immune proteins were transferrin variant C, lysozyme g, annexin A11, 26S proteasome non-ATPase regulatory subunit 8, hypothetical protein ROHU_000884, 60S ribosomal L7a, calpain-2 catalytic subunit-like protein, calpain-9-like protein, complement component C9, complement C3, cathepsin S, cathepsin Z, 14 kDa apolipo, heat shock protein and intelectin, whereas, leukocyte elastase inhibitor, annexin A11, C-factor-like protein, biotinidase isoform X1 and epidermal growth factor receptor substrate 15-like were the downregulated proteins. Moreover, we for the first-time report proteins such as coactosin, lamin-B2 and kelch 12, which were never reported in fish. Our study directly pointing out the possible immunological biomarkers in the skin mucus of *C. idella* after *A. hydrophila* treatment. Each of the protein we report in this study could be used as base to establish their mechanism of action during bacterial infection that may contribute to the strategies against bacterial prevention and control in fishes.

Introduction

Bacterial diseases have limited the development and production of the global fish industry. It causes mortality particularly in farmed fish with consequent economic losses [1]. *Aeromonas hydrophila* is a common and major bacterial pathogen in many fish species of the world [2]. *Aeromonas hydrophila* causes abdominal dropsy, hemorrhagic septicemia, and skin lesions in both freshwater and marine fish, and can specifically infect members of the family Cyprinidae [3]. The exact treatments and control of bacterial infection in fish have not yet been discovered [4]. However, different immune components have been categorized with possible roles against several bacteria [5,6]. Particularly, the roles of fish skin mucus against bacterial infections have been focused and signified [7–9].

Fish skin mucus is composed of lipids, glycoproteins, DNA, RNA and secondary metabolites [10]. It is the first biochemical barrier between the fish and its surrounding that works through skin surface against pathogenic bacteria [11]. The mucus of fish contains a wide range of antimicrobial peptides, proteins, histones, ribosomal proteins, lysozyme, peptidases, actin, complement factors, hemoglobin, lectin,

* Corresponding author.
** Co-corresponding author.
E-mail addresses: farmandawar@kust.edu.pk (F.U. Dawar), zhezhao@hhu.edu.cn (Z. Zhao).
[1] These authors contributed equally to this work

https://doi.org/10.1016/j.fsirep.2023.100122
Received 14 September 2023; Received in revised form 25 October 2023; Accepted 31 October 2023
Available online 1 November 2023

immunoglobulins, which contribute to the fish innate immunity [12]. In the skin mucus of *Danio rerio* (*D. rerio*) [13] and *Epinephelus coioides* (*E. coioides*) [14], immunological proteins such as complements, chemokines, and antigen processing proteins were found against bacterial infection. Recently we have shown several immunological proteins including Vitellogenin, Toll-like receptors (TLRs13), Alpha-2 Macroglobulin-like protein, Keratin-like proteins, Calpains, and heat shock proteins in the skin mucus of *Labeo rohita* against *A. hydrophila* infection [15]. However, less information is available about the molecular response of skin mucus of fish against bacterial infections and thus it needs further studies.

Ctenopharyngodon idella (*C. idella*) is an economically important and highly produced farmed freshwater species that is widely distributed in various regions of Asia [2]. Bacterial infections in *C. idella* are evidenced in times and is a threat to their population [16,17]. To understand bacterial infections, host fish mucosal immunity has been focused and explored [11,18–20], for fish mucus is rich in immunological and antibacterial proteins [21]. Therefore, this study analyzed the proteomic profile of the skin mucus of *C. idella* after infection with *A. hydrophila*. This comprehensive report will be used as a base for further functional studies of each newly identified protein in the skin mucus of fish. Overall, the study is an effort to understanding of mucosal immune response of fish against bacterial infection that may contribute to the strategies of prevention and control of bacterial disease in fish.

Materials and methods

Collection and maintenance of fish

Initially, all procedures used in this research were approved from the Ethical and Research Committee of Kohat University of Science and Technology (KUST) Kohat, under letter number, KUST/Ethical Committee/1447). Fish with an average weight of 949±10 g were purchased from a fish farm in district Kohat and were acclimatized for two weeks in the Laboratory of Fisheries and Aquaculture, Department of Zoology, KUST. The fish were fed with commercial pellet diet for grass carp and the standard physiochemical parameter of water were maintained. For experimentation, 36 fish were distributed in six rectangular glass aquaria (each with 200 liters + 6 fish) where 3 aquaria (with 18 fish) were considered as a control and three aquaria (with 18 fish) were considered as a treated group. The feces from each aquarium were regularly removed daily and fish were examined for any clinical symptoms.

Bacterial treatment and mucus collection

Previously stored *A. hydrophila* (ON920871) [7] were cultured in Luria Bertani (LB) media overnight at 37 °C, and Lethal Dose (LD_{50}) of *A. hydrophila* was determined as per our pervious method [15]. Around 1.5×10^9 Colony Forming Unit (CFU) was injected intraperitonially to each fish in treated group and an equal volume of Phosphate Buffer Saline (PBS) was injected to each fish in the control group. Skin and body lesions and hemorrhages were observed in the bacterial treated fish whereas no such symptoms of infection were observed in the fish of control group.

After 48 h, fish in each group were anesthetized with 200 mg/L tricaine methane sulfonate (MS-222) (Sigma-Aldrich; purchased from Afnan Traders, Rawalpindi, Pakistan) washed twice with dd water, and were put in a sterile tray. The lateral sides of fish were gently scraped with a sterile slide with no skin dent or debris. Skin mucus from 18 fish (6 fish per aquarium) treated with *A. hydrophila* was the treated sample and 18 fish (6 fish per aquarium) treated with PBS was the nontreated sample. Mucus samples were collected in replicates (3 samples of each treated and control group) and labeled as T-CI 1-3, and NT-CI 1-3 respectively. The sample was flash frozen in liquid nitrogen and sent to Malaysia Genome and Vaccine Institute (MGVI) Jalan Bangi, 43,000 Kajang, Selangor, Malaysia for lyophilization.

Protein extraction

For protein extraction, the preserved mucus was diluted with 1 mL of PBS and were sonicated (2 × 5 s) (SONICS Vibracell VCX750, USA). Then the sample was centrifuged for 30 min at 15,000 X g and the supernatant was collected. A mixture of 10% w/v trichloroacetic acid (TCA) and 0.1 % DL-Dithiothreitol (DTT, Sigma, USA) was added into the sample and incubated for 2 h at −20 °C. The sample was again centrifuged at 10,000 X *g* for 30 min and cold acetone containing 0.1 % DTT was added and incubated for 1 hour at −20 °C. The sample was centrifuged (4 °C) at 10,000 X *g* for 30 min, and the pellets obtained were dried for 2–3 min and suspended in rehydration buffer (9.8 M urea, 20 mM DTT).

Protein quantifications and In gel digestion

Protein concentration was determined through Bradford method [22] where the sample was initially run through SDS-PAGE (Fig.-1). For In gel digestion, 50 μg / hole protein sample was loaded to 15 % polyacrylamide gel and the aggregated protein bands were separated from the stacking gel after 15 min. The bands were further sliced into 1 mm × 1 mm size and were placed in a clean microcentrifuge tube. To wash off the Coomassie staining, 100 μL (50 % acetonitrile (ACN)) in 50 mM ammonium bicarbonate (ABC) were added into the tube with excised gel, vortex and shake for 15 min. Further, 300 μL of 10 mM Dithiothreitol (DTT) in 100 mM ABC was added into the sample and incubated for 30 min at 60 °C. After this reduction process, 300 μL of 55 mM indole acetic acid (IAA) in 100 mM ABC was added for proteins alkylation and the sample was incubated for 20 min at room temperature in the dark. The reduced and alkylated gel was washed with 1000 μL of 50 % ACN in 100 mM ABC for 20 min and vortex for 15 min and again 100 μL of 100 % CAN was added.

The overnight incubated gel was rehydrated at 37 °C using 50 μL of 6 ng/μL trypsin in 50 mM ABC along with 50 μL of 50 mM ABC. After incubation, the trypsin-digested gel was vortexed and 100 μL of 100 % ACN was added and shaken for 15 min. After adding 100 μL of 50 % ACN and shaking the sample for 15 min, the liquid was collected in the same tube. The digested samples were dried completely under speedvac and then stored at −20 °C for further analysis.

