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Acetylome Profiling of *Vibrio alginolyticus* Reveals Its Role in Bacterial Virulence

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Abstract: It is well known that lysine acetylation (Kace) modification is a common post-translational modification (PTM) that plays an important role in multiple biological and pathological functions in bacteria. However, few studies have focused on lysine acetylation modification in aquatic pathogens to date. In this study, the acetylome profiling of fish pathogen, *Vibrio alginolyticus* was investigated by combining affinity enrichment with LC MS/MS. A total of 2883 acetylation modification sites on 1178 proteins in this pathogen were identified. The Kace modification of several selected proteins were further validated by Co-immunocoprecipitation combined with Western blotting. Bioinformatics analysis showed that seven conserved motifs can be enriched among Kace peptides, and many of them were significantly enriched in metabolic processes such as biosynthesis of secondary metabolites, microbial metabolism in diverse environments, and biosynthesis of amino acids, which was similar to data previously published for *V. parahaemolyticus*. Moreover, we found at least 102 acetylation modified proteins predicted as virulence factors, which indicate the important role of PTM on bacterial virulence. In general, our results provide a promising starting point for further investigations of the biological role of lysine acetylation on bacterial virulence in *V. alginolyticus*.

Keywords: *Vibrio alginolyticus*; Acetylome; Bacterial virulence; Proteomics

1. Introduction

With the development of high-resolution mass spectrometry and high affinity purification technology for lysine acetylated peptides, lysine acetylation (Kace) modification of proteins has been well validated to be a widely distributed post-translational modification (PTM) in both eukaryotes and bacteria [1, 2]. Moreover, this PTM was reported to play important roles in maintaining the physiological and pathologic function. For example, in mammalian cells, some Kace modified proteins such as HDAC6 and Tau, were found to be involved in human diseases such as inflammation and Alzheimer's disease [3, 4]. In bacterial cells, the distribution of Kace modification has been well documented in dozens of species, including *Vibrio parahaemolyticus*, *Escherichia coli* and *Mycobacterium tuberculosis* [5-7], and was reported to be involved in many basal physiological functions such as chemotaxis, virulence, and antibiotic resistance [8-10]. Due to the diverse nature of this PTM, it has been suggested that lysine acetylation could provide a new target for vaccine and drug development based on an understanding of the regulatory mechanisms of this modification [11, 12]. However, although there are more studies reporting the roles of Kace modification on diverse cellular functions, the profile and physiological functions of this PTM still remain elusive in many species, especially fish pathogens. Until now, few acetylome profiles in fish pathogens have been reported e.g. *V. parahaemolyticus* and *Aeromonas hydrophila* [5, 13]. Given the importance of infectious diseases in aquaculture, it is necessary to carry out large scale identification of this PTM and compare various Kace modification profiles in more aquatic pathogens.

Vibrio alginolyticus a well known fish pathogen in the aquaculture industry which can infect orange-spotted grouper (*Epinephelus coioides*) [14], *Lutjanus erythopterus*(Bloch) [15], and large yellow croaker (*Larimichthys crocea*) [16] fish. Each year, the fish diseases caused by this pathogen leads to an economic loss of approximately $150 million. Recently, several proteins such as HopPmaJ [17], Acfa [18] and VscO [19] have been reported to be involved in invasion of the host as a virulence effector, as well as protein RpoS affect on adhesion to bacteria, growth, hemolysis, biofilm production, movement [20], and SodB protein influence of bacterial motility and adhesion, leading to changes in bacterial virulence [21]. However, the intrinsic virulence mechanisms are still largely unknown, especially for the role of Kace modification.

In this study, the acetylome profile of the pathogenic *V. alginolyticus* was performed by combining high-accuracy MS and a highly specific Kace affinity method, and results were compared with previously published *V. parahaemolyticus* acetylome data. Bioinformatic analysis of the resulting proteins revealed that these Kace proteins are involved in various biological processes and central metabolism pathways, which is similar with that seen in *V. parahaemolyticus*. Moreover, we also found that several virulence effectors, such as HemL, FabB, FabD, FabF-3 and PhoR, are acetylated, which indicate PTM of these proteins may play an important role in bacterial-host interactions and should be further investigated in the future. Our data enrich the

understanding of Kace modification profiling in prokaryotes and provides new insights into the potential role of PTMs in fish pathogens.

2. Materials and methods

2.1 Bacterial strains and Sample preparation

V. alginolyticus strain HY9901, isolated from *Lutjanus erythopterus* [15], was used in this study. A single colony was cultured overnight in Luria-Bertani (LB) broth at 28° C on a shaker at 200 rpm, and then diluted to 1:100 in fresh LB medium.This was cultured to logarithmic growth stage, the cells collected by centrifugation at 8000 rpm, 4° C for 10 min, and washed twice with cold PBS (phosphate buffer saline). The pellets were resuspended with 8 M Urea and 0.2% SDS in 50 mM Tris-HCl (pH=8) and then broken by ultrasonication in an ice bath for 5 min before centrifugation at 12000 g for 15 min at 4°C. The supernatant was collected and the protein concentration was determined using the Bradford assay. The supernatant was incubated with 2 mM DTT at 56°C for 1 hour followed with adding 20 mM iodoacetamide (IAA) for 1 hour at room temperature in the dark as previously described [22]. Four volumes of pre-chilled acetone were added to the supernatant to precipitate protein at -20°C for 2 h and the precipitate was then washed twice with cold acetone. The pellet was dissolved in 8 M urea in 0.1 M triethylammonium bicarbonate (TEAB, pH=8.5) and the protein concentration was determined again before further procedures.

2.2. Immunoaffinity enrichment of lysine-acetylated peptides

10 mg of protein was digested with Trypsin Gold (Promega) at 1:20 (Trypsin: protein = 1:20) ratio at 37°C for 16 h as previously described [23]. The digested peptide was

desalted with C18 cartridge (Waters Inc., Milford, MA, USA) and dried by a CentriVap vacuum concentrator (Labconco Inc., Kansas City, MO, USA). Lyophilized peptides were dissolved in MOPS IAP buffer (50 mM MOPS, 10 mM KH_2PO_4 and 50 mM NaCl, pH 7), mixed with anti-acetyl-lysine beads (Cell Signaling Technology) for 2.5 h at 4°C, and then washed twice with MOPS IAP buffer by centrifugation for 30 s at 3000 g at 4°C. The Kace enriched peptides were eluted with 0.15% TFA (Trifluoroacetic acid) and then desalted using peptide desalting spin columns (Thermo Fisher) before MS analysis.

2.3. LC-MS/MS Analysis

The desalted peptides were analyzed using an EASY-nLCTM 1200 UHPLC system (ThermoFisher) coupled to an Orbitrap Q Exactive HF-X mass spectrometer (ThermoFisher) in the data-dependent acquisition (DDA) mode [24]. Briefly, peptides in 0.1% FA (Formic acid) were injected onto a Acclaim PepMap100 C18 Nano-Trap column (2 cm×100 μm, 5 μm) and separated on a Reprosil-Pur 120 C18-AQ analytical column (15 cm×150 μm, 1.9 μm) with a 120 min linear gradient from 5 to 100% buffer B (0.1% FA in 80% ACN) in buffer A (0.1% FA in H_2O) at a flow rate of 600 nL/min. The solvent gradient was set as follows: 5-10% B, 2 min; 10-40% B, 105 min; 40–50% B, 5 min; 50–90% B, 3 min; 90–100% B, 5 min.

For DDA, the Q-Exactive HF-X mass spectrometer was operated in positive mode with spray voltage of 2.3 kV and the capillary temperature was 320°C. Full MS scans

from 350 to 1500 m/z were acquired at a resolution of 60000 (at 200 m/z) with an AGC target value of 3×10^6. From the full MS scan, a maximum number of 40 of the most abundant precursor ions were selected for higher energy collisional dissociation (HCD) fragment analysis at a resolution of 15000 (at 200 m/z), a maximum ion injection time of 45 ms, a normalized collision energy of 27%, an intensity threshold of 8.3×10^3, and the dynamic exclusion parameter set at 60 s.

2.4 Bioinformatics

The Gene Ontology (GO) annotation and KEGG pathway were enriched by Omicsbean online software (http://www.omicsbean.cn/) [25]. STRING software (https://string-db.org/cgi/input.pl?sessionId) combined with Cytoscape were used to predict protein-protein interaction network [26], and motif analysis was performed by the Motif-X software. Virulence factors (VFs) retrieval was carried out using the Virulence Factor Database (VFDB) database (http://www.mgc.ac.cn/VFs/main.htm), which is a comprehensive public store and online platform for deciphering bacterial pathogenesis. The VFs homologs were identified using Blast analysis and using E-value $< 1.0^{-5}$ as cutoff [27].

2.5 CO-IP and Western blot

Specific polyclonal antibodies to D0X475 (Uncharacterized protein) and OmpH (Chaperone protein Skp) were used to precipitate target proteins. *V. alginolyticus*strain cell lysates (500 μg) were interacted with D0X475 and OmpH

antibody at 4°C overnight. Protein A/G beads washed three times with PBS buffer were added to the lysates at 4°C for 1-3 h [28]. The beads were pelleted at 4°C, followed by five washes with PBS buffer. 50μL of loading sample buffer (250 mM Tric-HCl PH=6.8, 10% SDS, 0.5% bromophenol blue 50% glycerol and 5% β-mercaptoethanol) was added to the pellet, boiled for 5 min, and subsequently analyzed by SDS-PAGE and western blot.

For western blot, proteins were run 12% 1-DE gels and transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were blocked in TBS (Tris buffered saline) containing 0.05% (v/v) Tween 20 with 5% (w/v) skim milk and incubated 1 h at room temperature. The primary antibodies used in the western blot were anti-Kace (1:1000), anti-D0X475 (1:4000) and anti-OmpH (1:4000) and incubated overnight at 4°C. Horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG was used as the secondary antibody at a 1:5000 dilution. Finally, the membrane was visualized using the ECL system (Bio-Rad, Hercules, CA, USA), and recorded by the ChemiDoc™ MP (Bio-Rad, Hercules, CA, USA) imaging system [29].

3. Results and Discussion

3.1 Quantitative proteomic profiling of lysine acetylation peptides in V. alginolyticus

In order to map lysine acetylation sites in *V. alginolyticus*, proteins were isolated from exponentially growing cells. Acetylated peptides were generated through purification

and digestion of these protein samples, followed by affinity enrichment and purification. Peptides were further separated and identified by EASY-nLC™ 1200 UHPLC system coupled to an Orbitrap Q Exactive™ HF-X mass spectrometer (**Figure 1A**). Finally, we performed the first largescale analysis of lysine acetylated proteins in *V. alginolyticus*. The results yielded a total of 2883 peptides and 2537 acetylated sites on 1178 proteins (Supplemental Table S1 and S2). As shown in **Figure 1B,** the mass error of most acetylated peptides ranged from -5 to 5 ppm, indicating an expected error control from the MS dataset. The mass of most acetylated proteins was in the range of 10-60 kDa (**Figure 1C**). The length of most of the peptides ranged from 7-20 amino acids, and exhibited distinct abundance depending on their lengths (**Figure 1D**), well in agreement with the property of peptides and the preparation standard of protein sample. Moreover, the numbers of modification sites for each protein is mainly from 1 to 10, and more than 45.2% of proteins contained only one acetylation sites, and the 22.7%, 11.9%, 6.1%, and 14.1% of proteins with two, three and four or more modification sites, respectively (**Figure 1E**), which was in common with many other studies [30, 31]. In this study we found that D0WZ79 (*VMC_24790*), the component of the pyruvate dehydrogenase (PDH) complex as the key enzyme in the pyruvate cycle has the highest number of acetylation sites (24 acetylation sites). A previous study has showed that pyruvate cycle provides respiratory energy in bacteria, and protect bacteria from stressful environments, which indicate the important role of this protein on intracellular metabolic regulation [32]. In addition, there were many translation-related PTM proteins on 50S (except for S12, S14 and S20) and 30S

(except for L7, L8, L26 and L32) ribosome subunits. The results indicated that acetylation may be involved in bacterial protein translation. In addition, 30S ribosome subunits D0WW34 (*rpoB*) and D0WW35 (*rpoC*) have 20 and 17 modified sites, respectively, and a previous study reported that knockout of *rpoB* gene reduced the *rpoB*-*rpoC* mRNA level by two-fold and may lead to cell defects [33].