Liquid chromatography mass spectrometry (LC–MS/MS)

Initially, samples were quantified by Scopes protocol [23]. The LC–MS/MS was performed by loading 1 μg of the peptide sample into the column and all samples (biological replicates) were run with a LC gradient of 120 min. Orbitrap Fusion Tribrid mass spectrometer was used for data acquisition connected to nano liquid chromatography system (Easy-nLC) 1200. Peptide samples were loaded onto EASY-spray column Acclaim pep map TM C18 100 A^0, 50 μm id x 15 cm, 2 μm particle size at a flow rate of 5 μL/minute. In Acclaim spray column, peptides were converted into liquid for 2 h in a gradient with a flow rate of 250 nL/minute in solvent B (80 % ACN containing 0.1 % formic acid). The Orbitrap mass analyzer was exploited for MS acquisition in Data-Dependent Acquisition (DDA) mode with 60,000 mass resolution and scanning range was 310–1800 *m/z*. The mass window was set at 10 ppm with a 20-second dynamic exclusion period. Each MS spectra were attained using Collision-Induced Dissociation (CID) and High-Energy Collision Dissociation (HCD) methods for shattering at the MS1–2 levels, with AGC targets set at 10,000 and 400,000, respectively. For positive internal calibration, a lock mass of 445.12003 *m/z* was utilized.

Label-free quantification and identification

Thermo Scientific TM Proteome Discoverer Version 2.1 was used for

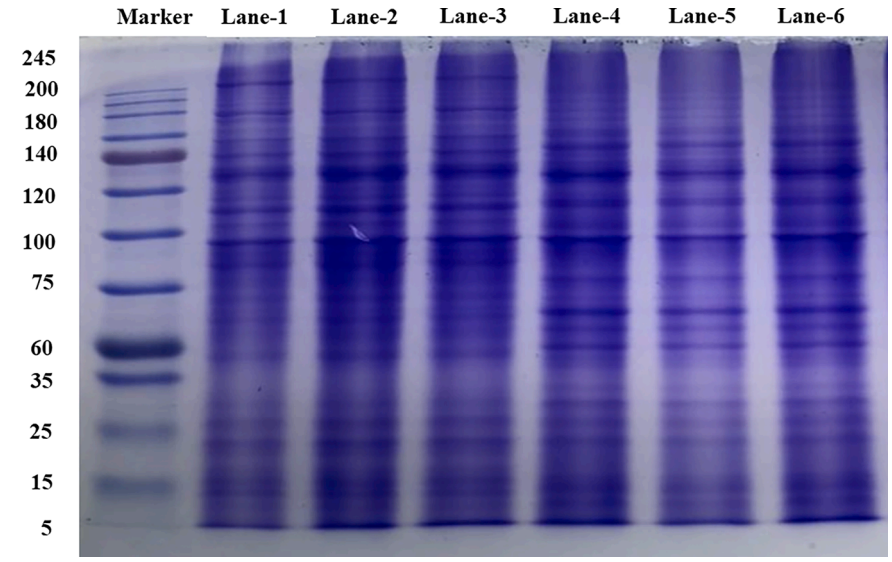

Fig. 1. SDS-PAGE showed the separation of proteins extracted from *C. idella* skin mucus with 3-biological replicates of nontreated (NT-CI 1–3) and treated (T-CI 1–3) with *A. hydrophila*: Lane 1 (NT-CI-1), Lane 2 (NT-CI-2), Lane 3 (NT-CI-3), Lane 4 (T-CI-1), Lane-5 (T-CI-2), Lane-6 (T-CI-6). Smobio Enhanced 3-color High Range Protein Marker (5–245 kDa) was used as a standard. Approximately 10 μg protein was loaded per lane.

analysis of raw mass spectrometry data against the NCBI protein database for *C. idella* (taxonomy ID - 7959, retrieved 10/20/2022) using the National Center for Biotechnology Information (NCBI) integrated search engine. Orbitrap fusion mode was chosen as the device by using LFQ. Enzyme trypsin was selected as the protease and for whole analysis, 2-missed cleavages were permitted. Oxidation on methionine was selected as variable while carbamidomethylation on cysteine was chosen as the fixed modification. The reverse mode was selected as the decoy mode, and proteins were detected based merely on their unique peptide.

Statistical analysis

Perseus software was used for statistical and bioinformatics analysis, with an output file from Proteome discoverer. To identify DEPs, R programming language was executed for statistical assessment. The label-free quantification (LFQ) concentrations were then converted to logarithmic values and the data missing were replaced according to a standard distribution. The rows were the filter with valid values (with at least three values in one group). After that, all the missing values were overwritten using the imputation function to make normal distribution. To obtain variabilities estimation between the biological replicates of both groups, DE analysis was used by applying RStudio. For validation of peptides, the percolator® algorithm based on q-value (smaller than one percent) False Discovery Rate (FDR) was used.

Functional annotations

The DEPs selected from the data after Perseus were fictionally annotated. Initially, the DEPs were integrated into Ubuntu and the sequence for each accession number was obtained, then were submitted to Euro-galaxy and the sequence was blast. The blastp.xml files were subjected to BLAST2GO for Gene Ontology (GO). For the comparison of Gene ontology between the DEPs, Fisher's exact test and KEGG analysis were performed (P-value ≤ 0.05).

Results

Initial identification of DEPs

Using Discovery Proteome software, proteins with expression difference (P-value ≤ 0.05) were considered significant proteins to distinguish between the nontreated and treated groups. Firstly, 1,466,484 raw spectra were identified, including 75,100 clean spectra and 7979 peptides. From 7979 peptides, 1910 proteins were identified in nontreated and 1452 proteins in treated groups.

Further, 126 proteins were identified as DEPs through Perseus analysis, of which 89 proteins were upregulated and 37 proteins were downregulated, where the downregulated proteins were found mostly in nontreated group (Table 1). Hierarchical cluster analysis (Heat map) of top 27 proteins was made for the differentiation between the DEPs of treated and nontreated groups (Fig.-2) and found significant data points with permutation-based FDR calculations. Furthermore, RStudio (DE analysis) showed the fold change between the treated and nontreated group with ≥ 1 and ≤ -1 (Table-1). Some of the upregulated proteins were overexpressed based on fold change (FC) as transferrin variant C (23-FC), calpain-2 catalytic subunit-like protein (22-FC), calpain-9-like protein (20-FC), Lysozyme g (20-FC), Annexin A11 (19-FC), 26S proteasome non-ATPase regulatory subunit 8 (18-FC), hypothetical protein ROHU_000884 (18-FC), natural killer cell enhancing factor (16-FC), type I cytoskeletal 19-like protein (17-FC) and complement component C9 (15-FC) (Table 1).

Functional annotations of DEPs

The DEPs identified from both groups were enriched with specific functions (BLAS2GO and OMICSBOX). The top GO term were cellular process, metabolic process, and response to stimulus (Fig.-3). Proteins associated with cellular process includes calcium calmodulin-dependent kinase type II subunit beta isoform X18- X19, type I cytoskeletal 19-like protein, tubulin beta-4B chain, Keratin, Myosin-9, type II cytoskeletal 8, and tropomyosin alpha-3 chain isoform X6. Some immune related and antibacterial proteins were upregulated including transferrin variant C, Lysozyme g, Annexin A11, 26S proteasome non-ATPase regulatory subunit 8, hypothetical protein ROHU_000884, 60S ribosomal L7a, calpain-2 catalytic subunit-like protein, calpain-9-like protein, complement component C9, complement C3, Cathepsin S, Cathepsin Z, 14 kDa apolipo, heat shock protein and Intelectin (Table 1), whereas, Leukocyte elastase inhibitor, Annexin A11, C-factor-like protein, biotinidase isoform X1, epidermal growth factor receptor substrate 15-like were downregulated.

Table 1
Representative differentially expressed proteins (DEPs) in the skin mucus of *C. idella* infected with *A. hydrophila*.