3.2 Functional annotation and enrichment of acetylated proteins in V. alginolyticus

To better understand the role and distribution of the acetylated modification in *V. alginolyticus*, the GO functional annotations, KEGG pathway analysis and protein domains of acetylation-modified proteins were analyzed. The GO analysis was performed to gain insight into the potential functional role. In total, 1178 of the acetylated proteins were analyzed according to their biological process, cellular component and molecular function using OmicsBean online software. In the biological process (BP) category, many of acetylated proteins were significantly enriched in metabolic process, especially in organic substance metabolic process and cellular metabolic process (**Figure 2A**). Consistently, in the cellular component (CC) category, most of the detected lysine-acetylated proteins were involved in the cell part, cell, intracellular, cytoplasm and intracellular part (**Figure 2B**). The molecular function (MF) category, mainly comprised ion binding, small molecule binding, nucleoside binding, nucleoside phosphate binding, and anion binding (**Figure 2C**). The GO analysis results are consistent with previous results that showed that most lysine acetylated

proteins are categorized as involved in metabolic and biosynthetic processes and located in the cytoplasm [5, 10, 34].

To gain further insights into the processes regulated by the acetylation, we mapped the acetylated proteins to KEGG pathways (**Figure 2D**). The results showed that the acetylated proteins identified were assigned to several groups, including metabolism pathways, biosynthesis of secondary metabolites, biosynthesis of antibiotics, microbial metabolism in diverse environments, and biosynthesis of amino acids. In general, lysine acetylation occurs on many enzymes involved in metabolism, and these findings were consistent with previous studies in *V. parahaemolyticus* [5].

Proteins are composed of domains, which are the units of protein structure, function and evolution. In this study, the protein domain enrichment analysis showed that proteins with P-loop containing nucleoside triphosphate hydrolase, nucleic acid-binding, OB-fold, NAD(P)-binding domain superfamily and NAD(P)-binding domain have a higher tendency to be acetylated (**Figure 2E**). The domain P-loop containing nucleoside triphosphate hydrolase has been shown to have a significant role in different forms of signal transduction, intracellular trafficking, and cytoskeletal re-organization [35]. Previous studies have reported that the NAD(P)-binding domain superfamily and NAD(P)-binding domain are particularly well suited to regulate transcriptional activity in response to changes in cellular redox balance [36]. Our results indicate that Kace modification may be involved in complicated biological

processes including protein biosynthesis and nucleic acid-binding.

3.3 Analysis of acetylated-lysine sequence motifs

In this study, we used the Motif-X extractor web tool [37] (a software that was designed to extract overrepresented patterns from any sequence data set) to determine the sequence motifs from all of the acetylated peptides in *V. alginolyticus*. The analysis of the frequency of amino acids sequences from -10 to +10 surrounding the acetylated lysine. Seven conserved motifs that can be enriched among acetylated peptides, such as EK^{ac}, AK^{ac}, LK^{ac}, K^{ac}**K, DK^{ac}, L*K^{ac}****R, GDK^{ac} are shown in **Figure 3**. The results showed that the EK^{ac}, AK^{ac}, LK^{ac}, K^{ac}**K, DK^{ac} were significantly preferred, suggesting that these conserved sites may be functionally important for acetylation in *V. alginolyticus*. Similar results have been found in *Thermus thermophilus* and *Mycobacterium tuberculosis* [7, 38]. LK^{ac}, EK^{ac} and DK^{ac} have also been identified in the consensus motif, and LK^{ac} motif was most abundant in *Bacillus amyloliquefaciens* [10], indicating that the acetylation sites of lysine are conserved. Interestingly, it could be observed that the residue preferences for acetylated peptides are -1 position, such as residue E, A, L and D, indicating the conservation of Kace modification motifs.

3.4 Protein-protein interaction network of the V. alginolyticus acetylome

In order to investigate cell life activity involving acetylated proteins in *V. alginolyticus*, the protein-protein interaction (PPI) network was established (**Figure 4**).On the basis

of STRING database analysis, we found that 36 ribosomal subunit proteins were highly interconnected, indicating that the translation process may be regulated by acetylation in *V. alginolyticus*, in accordance with *V. parahaemolyticus* and *M. tuberculosis* results [5, 7]. Interestingly, a large number of ribosomal subunit proteins were also succinylated in *A. hydrophila*, suggesting that ribosomal proteins have "cross-talk" phenomenon [22]. Furthermore, most of the acetylated proteins were involved in metabolic pathways, including the microbial metabolism in diverse environments, carbon metabolism, biosynthesis of amino acids, and aminoacyl-tRNA biosynthesis. These PPI networks, suggest that protein acetylation is a key PTM in *V. alginolyticus* that contributes to cooperation and coordination of metabolic pathways.

3.5 Validation of D0X475 and OmpH lysine-acetylated proteins using Co-Immunoprecipitation and Western blotting.

To further validate the identified lysine-acetylated results, 2 Kace proteins (D0X475 and OmpH) were selected and analyzed by Co-IP and Western blotting. The D0X475 and OmpH proteins were captured by their respective antibodies and then Western blotting was performed with anti-acetylation and anti-target protein antibody, respectively (**Figure 5**). The results showed that D0X475 and OmpH proteins exhibited acetylation modifications consistent with lysine-acetylated proteomic data, further validating our proteomics results.