Upregulated proteins Accession	Protein name	Coverage[a]	Sequence score[b]	Molecular weight[c]	*P*-value[d]	Fold change logFC[e]	Peptides[f]
RXN27874.1	transferrin variant C	36.84210526	1121.827203	60.848	1.84E−19	23.34554082	17
KAI2644335.1	Intelectin	14.28571429	139.2787138	34.597	0.018689628	22.2942135	4
RXN22517.1	calpain-2 catalytic subunit-like protein	9.137931034	113.7131982	64.087	0.027501319	22.05692515	4
RXN09117.1	malate cytoplasmic-like protein	24.62462462	545.2215532	36.315	0.013964075	21.74423925	6
KAI2645617.1	Histone H2B	21.88841202	747.2304788	51.789	0.013964075	20.87551116	13
KAI2646108.1	Lysozyme g	7.341269841	31.49613488	56.016	0.010679809	20.51383171	2
RXN09056.1	calpain-9-like protein	0.951248514	16.03316355	95.464	0.018689628	20.39816712	2
KAI2657647.1	Annexin A11	4.562043796	21.25099933	57.791	0.010679809	19.49724487	2
RXN12794.1	heat shock 70	17.88491446	423.3498425	70.451	0.010679809	18.89850887	8
RXN33734.1	E3 ubiquitin- ligase TRIM32	5.461767627	154.4876567	111.264	4.60E−25	18.89186664	4
RXN27399.1	F-actin-capping subunit alpha-1	23.42657343	227.4963439	32.852	1.87E−25	18.84535709	4
RXN31849.1	26S proteasome non-ATPase regulatory subunit 7	7.317073171	11.13398886	37.211	3.63E−26	18.76585733	2
RXN30003.1	calcium calmodulin-dependent kinase type II subunit beta isoform X19	7.743362832	91.1536901	51.198	4.19E−27	18.5733765	3
RXN38693.1	hypothetical protein ROHU_000884	0.903225806	182.3876158	177.287	3.01E−27	18.54267904	2
AHL20261.1	complement C3	6.930091185	327.3678728	184.09	0.013964075	18.38660061	9
KAI2648095.1	Glucose-6-phosphate isomerase	15.92128801	315.4677697	62.652	1.86E−28	18.30802968	6
KAI2653904.1	26S proteasome non-ATPase regulatory subunit 8	5.617977528	42.39479637	30.968	0.018689628	18.06628227	2
APB93352.1	vitellogenin 1	9.179104478	257.1228055	145.568	1.23E−30	17.91380916	10
RXN31235.1	calcium calmodulin-dependent kinase type II subunit beta isoform X18	4.323308271	55.68946135	59.523	6.82E−31	17.82582837	2
RXN27357.1	proteasome subunit alpha type-3	12.62135922	79.17107105	22.978	0.010679809	17.50323152	2
RXN35533.1	UDP-N-acetylhexosamine pyrophosphorylase isoform X2	1.904761905	3.876709104	58.854	5.05E−33	17.23466051	2
AAY43356.2	intelectin	16.03773585	196.2851093	35.241	0.013964075	17.22784023	5
RXN22518.1	calpain-2 catalytic subunit-like protein	22.97297297	160.9368401	8.864	0.027501319	17.19467729	2
AGS17147.1	natural killer cell enhancing factor	10.65989848	46.66932809	21.827	8.57E−35	16.2595628	2
ABD76396.1	immunoglobulin mu heavy chain	10.59027778	117.7610292	64.175	0.010679809	16.15067568	4
KAI2667762.1	Proteasome subunit beta type-5	2.103559871	9.538149357	69.286	1.02E−34	16.11893219	2
RXN24566.1	type I cytoskeletal 19-like protein	4.671968191	327.4075974	110.849	1.07E−34	16.07411186	3
RXN38413.1	TRPM8 channel-associated factor-like protein	2.407002188	49.00683606	102.021	1.08E−34	16.06996735	2
RXN17233.1	apoptosis inhibitor 5	1.754385965	31.12760317	95.474	1.36E−34	15.88076932	2
AII80321.1	complement component C9	3.153153153	3.766227841	71.892	1.51E−34	15.79458961	2
KAI2646821.1	Histone H4	25.29832936	868.5344418	46.687	0.005148023	4.441784363	13
RXN28542.1	tropomyosin alpha-3 chain isoform X6	4.838709677	6.11504674	28.827	0.005074414	3.8249998	2
KAI2654466.1	14-3-3 protein zeta/delta	32.24489796	356.0382429	27.83	0.005197883	3.790064637	7
RXN10328.1	60S ribosomal L7a	4.887218045	56.50464058	29.997	0.003112154	3.768403428	2
KAI2655256.1	14-3-3 protein beta/alpha-A	6.811145511	109.3536015	36.085	0.004463738	3.701594632	2
RXN03646.1	type I cytoskeletal 19-like protein	7.136715391	147.1744276	127.673	0.004675629	3.599864151	5
RXN24735.1	coactosin	31.69014085	85.82882822	16.084	0.005272049	3.437865177	3
RXN20813.1	lamin-B2	4.266211604	2.483951092	66.375	0.005793344	3.416595556	2
KAI2663271.1	Myosin-9	3.790238837	5.449974537	136.697	0.006175834	3.341401954	4
KAI2652920.1	Proteasome subunit beta type-2	4.435483871	10.68415427	27.861	0.006462422	3.304180891	2
RXN21681.1	urokinase plasminogen activator surface receptor-like protein	1.171605789	53.45086622	151.501	0.006552442	3.297719454	2
AVP32216.1	14-3-3 protein beta/alpha-A	42.21311475	420.4139014	27.616	0.021362488	3.171336113	9
RXN24768.1	epithelial cell adhesion molecule-like protein	5.096660808	82.86241126	64.769	0.01264059	3.164808064	2
KAI2649847.1	DNA primase small subunit	0.962861073	45.73483157	155.118	0.008564798	3.126406961	3
RXN20828.1	peptidyl-prolyl cis-trans isomerase-like protein	7.317073171	47.23935413	17.529	0.010686717	3.11309351	2
NP_001002468.1	peroxiredoxin-2	21.82741117	112.3246565	21.837	0.01057436	3.105368464	3
RXN27618.1	calcium uniporter mitochondrial	4.391891892	19.66965675	34.522	0.011188769	3.091415046	2
KAI2657905.1	Phosphoglycerate mutase 1	18.11023622	60.80386984	28.908	0.008327485	3.086279865	4
KAI2651808.1	Proteasome subunit alpha type-6	14.53900709	88.50388002	49.686	0.012234342	3.068895872	3
RXN16746.1	14-3-3 zeta delta-like protein	17.95918367	21.57276702	53.507	0.011083274	3.055170103	2
KAI2652551.1	SH3 domain-binding glutamic acid-rich-like protein 3	6.289308176	38.87750423	17.415	0.011468232	3.054822199	4
RXN26640.1	rho GDP-dissociation inhibitor 2-like isoform X2	5.069124424	49.15136027	27.587	0.009588524	3.032891426	2
RXN32036.1	annexin A1-like protein	10.52631579	141.2285335	37.693	0.013964075	2.99735262	3
KAI2650163.1	Profilin-2	7.262569832	8.151986122	19.455	0.012741644	2.992860709	2
RXN34607.1	type I cytoskeletal 13-like protein	14.25178147	143.731734	46.866	0.014028199	2.992486833	6
KAI2649895.1	Keratin, type II cytoskeletal 8	12.09540034	45.73483157	155.118	0.013084122	2.959071593	2
KAI2655169.1	Cathepsin S	0.974858902	13.30543518	215.547	0.009804199	2.957684482	2
KAI2668529.1	Acidic leucine-rich nuclear phosphoprotein 32 family member B	4.135338346	12.8926847	30.12	0.012841944	2.955201812	2
KAI2653775.1	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	5.917159763	13.66288483	19.557	0.015132921	2.919626117	5
RXN02410.1	antigen peptide transporter 2-like protein	1.103752759	57.04752529	100.202	0.01017069	2.905063399	2
RXN29259.1	tubulin beta-4B chain	21.7847769	9.976090908	24.512	0.014558426	2.903531071	2
KAI2657300.1	BTB/POZ domain-containing protein KCTD12	3.333333333	103.6426791	35.183	0.011758111	2.900546899	2
RXN22594.1	AP-2 complex subunit beta isoform X2	4.124860647	43.48040962	99.817	0.015070306	2.878703635	3
RXN32412.1	vacuolar sorting-associated 29 isoform X2	1.828410689	15.39575028	73.009	0.010679809	2.876414138	2
RXN10255.1	14 kDa apolipo	16.25441696	133.5789763	31.576	0.015009772	2.840432432	3

(*continued on next page*)

Table 1 (*continued*)

Upregulated proteins Accession	Protein name	Coverage[a]	Sequence score[b]	Molecular weight[c]	*P*-value[d]	Fold change logFC[e]	Peptides[f]
KAI2662814.1	RNA-binding protein 4.1	1.292407108	5.449974537	136.697	0.014861859	2.822794885	2
KAI2656133.1	Heat shock cognate 71 kDa protein	36.46258503	12.14869046	101.005	0.015976667	2.820827254	4
KAI2659941.1	14-3-3 protein gamma	21.95121951	172.9431078	27.915	0.017182286	2.810868326	5
AGM48543.1	mitochondrial voltage-dependent anino channel protein 1	25.795053	107.9554198	30.52	0.01689639	2.710896411	5
RXN31871.1	ELAV 1 isoform X1	8.027522936	57.15586817	48.04	0.018689628	2.69152109	3
RXN29152.1	guanine nucleotide-binding subunit beta-2-like 1	6.52360515	120.3453851	131.059	0.025144586	2.687335399	6
RXN23792.1	myosin regulatory light chain smooth muscle minor isoform-like protein	18.49710983	117.6459525	20.014	0.0173516	2.678876441	3
RXN35236.1	neuroblast differentiation-associated AHNAK isoform X1	0.632244468	17.93797851	404.439	0.018963142	2.675294023	2
ADA70351.1	heat shock protein	8.521303258	198.5128449	91.934	0.01937523	2.650702614	6
RXN17797.1	ras-related Rap-1b	8.387096774	18.92296886	17.563	0.016868929	2.646499719	2
RXN06676.1	rho GDP-dissociation inhibitor 1	10.31746032	40.52919555	48.172	0.018478706	2.639703221	2
KAI2651667.1	Thioredoxin-like protein 1	12.96296296	29.35901809	30.531	0.019508352	2.639375421	2
KAI2663847.1	Cathepsin Z	3.986710963	15.21337497	33.543	0.015880309	2.632568964	2
RXN20411.1	ras-related Rab-11B	15.59633028	33.24337554	24.524	0.01226128	2.609160807	3
KAI2657651.1	Catechol O-methyltransferase domain-containing protein 1	6.324110672	28.23900032	27.935	0.020355894	2.59671257	3
KAI2647796.1	Epidermal differentiation-specific protein	14.53488372	33.08467174	25.306	0.020718603	2.583746712	2
RXN24522.1	proliferating cell nuclear antigen	12.30769231	6.560997844	28.622	0.019316884	2.578430991	2
RXN12800.1	major vault	17.88491446	423.3498425	70.451	0.027501319	2.557959773	8
RXN17024.1	chloride intracellular channel 1-like protein	26.53061224	28.20981455	31.043	0.022050859	2.420972495	2
KAI2654949.1	Uroplakin-1a	6.374501992	38.26232696	27.83	0.01900567	2.320774157	6
KAI2663888.1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	25.05747126	482.2050284	48.115	0.026409645	2.308797767	9
RXN27466.1	leukotriene A-4 hydrolase	2.454991817	23.79613292	75.739	0.02098997	2.266814466	2
RXN05188.1	polyadenylate-binding 1	18.08118081	15.26016688	70.527	0.023129409	2.227904939	2
RXN31035.1	histone H2A-like protein	5.92654424	45.86285114	136.105	0.022655796	2.075955768	6
KAI2668313.1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial	7.374631268	11.1543324	23.722	0.026385831	2.062553503	2
Downregulated Proteins							
RXN16149.1	fructose-bisphosphate aldolase A	6.313645621	84.84654331	54.516	0.009026327	−3.237366215	3
KAI2655282.1	Ictacalcin	6.132075472	104.4842637	23.298	0.002417316	−3.782743613	3
RXN03935.1	C-factor-like protein	2.196193265	25.28502226	146.473	1.32E−34	−15.90294274	4
RXN07400.1	septin-6 isoform X3	5.25	56.02560985	46.262	1.22E−34	−15.97120162	2
RXN18384.1	succinyl-:3-ketoacid coenzyme A transferase mitochondrial	9.9609375	24.52016652	55.706	1.20E−34	−15.9800634	3
RXN28428.1	desmoplakin-like protein	2.984201287	80.99688613	197.694	1.19E−34	−15.99325517	4
KAI2664776.1	AP-1 complex subunit beta-1	2.495378928	27.017923	119.297	1.17E−34	−16.00198291	2
KAI2667448.1	Serotransferrin-1	9.577922078	38.79055774	68.052	1.14E−34	−16.02357436	4
KAI2664481.1	Flotillin-2a	4.761904762	12.02546144	62.285	1.13E−34	−16.03212135	2
KAI2657821.1	Nucleolysin TIAR	3.359173127	12.12674344	43.086	1.10E−34	−16.05746183	4
KAI2667771.1	Coatomer subunit beta'	3.58490566	29.90615165	119.633	1.10E−34	−16.05746183	3
KAI2659039.1	Inter-alpha-trypsin inhibitor heavy chain H3	1.340250757	42.10711753	260.459	1.07E−34	−16.07411186	2
RXN27114.1	dipeptidyl peptidase 3-like isoform X1	7.80141844	257.6708645	79.057	1.07E−34	−16.07411186	4
KAI2663734.1	RuvB-like 1	12.71929825	87.04271936	50.281	1.04E−34	−16.10279435	4
KAI2655412.1	Cellular retinoic acid-binding protein 2	23.23943662	62.57048035	15.82	1.55E−34	−16.66094974	2
KAI2665568.1	DNA-(apurinic or apyrimidinic site) endonuclease	3.225806452	22.16896808	34.989	1.82E−34	−16.68442929	4
KAI2664689.1	Coatomer subunit delta	9.021113244	116.5875901	58.089	1.03E−33	−16.95246004	4
RXN06938.1	adenylosuccinate synthetase isozyme 2-like protein	1.525658807	9.400920868	80.01	1.24E−33	−16.98336643	2
RXN02577.1	coronin-1A-like protein	10.17881706	244.7156126	81.488	1.56E−32	−17.36451438	5
RXN34516.1	4-trimethylaminobutyraldehyde dehydrogenase	23.81889764	257.9869008	55.254	1.80E−32	−17.37946568	8
KAI2644069.1	60S ribosomal protein L27a	7.182320442	11.26524425	20.434	1.19E−31	−17.59296229	3
KAI2657029.1	Catenin alpha-1	3.13253012	4.771321058	46.29	1.53E−31	−17.62391554	2
RXN18284.1	carboxylesterase 5A-like protein	7.1278826	86.02395344	52.884	3.04E−31	−17.71323504	2
KAI2668100.1	Phosphoglycerate mutase 1	7.374631268	41.96276414	38.424	3.95E−31	−17.74878971	2
KAI2662067.1	Host cell factor 1	0.616016427	19.25037062	199.105	1.09E−30	−17.89464421	4
RXN06496.1	aspartate cytoplasmic	6.829268293	37.75830507	45.755	1.14E−30	−17.90227333	2
RXN28083.1	aspartate–tRNA cytoplasmic	6.060606061	28.48619044	60.112	1.66E−30	−17.96075429	2
RXN03499.1	Actin-related 2 3 complex subunit 1A	5.1558753	133.9451725	93.136	1.90E−29	−18.14386326	3
KAI2648716.1	Leukocyte elastase inhibitor	13.55498721	103.5149035	43.987	2.16E−27	−18.51217904	4
KAI2662410.1	Aspartate aminotransferase, mitochondrial	8.333333333	11.90077233	49.206	1.27E−26	−18.68167492	2
RXN18438.1	kelch 12	15	669.3012153	111.734	1.42E−26	−18.69318991	10
KAI2642456.1	Alpha-aminoadipic semialdehyde dehydrogenase	10.3861518	224.1414804	81.994	1.48E−21	−19.42344571	6
KAI2657647.1	Annexin A11	4.562043796	21.25099933	57.791	3.26E−21	−19.49724487	2
RXN26579.1	GDP-mannose 4,6 dehydratase	18.88888889	154.1936966	29.937	1.45E−20	−19.8405514	3
RXN25133.1	epidermal growth factor receptor substrate 15-like 1	5.336832896	155.8060845	127.973	1.74E−20	−19.88747944	6
KAI2654016.1	von Willebrand factor A domain-containing protein 5A	6.580829757	86.40342057	75.988	1.96E−20	−19.91827016	4
KAI2663472.1	Protein disulfide-isomerase	17.71561772	164.2729784	47.722	2.61E−20	−19.9928644	6

[a] Coverage shows the protein sequence coverage.
[b] Sequence Score shows identification score of proteins.
[c] Molecular weight indicates the size of proteins.

[d] Proteins values considered significant ($P \leq 0.05$).
[e] Fold change are measured as ≥ 1 and ≤ -1.
[f] Peptide designates number of peptide sequence matched a protein.
[g]The proteins numbers are calculated: 37 (Downregulated); 89 (Upregulated) proteins.