3.6 Comparison of lysine acetylome between V. alginolyticus and V.

parahaemolyticus

V. alginolyticus and *V. parahaemolyticus* are both common aquatic microorganisms in the marine environment with considerably high homology. A previous study reported the lysine acetylome in *V. parahaemolyticus* and found 1413 acetylation sites on 656 proteins [5]. In this study, we compared the acetylated modified proteins in both pathogens. Results showed that 408 proteins have orthologs in the acetylome of *V. parahaemolyticus* (**Figure 6A**), indicating that they have considerable similarities in functions such as signal transduction, energy metabolism, cytoskeleton and transcription factor activity, to name a few. Furthermore, we analyzed the KEGG pathway of the overlap acetylated proteins. The results showed that the common pathways were related to biosynthesis of secondary metabolites, antibiotics, and amino acid, microbial metabolism in diverse environments, carbon metabolites, and ribosome. We also aligned *V. alginolyticus* acetylated peptides with *V. parahaemolyticus* and found 372 acetylated peptides conserved (**Figure 6B**). Further, the overlapping acetylated peptides for motif analysis, and the results showed that two conserved motifs were enriched, such as K^{ac}**E and D**K^{ac}, indicating that there are many conservation acetylation modification sequences in *Vibrio. spp*.

3.7 Virulence factors of acetylated proteins in V. alginolyticus

To date, a number of acetylated proteins were reported to be involved in virulence in various bacterial species, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *V. parahaemolyticus* [5, 39-41]. Virulence

factors (VFs) of *V. alginolyticus* were retrieved from the VFDB database in order to better understand the effect of Kace modification on *V. alginolyticus* virulence. A total of 102 proteins were detected, accounting for 8.66% of the total proteins (Supplementary Table S3). Further, analysis of the protein-protein interaction network of VFs revealed that 63 proteins were found to interact (**Figure 7**). The LysR-family transcriptional regulators are among the most widespread regulators in prokaryotes, and control a wide range of biological processes in order to achieve optimal bacterial survival and adaptation in adverse environments [42, 43], such as some LysR-family regulators, RovMin in *Yersinia pseudotuberculosis*, OxyR in *Klebsiella pneumoniae*, BexR in *Pseudomonas aeruginosa* have been shown to be important for regulating virulence [44-46]. In this study *N646_4035* had the most acetylation sites, and sequence analysis revealed that it belongs to the LysR family transcriptional regulator, which may be involved in the virulence of *V. alginolyticus*. In addition, N646_2304 and N646_2853 two GGDEF family proteins were acetylated, and studies in *Xanthomonas oryzae* reported that GGDEF-domain proteins are connected with virulence and motility [47, 48], indicating that N646_2304 and N646_2853 may affect the virulence of *V. alginolyticus*. However, it remains unknown how acetylation regulates virulence in *V. alginolyticus* and that warrants further study in the future.

4. Conclusion

It is well known that lysine acetylation (Kace) modification plays an important role in the multiple biological functions in bacteria whereas few reports focus on the lysine acetylation modification profiling in aquatic pathogens such as *V. alginolyticus*. In this study, using high-resolution mass spectrometry with a high affinity enrichment method, we successfully identified a total a total of 2883 acetylation modification sites on 1178 proteins in *V. alginolyticus*. Our results showed that the many of Kace proteins detected are involved in various metabolic pathways and translation processes, which is similar with other bacterial species, especially *V. parahaemolyticus*. Moreover, we also found a considerable number of Kace proteins were predicted as virulence factors and may play important roles in bacterial virulence, potentially providing targets for vaccine development. The virulence mechanisms and pathogen-host interactions of this important aquaculture pathogen should be further investigated.

Acknowledgments

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Legends and Figures

Figure 1. Outline of *V. alginolyticus* lysine acetylation proteomics. (A) Workflow of quantitative proteomics analysis of lysine acetylation in *V. alginolyticus.* (B) Distributions of mass errors for all identified lysine-acetylated peptides. (C) Distribution of protein mass in the acetylated proteins. (D) Distribution of acetylated peptides based on their length. (E) Distribution of acetylated sites in the acetylated proteins.

Figure 2. **Functional classification of acetylated proteins in *V. alginolyticus*.** (A-C) Biological Processes, Cell Components and Molecular Function enrichment analysis of the acetylated proteins. (D) KEGG pathway-based enrichment analysis of the acetylated proteins. (E) Domains enrichment analysis of the acetylated proteins.

Figure 3. Properties of the acetylated peptides. (A) Acetylation motifs and conservation of acetylation sites. The height of each letter corresponds to the frequency of that amino acid residue in that position. The central K refers to the acetylated lysine. (B) Number of peptides containing significantly enriched motifs as identified using MotifX.

Figure 4. Protein-protein interaction network of the *V. alginolyticus* acetylome. Protein-protein interaction network obtained with STRING (11.0) at medium confidence scores ≥ 0.4.

Figure 5. Validation of D0X475 and OmpH lysine-acetylated proteins in *V. alginolyticus* using Co-Immunoprecipitation and Western blotting. D0X475 and OmpH proteins were enriched by Co-IP with specific antibodies, followed by Western blotting with D0X475 and OmpH proteins specific antibodies (above), and Western blotting with anti-lysine acetylation antibodies (below).

Figure 6. Comparison of *V. alginolyticus* acetylproteome with *V. parahaemolyticus*. (A) Comparison of *V. alginolyticus* and *V. parahaemolyticus* overlapped acetylated proteins, and KEGG pathway enrichment analysis with overlapped proteins. (B) Comparison of *V. alginolyticus* and *V. parahaemolyticus* overlapped acetylated peptides, and motif analysis with overlapped peptides.