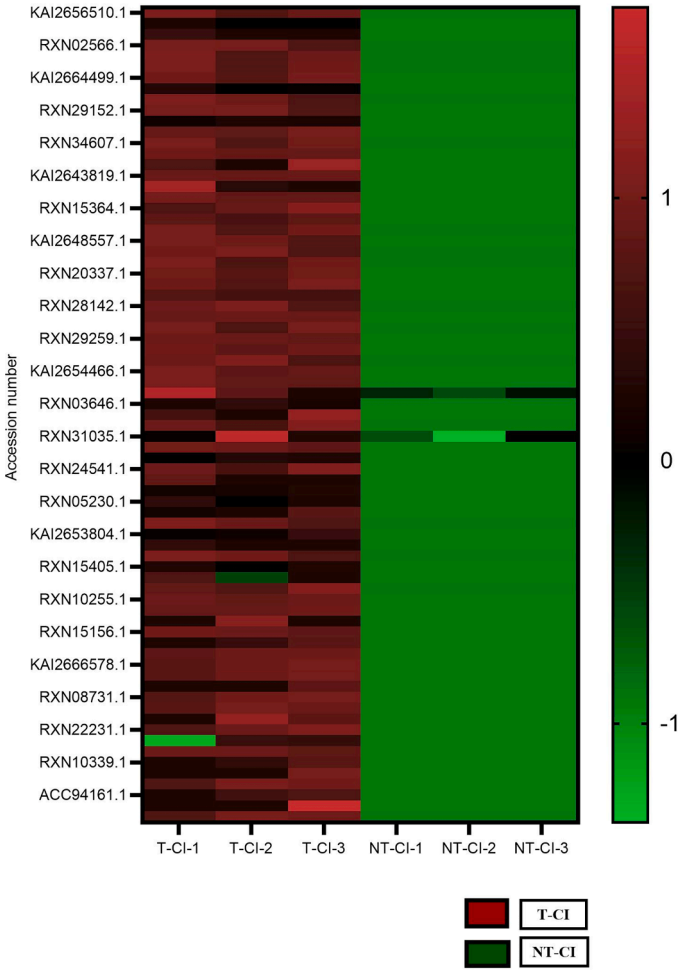

Fig. 2. Hierarchical clustering (Heat map) of significantly top 27 differentially expressed proteins (DEPs) identified between treated skin mucus (T-CI 1–3) and nontreated (N—CI 1–3) group. Lines are normalized with Z-scores of imputed values. Green color designates downregulated proteins, while red color designates upregulated proteins. Significant immune related proteins were upregulated in the treated group, while they were downregulated in the nontreated group in comparison.

Anti-oxidative proteins altered against A. hydrophila

Biotinidase protein was downregulated (−18 FC) in the mucus of fish from treated group against *A. hydrophila* (Table 1). Biotinidase reutilizes protein bound biotin which is crucial for the metabolism of fat, protein, and carbohydrate. Biotin deficiency has been altered normal immune functions. Also, Alpha-aminoadipic semialdehyde dehydrogenase, a multifunctional enzyme, was downregulated (−19 FC).

Fisher exact test and KEGG pathways

Fisher exact test was performed to differentiate among the DEPs after GO terms analysis (Fig.-4) based on the pathways they were involved. The KEGG analysis of the DEPs showed that these pathways were generally correlated to proteasome, Histidine metabolism, calcium signaling pathways, metabolic pathways, biosynthesis of amino acids. Moreover, proteasome, Histidine metabolism and metabolic pathways, were the significant pathways, which have important role in immune response (Fig.-5).

Discussion

Fish skin mucus is immunological barrier against invading bacteria [11,24], and has been studied at proteomic level in several fishes [13,14]. The biochemical composition of fish skin mucus is stimulus-specific and greatly varies from species to species. Therefore, proteomic composition of skin mucus from fishes inhabiting different environments has always been unique and interesting [6,15,25]. With this background and the important functions of immunological enzymes in the innate immune system of fish [26], this study for the first established the complete proteome-sketch of skin mucus from *C. idella* against *A. hydrophila* infection.

The important immune related proteins, this study reports, are the complement proteins, which directly interact with bacteria and function in fish immunity. Complement proteins are involved in inflammatory response, phagocytosis, eliminates of immune complexes, and assist in the production of antibodies [27]. The expression of complement proteins in the gills and skin of *D. rerio* was significantly upregulated after *Citrobacter freundii* and *A. hydrophila* infections [28,29]. The present study identified complement proteins C3 and C9 which were significantly upregulated against *A. hydrophila* infection. These results showed that complement cascade in *C. Idella* may be used to establish the Membrane Attack Complex (MAC) after being treated with *A. hydrophila*. MAC is imperative outcome complex of innate immune system, which can form holes on the bacterial cell surface, which make a route for the entry of calcium ions, modify the cell membrane permeability, and leads directly to cell dissolution [30]. Thus, C9 acts as an important protein components among the Terminal Complement Complex (TCC) proteins, and plays part in the innate resistance against bacterial pathogens [31].

Cathepsins is involved in antigen processing and maturation of the major histocompatibility complex class II molecules in fish. Cathepsin L was upregulated in the kidney of Japanese Flounder after exposure to *Edwardsiella tarda* and inhibited significantly colonization of this bacteria [11,32]. While cathepsin D was upregulated in the liver of Japanese Flounder, which revealed their role in immune against *Edwardsiella tarda* [32]. We found that Cathepsins S and Cathepsins Z, which are important lysosomal cysteine protease, were upregulated in the skin mucus of treated group of *C. Idella*, indicating their possible role in maintaining homeostasis and the physiological mechanisms of immune cells. Bacterial infections modify the normal functions of cellular, molecular, and metabolic homeostasis. In our study, calcium calmodulin-dependent kinase type II subunit beta isoform X18 and beta isoform X19 were upregulated (17 and 18 FC respectively) in the skin mucus of treated *C. idella*. Calmodulin is a calcium-binding protein that contribute in the polymerization of actin filaments [33]. Extracellular Calmodulin regulates fish skin permeability to ions and water [34]. Upregulation of Calmodulin in the treated group of *C. idella* revealed its possible role in response to bacteria.

Proteosome is a protease complex that is crucial for breakdown of proteins in an ATP dependent manner and helps in regulating the lipopolysaccharide induced signal transduction [4]. Proteasome subunit beta type-5, alpha type-3 and 6, 26S proteasome non-ATPase regulatory subunits 7 and 8 were upregulated in the skin mucus of *A. hydrophila* treated group in the present study. 20S proteasome complex with subunits alpha and beta were interacted in Rainbow Trout against *Aeromonas salmonicida* infection which together hints their role against bacterial infection [35].

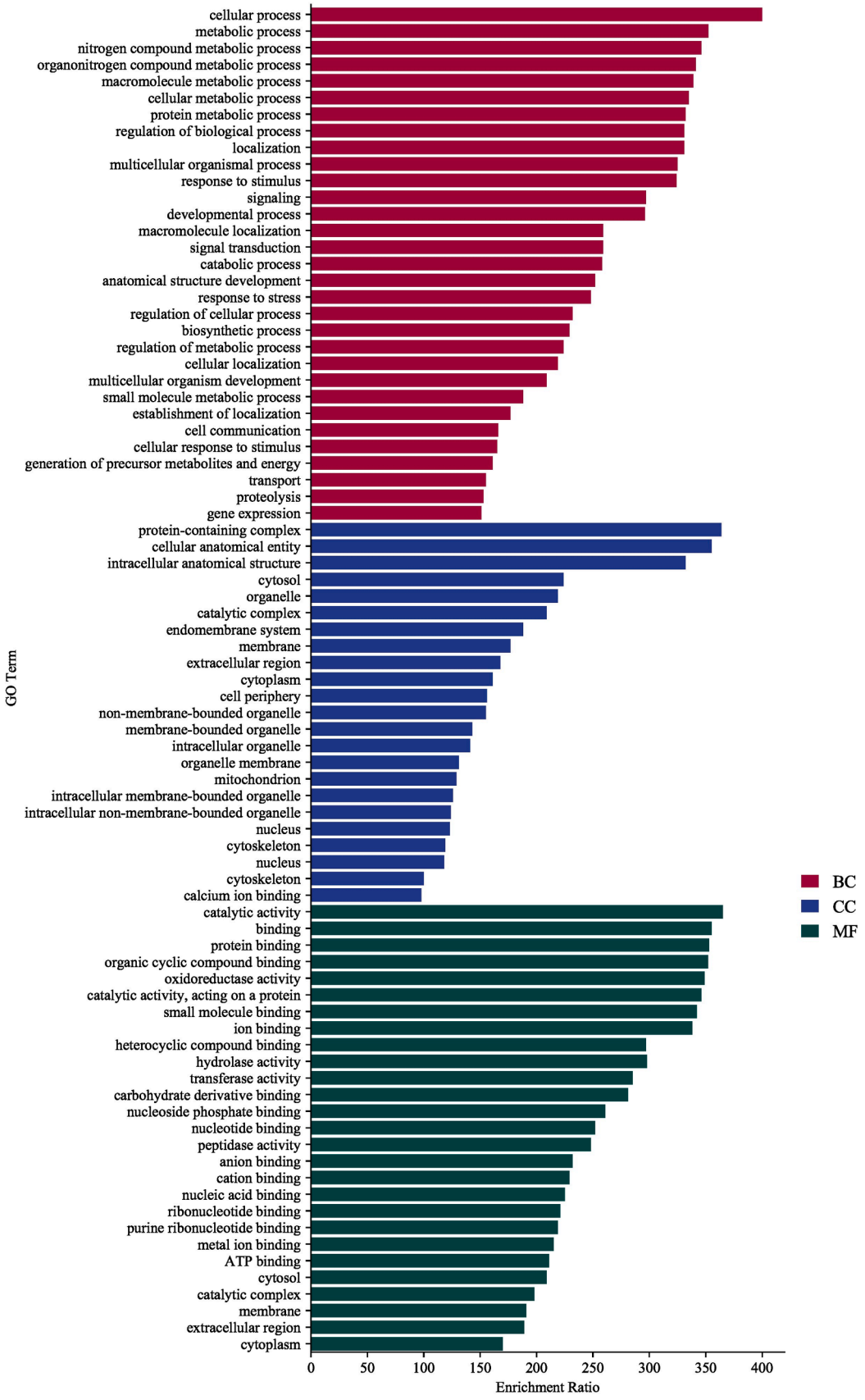

Fig. 3. Gene Ontology annotations of the DEPs in the skin mucus of *C. idella* upon exposure to *A. hydrophila.* The result is based on the BLAST2GO Pie graph 4levelsl ($P \leq 0.05$), which were characterized into 3 main parts. It includes **GO terms (a): Biological Process, (b): Cellular Component and (c): Molecular Function**.

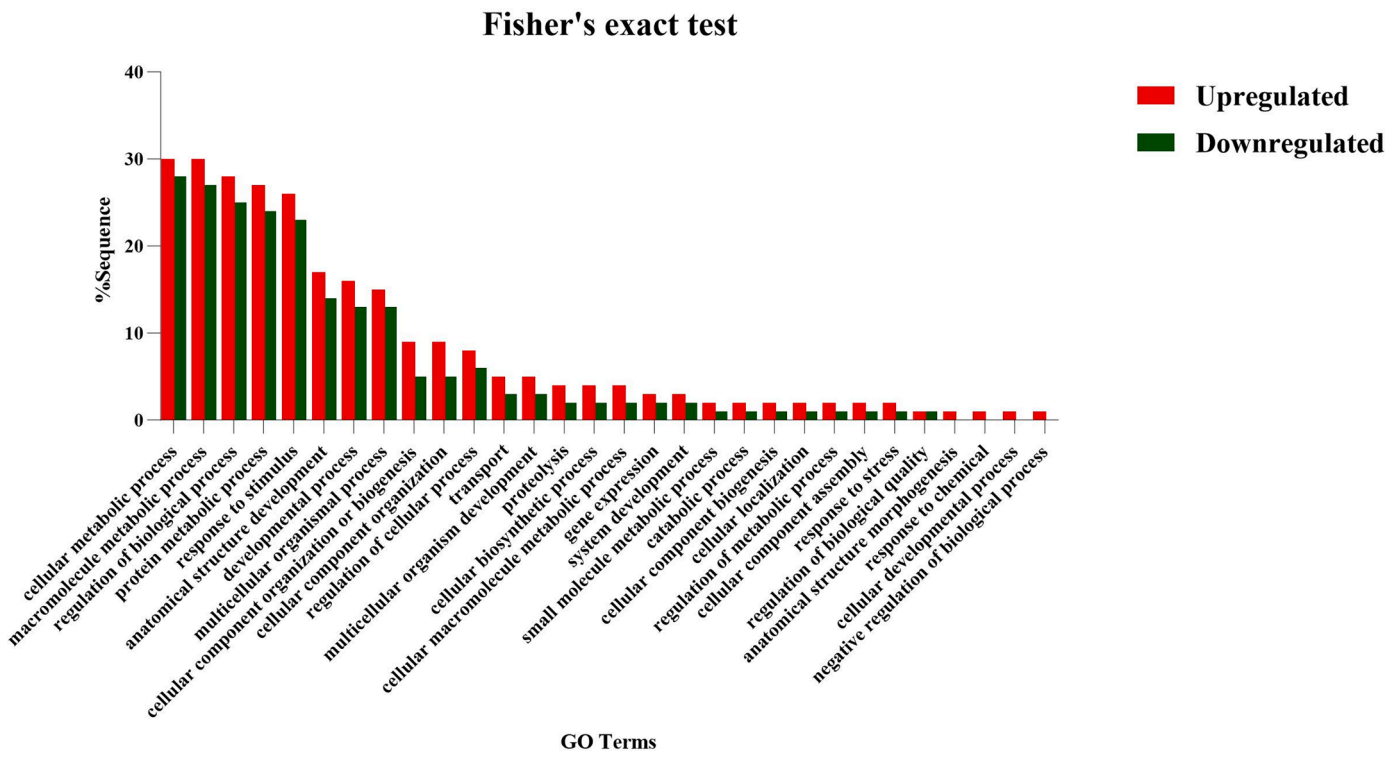

Fig. 4. Fisher Exact Test; GO terms showing significant enrichment in the treated *C. idella* (T-CI) compared with those in the nontreated *C. idella* (NT-CI) skin mucus. All levels of GO terms in biological process, molecular function and cellular component categories were compared using a two-tailed Fisher exact test (FDR < 0.05) with Blast2GO software.

Cytoskeleton related proteins were upregulated in the skin mucus of *C. Idella* including keratin, type II cytoskeletal 8, type I cytoskeletal 13 and 19-like protein, heat shock cognate 71 kDa protein, Myosin-9, tubulin beta-4B chain, 14 kDa apolipo, intelectin, which function in various cellular and in biological processes. Fish cytoskeleton related proteins function in bacterial resistance and viral infections [36,37]. Identification of modifications in the macrophage cellular proteome persuaded by calcium oxalate monohydrate, including F-actin protein and a-tubulin in increased phagocytic activity, and association of heat shock proteins and F-actin is imperative for phagosome formation [38]. Similarly, beta tubulin was found to be upregulated in skin mucus of *Gadus morhua* treated with *Vibrio anguillarum* probably due to its increased expression in the mucosal cells related with phagocytic processes [39]. Remarkably, DEPs (i-e, tubulin, actin, HSP90) were also identified in the gills of zebrafish treated with *A. hydrophila*, signifying that it possibly stimulates the phagosome pathways in local immune responses in gills. Overall, the upregulation of cytoskeleton proteins in these studies might be their involvement in the immune response of skin mucus against *A. hydrophila*.

Biotinidase is a crucial enzyme in mammals that cleaves vitamin, biotin from the biocytin and from the dietary protein sources [40]. The deficiency of biotinidase causes hypotonia, lethargy, eczema, alopecia, lethargy, optic atrophy, ataxia, loss of hearing, and cognitive retardation and it is usually inherited in patients [41]. The role of biotinidase in the immunity of vertebrates has been evident [42]. In Zebra fish, biotinidase was upregulated at transcriptional level against Cysteamine, which is a sulfhydryl compound and causes toxicity [43]. In our study, biotinidase isoform X1 enzyme was found to be downregulated in the skin mucus of *C. Idella* in the treated group. Similarly, biotinidase was also reported downregulated in the liver of *Labeo rohita* after exposure to *A. hydrophila* [4]. In *C. idella* (skin, head and kidney) deficiency of biotin decreased the mRNA level of bactericidal compounds i-e mucin, defensin and hepcidin while elevate the ratio of pro-inflammatory cytokines as interferon (IFN-γ2), tumor necrosis factor (TNF-α), and interleukin (IL-1, 6 and 8) [44]. Together all these reports speculate the role of biotinidase in immune response against bacterial infection in fish, which will surely need functional based studies on the mechanism of their action.