Figure 7. Protein-protein interaction network of acetylated virulence factors in *V. alginolyticus*. The size of the circle represents the number of modification sites.

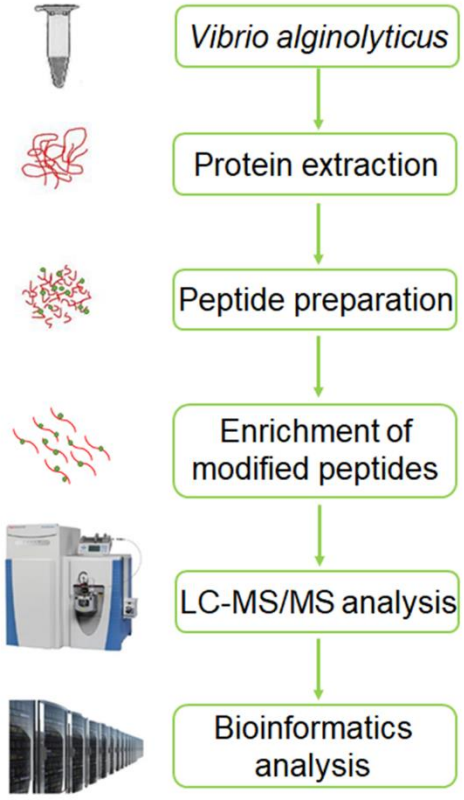
Supplemental Table S1. The identified acetylated proteins and sites in *V. alginolyticus*.

Supplemental Table S2. The identified peptides in *V. alginolyticus*.

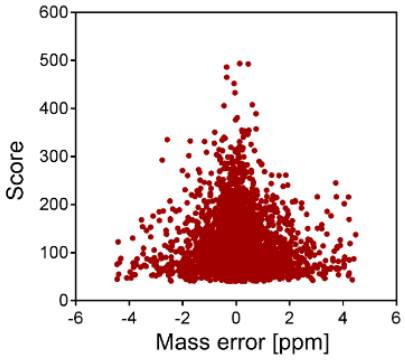
Supplemental Table S3. List of *V. alginolyticus* virulence factors. The list was

generated based on VFDB database.

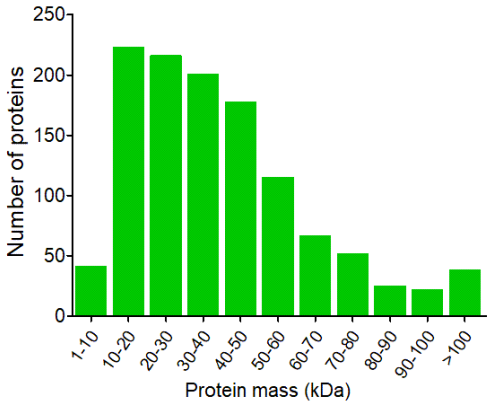
Fig 1


Vibrio alginolyticus
Protein extraction
Peptide preparation
Enrichment of modified peptides
LC-MS/MS analysis
Bioinformatics analysis

A


Score
Mass error [ppm]

B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Protein mass (kDa)
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10-20
20-30
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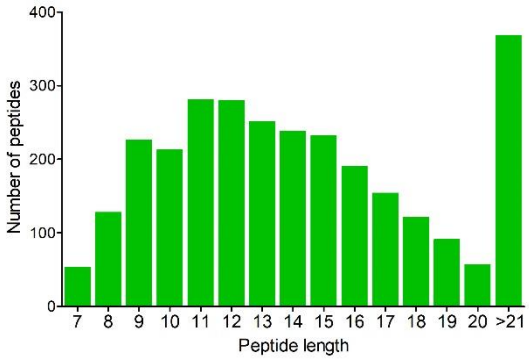
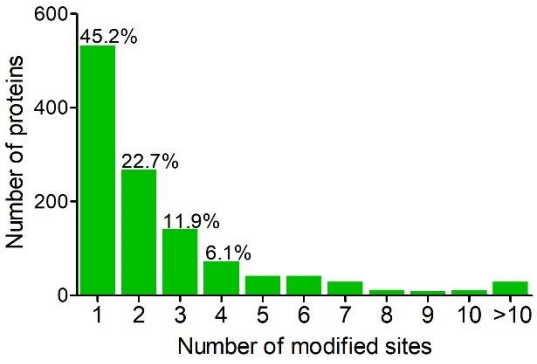
40-50
50-60
60-70
70-80
80-90
90-100
>100