Transferrin is one of the essential serum proteins in fish with role in iron metabolism [45]. Transferrin is primarily produced in the liver of fish and circulate into the blood, however they also express in other tissues [46]. Transferrin binds to iron, thus prevents its production, and consequently prevent bacterial infections. Though, in stress conditions, transferrin is identified mostly as a negative acute phase protein, however transferrin can be a positive acute phase protein [47,48]. In our study, transferrin variant C was upregulated (23 FC) in the skin mucus of *C. idella* against *A. hydrophila*. Accordingly, transferrin was significantly upregulated in the skin, spleen, and blood of Rough Skin Sculpin after lipopolysaccharide exposure [49]. Transferrin was recently shown to have role in immune response of Goldfish against acute inflammation [50]. Transferrin receptor-1 was downregulated in rainbow trout against *A. salmonicida* [51]. Though, cleaved transferrin products have recently been shown to activate macrophages in inflammatory conditions. Here we assume the defensive role of transferrin in the skin mucus of fish against bacteria. However, further question the mechanism by which transferrin had dealt with iron, macrophages, and bacteria at a time. Thus, detailed studies focused on the precise role of transferrin in the skin mucus of fish and particularly during bacterial infection, are recommended.

Conclusions

This study concludes that skin mucus of *C. idella* is rich at proteomic level. The proteins discovered here were mostly immunological including transferrin variant C, lysozyme g, annexin A11, 26S proteasome non-ATPase regulatory subunit 8, hypothetical protein ROHU_000884, 60S ribosomal L7a, calpain-2 catalytic subunit-like protein, calpain-9-like protein, complement component C9, complement C3, cathepsin S, cathepsin Z, 14 kDa apolipo, heat shock protein,

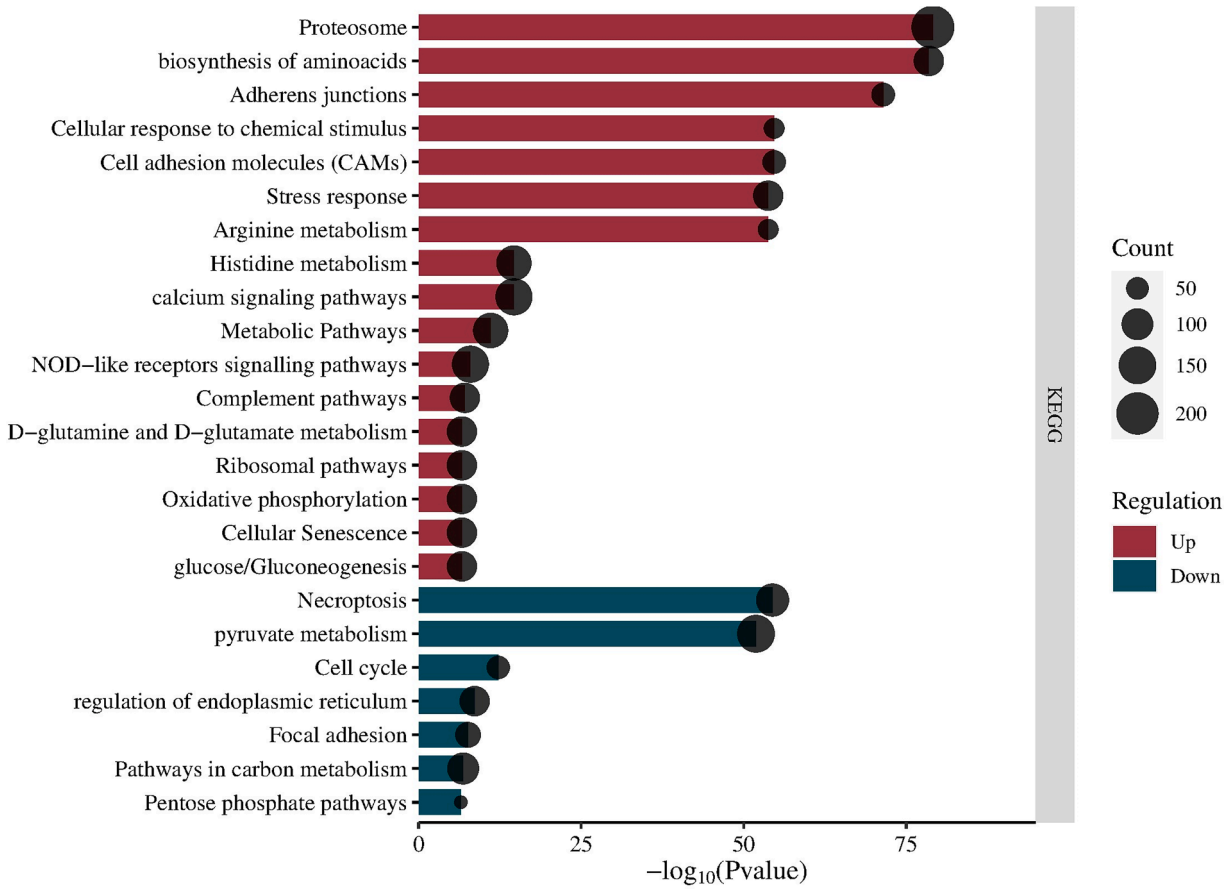

Fig. 5. KEGG Pathways; Each row represents an enriched function, and the length of the bar represents the enrich ratio, which is calculated as "input gene number". The color of the bar represents Up and down-regulated pathways.

intelectin, leukocyte elastase inhibitor, annexin A11, C-factor-like protein, biotinidase isoform X1 and epidermal growth factor receptor substrate 15-like and were expressed at differential levels against *A. hydrophila* infection. The proteins this study presented from the skin mucus of fish could be potential biomarkers and may be targeted for understanding bacterial infection and its control in fishes.

CRediT authorship contribution statement

Shandana Ali: Conceptualization, Formal analysis, Project administration, Software, Validation, Writing – original draft. **Farman Ullah Dawar:** Conceptualization, Resources, Supervision, Writing – review & editing. **Waheed Ullah:** Methodology. **Maizom Hassan:** Data curation, Visualization. **Kalim Ullah:** Investigation. **Zhe Zhao:** Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no competing interests

Data availability

No data was used for the research described in the article.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fsirep.2023.100122.

References

[1] T Lieke, T Meinelt, SH Hoseinifar, et al., Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases, Reviews in Aquaculture 12 (2020) 943–965.
[2] X Song, J Zhao, Y Bo, et al., Aeromonas hydrophila induces intestinal inflammation in grass carp (Ctenopharyngodon idella): an experimental model, Aquaculture 434 (2014) 171–178.
[3] Q Mu, Z Dong, W Kong, et al., Response of immunoglobulin M in gut mucosal immunity of common carp (Cyprinus carpio) infected with Aeromonas hydrophila, Frontiers in Immunology 13 (2022).
[4] M.U. Nissa, N. Pinto, B. Ghosh, U. Singh, M. Goswami, S. Srivastava, Proteomic analysis of liver tissue reveals Aeromonas hydrophila infection mediated modulation of host metabolic pathways in Labeo rohita, Journal of proteomics 279 (2023), 104870, https://doi.org/10.1016/j.jprot.2023.104870.
[5] FU Dawar, S Babu, H Kou, et al., The RAG2 gene of yellow catfish (Tachysurus fulvidraco) and its immune response against Edwardsiella ictaluri infection, Developmental & Comparative Immunology 98 (2019) 65–75.
[6] FU Dawar, J Tu, Y Xiong, et al., Chemotactic activity of cyclophilin a in the skin mucus of yellow catfish (Pelteobagrus fulvidraco) and its active site for chemotaxis, International Journal of Molecular Sciences 17 (2016) 1422.
[7] S Ali, MNK Khattak, W Ullah, et al., Bactericidal activities and biochemical analysis of skin mucus of Cyprinid Fish, Journal of King Saud University-Science (2023) 102731.
[8] S Ali, SAUR Shah, M Rauf, et al., Bactericidal role of epidermal mucus of freshwater fish treated with Aeromonas hydrophila, Journal of fish diseases (2023).
[9] J.P. Firmino, J. Galindo-Villegas, F.E. Reyes-López, E. Gisbert, Phytogenic Bioactive Compounds Shape Fish Mucosal Immunity, Frontiers in immunology 12 (2021), 695973, https://doi.org/10.3389/fimmu.2021.695973.
[10] KL Shephard, Functions for fish mucus, Reviews in fish biology 4 (1994) 401–429.
[11] S Dash, S Das, J Samal, et al., Epidermal mucus, a major determinant in fish health: a review, Iranian journal of veterinary research 19 (2018) 72.
[12] M Reverter, N Tapissier-Bontemps, D Lecchini, et al., Biological and ecological roles of external fish mucus: a review, Fishes 3 (2018) 41.
[13] AJ Lü, XC Hu, Y Wang, et al., Skin immune response in the zebrafish, Danio rerio (Hamilton), to Aeromonas hydrophila infection: a transcriptional profiling approach, Journal of Fish Diseases 38 (2015) 137–150.