C

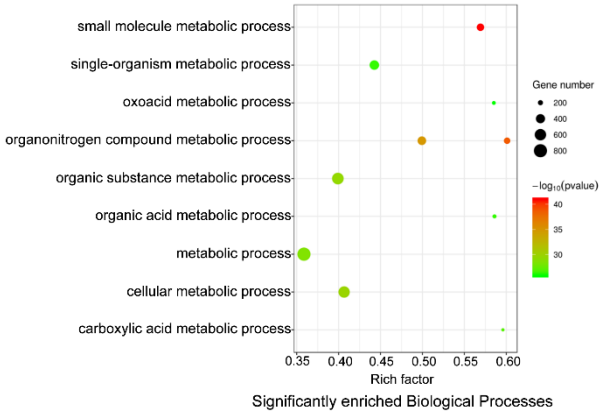

Number of peptides
Peptide length

D


Number of proteins
45.2%
22.7%
11.9%
6.1%
Number of modified sites

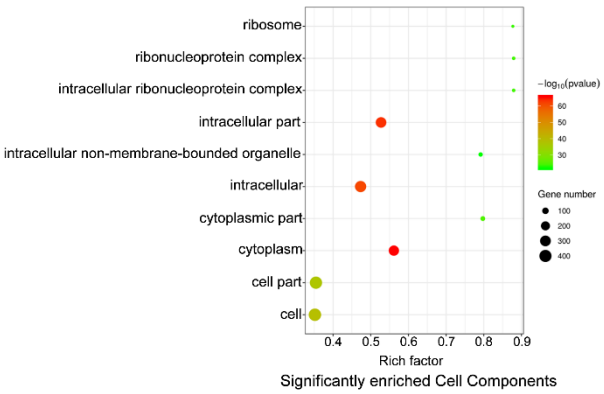
E

Fig 2



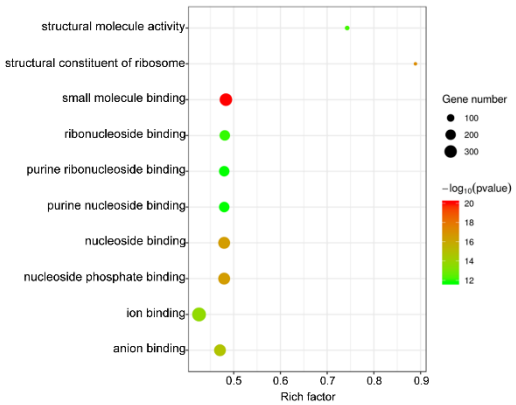
Significantly enriched Biological Processes

A



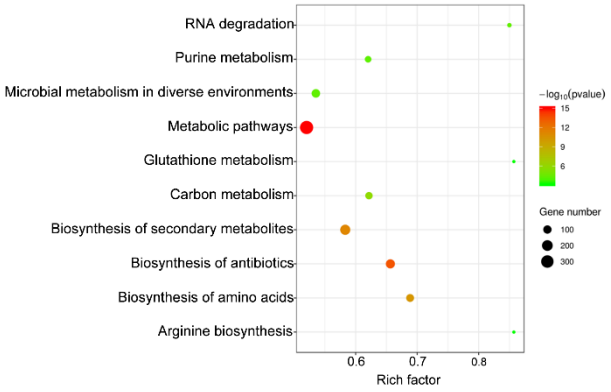
Significantly enriched Cell Components

B



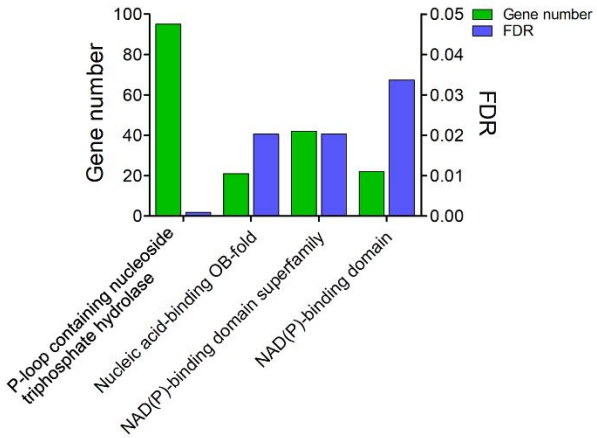
Significantly enriched Molecular Functions

C



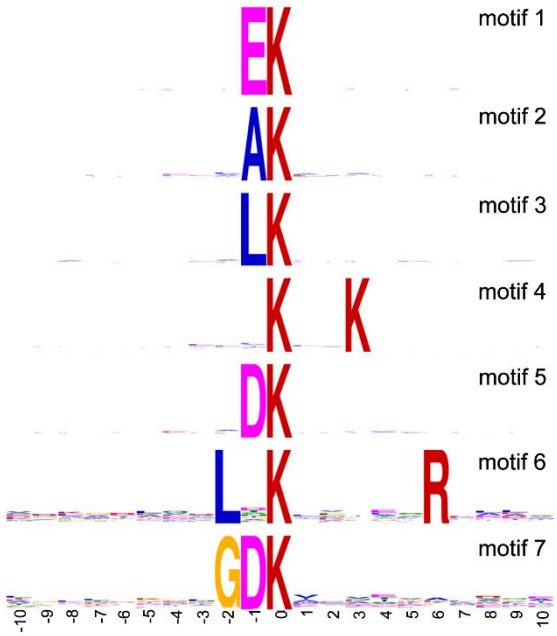
Distribution of enriched KEGG Pathway

D

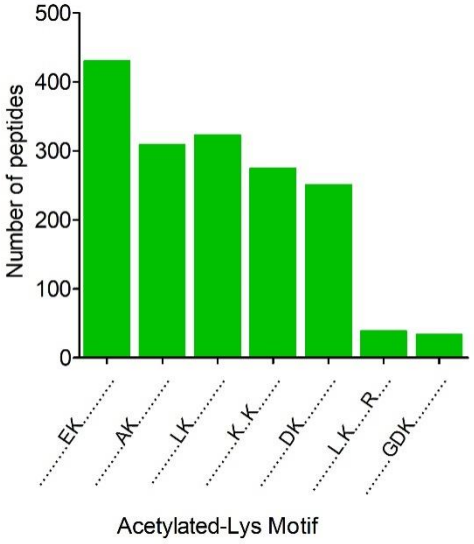


E

Fig 3


motif 1
motif 2
motif 3
motif 4
motif 5
motif 6
motif 7
-10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10

A


Number of peptides
500
400
300
200
100
0
.....EK.....
.....AK.....
.....LK.....
.....K..K.....
.....DK.....
.....L.K...R...
.....GDK.....
Acetylated-Lys Motif

B

Fig 4

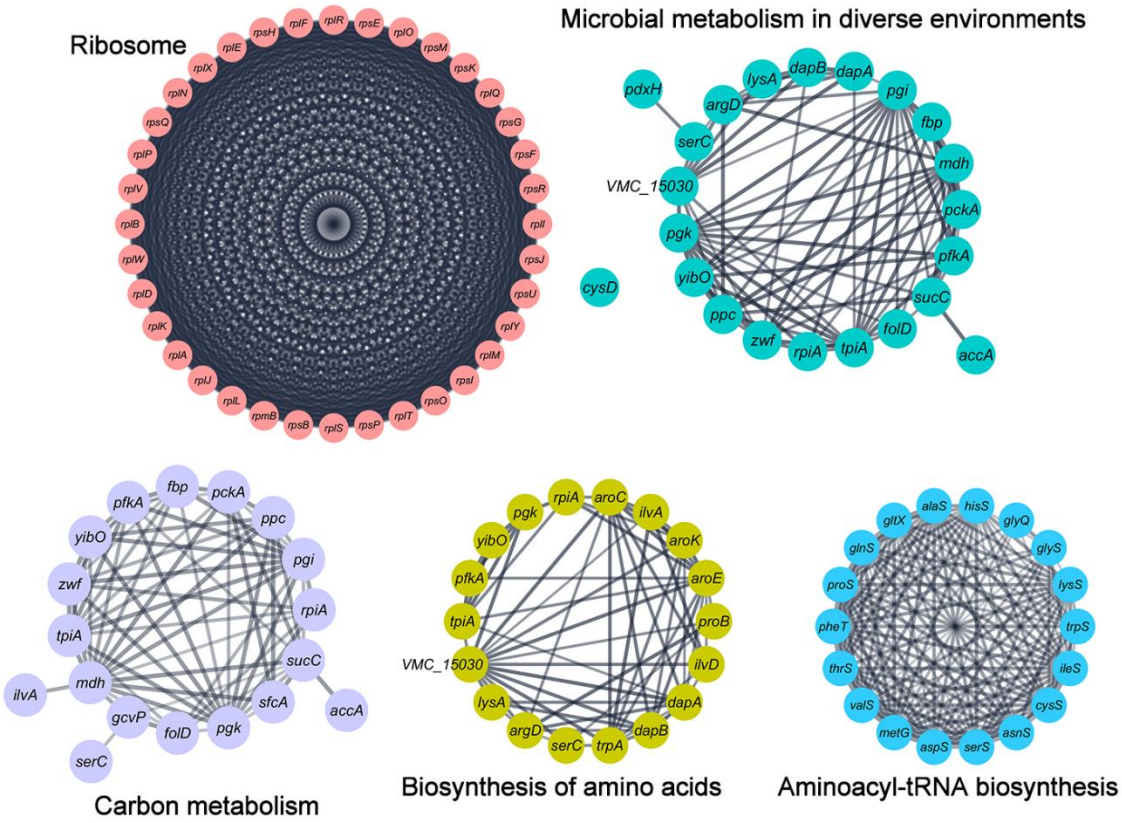

Ribosome
rplF rplR rpsE rplO rpsM rpsK rplQ rpsG rpsF rpsR rplI rpsJ rpsU rplY rplM rpsI rpsO rplT rpsP rplS rpsB rpmB rplL rplJ rplA rplK rplD rplW rplB rplV rplP rpsQ rplN rplX rplE rpsH
Microbial metabolism in diverse environments
pdxH lysA dapB dapA pgi fbp mdh pckA pfkA sucC folD accA tpiA rpiA zwf ppc yibO pgk VMC_15030 serC argD cysD
fbp pckA pfkA ppc yibO pgi zwf rpiA tpiA sucC mdh sfcA ilvA accA gcvP folD pgk serC
Carbon metabolism
rpiA aroC ilvA aroK aroE proB ilvD dapA dapB trpA serC argD lysA VMC_15030 tpiA pfkA yibO pgk
Biosynthesis of amino acids
alaS hisS glyQ glyS lysS trpS ileS cysS asnS serS aspS metG valS thrS pheT proS glnS gltX
Aminoacyl-tRNA biosynthesis

Fig 5

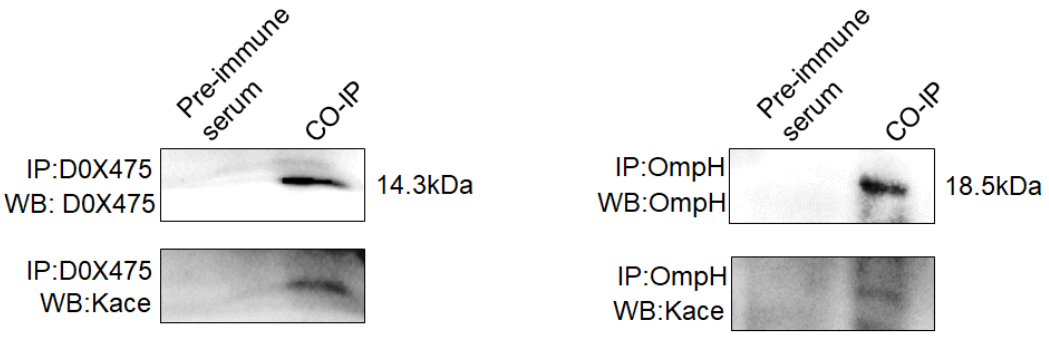

Pre-immune serum
CO-IP
IP:D0X475
WB: D0X475
14.3kDa
IP:D0X475
WB:Kace
Pre-immune serum
CO-IP
IP:OmpH
WB:OmpH
18.5kDa
IP:OmpH
WB:Kace