[14] Y Hu, A Li, Y Xu, et al., Transcriptomic variation of locally-infected skin of Epinephelus coioides reveals the mucosal immune mechanism against Cryptocaryon irritans, Fish and shellfish immunology 66 (2017) 398–410.
[15] S Ali, W Ullah, AFS Kamarulzaman, et al., Proteomic Profile of Epidermal Mucus from Labeo rohita Reveals Differentially Abundant Proteins after Aeromonas hydrophila Infection, Fish Shellfish Immunology Reports (2023), 100115.
[16] B Cudmore, NE Mandrak, Biological synopsis of grass carp (Ctenopharyngodon idella), Canadian manuscript report of fisheries and Aquatic Sciences 2705 (2004) 1–44.
[17] B Maiti, S Dubey, HM Munang'Andu, et al., Application of outer membrane protein-based vaccines against major bacterial fish pathogens in India, Frontiers in immunology 11 (2020) 1362.
[18] G Di, H Li, C Zhang, et al., Label-free proteomic analysis of intestinal mucosa proteins in common carp (Cyprinus carpio) infected with Aeromonas hydrophila, Fish and shellfish immunology 66 (2017) 11–25.
[19] Hu, X., Bai, J., Liu, R., & Lv, A. (2022). Comprehensive transcriptomics and proteomics analysis of Carassius auratus gills in response to Aeromonas hydrophila. Fish and shellfish immunology reports, 4, 100077. 10.1016/j.fsirep.2022.100077.
[20] D. Xu, L. Song, H. Wang, X. Xu, T. Wang, L. Lu, Proteomic analysis of cellular protein expression profiles in response to grass carp reovirus infection, Fish & shellfish immunology 44 (2) (2015) 515–524, https://doi.org/10.1016/j.fsi.2015.03.010.
[21] I Sanahuja, A Ibarz, Skin mucus proteome of gilthead sea bream: a non-invasive method to screen for welfare indicators, Fish and shellfish immunology 46 (2015) 426–435.
[22] F. He, Bradford protein assay, Bio-protocol (2011) e45. -e45.
[23] R. Scopes, Measurement of protein by spectrophotometry at 205 nm, Analytical biochemistry 59 (1974) 277–282.
[24] J Fan, Q Shan, J Wang, et al., Comparative pharmacokinetics of enrofloxacin in healthy and Aeromonas hydrophila-infected crucian carp (Carassius auratus gibelio), Journal of Veterinary Pharmacology and Therapeutics 40 (2017) 580–582.
[25] ALB Canellas, WF Costa, J Freitas-Silva, et al., In sickness and in health: Insights into the application of omics in aquaculture settings under a microbiological perspective, Aquaculture (2022), 738132.
[26] Á Fernández-Montero, S Torrecillas, D Montero, et al., Proteomic profile and protease activity in the skin mucus of greater amberjack (Seriola dumerili) infected with the ectoparasite Neobenedenia girellae—an immunological approach, Fish and shellfish immunology 110 (2021) 100–115.
[27] H Boshra, J Li, J Sunyer, Recent advances on the complement system of teleost fish, Fish and shellfish immunology 20 (2006) 239–262.
[28] A. Lü, X. Hu, Y. Wang, X. Shen, X. Li, A. Zhu, J. Tian, Q. Ming, Z. Feng, iTRAQ analysis of gill proteins from the zebrafish (Danio rerio) infected with Aeromonas hydrophila, Fish & shellfish immunology 36 (1) (2014) 229–239, https://doi.org/10.1016/j.fsi.2013.11.007.
[29] A Lü, X Hu, J Xue, et al., Gene expression profiling in the skin of zebrafish infected with Citrobacter freundii, Fish and shellfish immunology 32 (2012) 273–283.
[30] LJ Barkai, E Sipter, D Csuka, et al., Decreased ficolin-3-mediated complement lectin pathway activation and alternative pathway amplification during bacterial infections in patients with type 2 diabetes mellitus, Frontiers in immunology 10 (2019) 509.
[31] MCH Holland, JD. Lambris, The complement system in teleosts, Fish and shellfish immunology 12 (2002) 399–420.
[32] L Wang, C Shao, W Xu, et al., Proteome profiling reveals immune responses in Japanese flounder (Paralichthys olivaceus) infected with Edwardsiella tarda by iTRAQ analysis, Fish and shellfish immunology 66 (2017) 325–333.
[33] N. Pinto, Stress and immune relevant proteins in lumpsucker (Cyclopterus lumpus L.) skin-establishing basic knowledge, Nord universitet, 2017.
[34] G Zaccone, S Fasulo, L Ainis, et al., Localization of calmodulin positive immunoreactivity in the surface epidermis of the brown trout, Salmo trutta, Histochemistry 91 (1989) 13–16.
[35] M Long, J Zhao, T Li, et al., Transcriptomic and proteomic analyses of splenic immune mechanisms of rainbow trout (Oncorhynchus mykiss) infected by Aeromonas salmonicida subsp. salmonicida, Journal of proteomics 122 (2015) 41–54.
[36] A Lü, X Hu, Y Wang, et al., Proteomic analysis of differential protein expression in the skin of zebrafish [D anio rerio (Hamilton, 1822)] infected with A eromonas hydrophila, Journal of Applied Ichthyology 30 (2014) 28–34.
[37] P Zhang, C Li, Y Li, et al., Proteomic identification of differentially expressed proteins in sea cucumber Apostichopus japonicus coelomocytes after Vibrio splendidus infection, Developmental & Comparative Immunology 44 (2014) 370–377.
[38] N Singhto, R Kanlaya, A Nilnumkhum, et al., Roles of macrophage exosomes in immune response to calcium oxalate monohydrate crystals, Frontiers in Immunology 9 (2018) 316.
[39] B. Rajan, J. Lokesh, V. Kiron, M.F. Brinchmann, Differentially expressed proteins in the skin mucus of Atlantic cod (Gadus morhua) upon natural infection with Vibrio anguillarum, BMC veterinary research 9 (2013) 103, https://doi.org/10.1186/1746-6148-9-103.
[40] B. Wolf, R.E. Grier, R.J. Allen, S.I. Goodman, C.L. Kien, Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency, Clinica chimica acta; international journal of clinical chemistry 131 (3) (1983) 273–281, https://doi.org/10.1016/0009-8981(83)90096-7.
[41] A. Akgun, A. Sen, H. Onal, Clinical, biochemical and genotypical characteristics in biotinidase deficiency, Journal of pediatric endocrinology & metabolism: JPEM 34 (11) (2021) 1425–1433, https://doi.org/10.1515/jpem-2021-0242.
[42] K. Pindolia, H. Li, C. Cardwell, B. Wolf, Characterization and functional analysis of cellular immunity in mice with biotinidase deficiency, Molecular genetics and metabolism 112 (1) (2014) 49–56, https://doi.org/10.1016/j.ymgme.2014.02.008.
[43] C Chen, Y Zuo, H Hu, et al., Hepatic lipid metabolism disorders and immunotoxicity induced by cysteamine in early developmental stages of zebrafish, Toxicology 493 (2023), 153555.
[44] P He, W-D Jiang, X-A Liu, et al., Dietary biotin deficiency decreased growth performance and impaired the immune function of the head kidney, spleen and skin in on-growing grass carp (Ctenopharyngodon idella), Fish and Shellfish Immunology 97 (2020) 216–234.
[45] Neves JV, Wilson JM, Rodrigues PNJD, et al. (2009) Transferrin and ferritin response to bacterial infection: the role of the liver and brain in fish. 33: 848-857.
[46] LA Lambert, H Perri, PJ Halbrooks, et al., Evolution of the transferrin family: conservation of residues associated with iron and anion binding, Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 142 (2005) 129–141.
[47] CJ Bayne, L Gerwick, The acute phase response and innate immunity of fish, Developmental and Comparative Immunology 25 (2001) 725–743.
[48] JL Congleton, EC Wagner, Acute-phase hypoferremic response to lipopolysaccharide in rainbow trout (Oncorhynchus mykiss), omparative Biochemistry and Physiology Part A: Physiology 98 (1991) 195–200.
[49] Y. Liu, S. Yu, Y. Chai, Q. Zhu, Transferrin gene expression in response to LPS challenge and heavy metal exposure in roughskin sculpin (Trachidermus fasciatus), Fish & shellfish immunology 32 (1) (2012) 223–229, https://doi.org/10.1016/j.fsi.2011.10.023.
[50] M Trites, D Barreda, Contributions of transferrin to acute inflammation in the goldfish, C. auratus, Developmental and Comparative Immunology 67 (2017) 300–309.
[51] DR Causey, MA Pohl, DA Stead, et al., High-throughput proteomic profiling of the fish liver following bacterial infection, Journal of BMC genomics 19 (2018) 1–17.