Fig 6

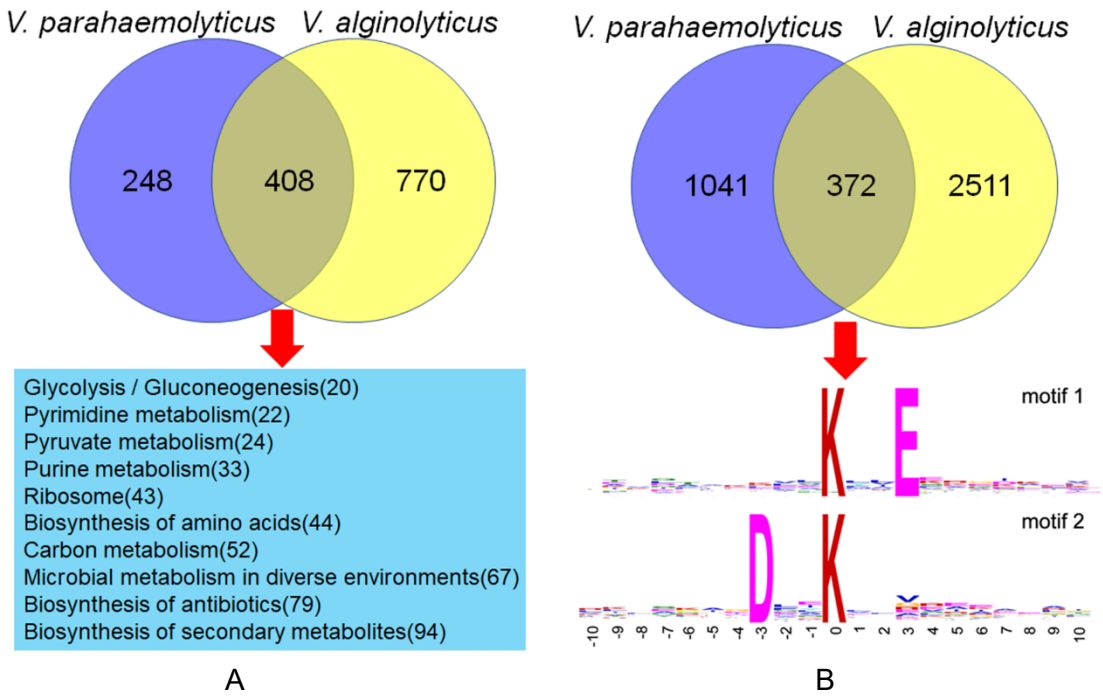
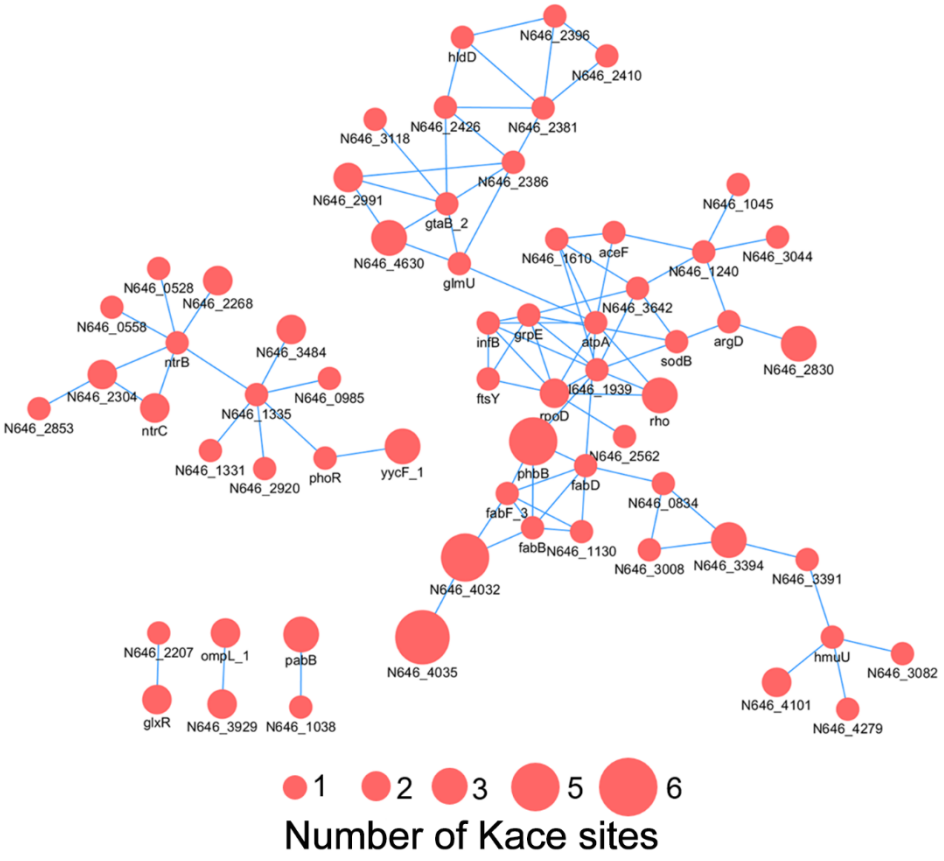

V. parahaemolyticus
V. alginolyticus
248
408
770
Glycolysis / Gluconeogenesis(20)
Pyrimidine metabolism(22)
Pyruvate metabolism(24)
Purine metabolism(33)
Ribosome(43)
Biosynthesis of amino acids(44)
Carbon metabolism(52)
Microbial metabolism in diverse environments(67)
Biosynthesis of antibiotics(79)
Biosynthesis of secondary metabolites(94)
A
V. parahaemolyticus
V. alginolyticus
1041
372
2511
motif 1
motif 2
B

Fig 7


N646_2396
hldD
N646_2410
N646_2426
N646_2381
N646_3118
N646_2386
N646_2991
gtaB_2
N646_4630
glmU
N646_1045
N646_1610
aceF
N646_3044
N646_1240
N646_0528
N646_2268
N646_0558
N646_3642
infB
grpE
atpA
argD
sodB
N646_2830
N646_3484
ntrB
N646_2304
N646_0985
N646_2853
ntrC
N646_1335
ftsY
N646_1939
rho
rpoD
N646_2562
N646_1331
N646_2920
phoR
yycF_1
phbB
fabD
N646_0834
fabF_3
fabB
N646_1130
N646_3008
N646_3394
N646_3391
N646_4032
N646_4035
N646_2207
ompL_1
pabB
hmuU
N646_3082
glxR
N646_3929
N646_1038
N646_4101
N646_4279
1
2
3
5
6
Number of Kace sites

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