Arch Microbiol
DOI 10.1007/s00203-016-1326-1

ORIGINAL PAPER

Chitin degradation and utilization by virulent *Aeromonas hydrophila* strain ML10-51K

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Received: 25 September 2016 / Revised: 28 November 2016 / Accepted: 7 December 2016

Abstract Virulent *Aeromonas hydrophila* (vAh) is one of the most important bacterial pathogens that causes persistent outbreaks of motile *Aeromonas* septicemia in warm-water fishes. The survivability of this pathogen in aquatic environments is of great concern. The aim of this study was to determine the capability of the vAh strain ML10-51K to degrade and utilize chitin. Genome-wide analysis revealed that ML10-51K encodes a suite of proteins for chitin metabolism. Assays in vitro showed that four chitinases, one chitobiase and one chitin-binding protein were secreted extracellularly and participated in chitin degradation. ML10-51K was shown to be able to use not only *N*-acetylglucosamine and colloidal chitin but also chitin flakes as sole carbon sources for growth. This study indicates that ML10-51K is a highly chitinolytic bacterium and suggests that the capability of effective chitin utilization could enable the bacterium to attain high densities when abundant chitin is available in aquatic niches.

Communicated by Erko Stackebrandt.

Electronic supplementary material The online version of this article (doi:10.1007/s00203-016-1326-1) contains supplementary material, which is available to authorized users.

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Keywords Virulent *Aeromonas hydrophila* · Chitinase · Chitinolytic enzymes · Chitin degradation · Chitin utilization

Introduction

Aeromonas hydrophila, a Gram-negative bacterium, is the causal agent of motile *Aeromonas* septicemia (MAS) in catfish and other warm-water fishes (Plumb and Hanson 2011). A severe outbreak of MAS in the Southeastern United States was reported in 2009 (Hemstreet 2010); the disease has since resulted in loss of millions of pounds of market-size catfish annually (Bebak et al. 2015; Hossain et al. 2014). Emergence of a new pathotype of virulent *A. hydrophila* (vAh) was associated with the outbreaks (Griffin et al. 2013; Hossain et al. 2014) but certain aquaculture practices also had an impact on severity of disease severity (Bebak et al. 2015). Earlier studies found that *A. hydrophila* was more abundant in waters with a high organic load than in relatively unpolluted water (Jeney and Jeney 1995) and MAS outbreaks caused by vAh in crucian carp in China occurred primarily in fish ponds with high organic loads (Nielsen et al. 2001). Since chitin is the major component of organic matter in aquatic ecosystems (Beier and Bertilsson 2011), understanding the role of chitin in vAh pathogenesis is of great importance.

Chitin is a linear chain polymer composed of β-(1, 4)-linked *N*-acetyl-D-glucosamine residues, the most abundant of which comes from exoskeletons of arthropods (mainly crustaceans) in aquatic ecosystem (Bai et al. 2016). Bacterial chitinases play a fundamental role in chitin degradation and recycling (Beier and Bertilsson 2013) and may serve as virulence factors (Frederiksen et al. 2013). Within the genus *Aeromonas*, several species of bacteria

Published online: 28 December 2016

are known to produce extracellular chitinases (Pemberton et al. 1997). Chitinases of two environmental strains of *A. hydrophila* have been partially characterized (Chen et al. 1991; Lan et al. 2006). Recently, a chitinase was identified in the extracellular proteins (ECP) produced by vAh strain ML10-51K, which was recognized by antibodies in catfish anti-ECP serum (Zhang et al. 2014). The chitinase gene derived from ML10-51K was heterologously produced in *E. coli*, and the recombinant chitinase was shown to be able to hydrolyze both chitosan and colloidal chitin (Zhang et al. 2015).

The aim of this study was to gain insight into the genome of ML10-51K with regard to chitin utilization and metabolism proteins/enzymes in order to identify chitin-binding proteins in ECP and ECP-mediated chitinolytic products and to evaluate the capability of this bacterium to use chitin as a sole carbon source.

Materials and methods

Virulent *A. hydrophila* strain ML10-51K

Aeromonas hydrophila strain ML10-51K was isolated from the kidney of a diseased channel catfish during a 2010 outbreak of MAS in west Alabama, USA. Recent investigation indicates that ML10-51K is one of the virulent strains (vAh) affiliated with sequence type 251 (ST251) described by Pang et al. (2015). The vAh strains caused significantly higher fish mortality than non-vAh isolates share >99% of average nucleotide identity values and form a monophyletic group based on core phylogenetic analysis of conserved sequences (Rasmussen-Ivey et al. 2016). The strain was deposited in fish pathogen collections at Aquatic Animal Health Research Unit, US Department of Agriculture; it is available upon request under Material Transfer Agreement.

Genome subsystem annotation and identification of chitin utilization-associated proteins

Draft genome sequencing and sequence data of vAh strain ML10-51K were described by Rasmussen-Ivey et al. (2016) with the National Center for Biotechnology Information (NCBI) accession number of SAMN05223363. Metabolic subsystems of the genome were annotated with the National Microbial Pathogen Data Resource (NMPDP)-Rapid Annotations Using Subsystems Technology (RAST) version 2.0 server. Proteins associated with chitin and *N*-acetylglucosamine (GlcNAc) metabolism, revealed from RAST analysis, were individually compared with protein databases using NCBI's BLASTp program. Additional protein sequence analysis was performed using the Vector NTI program (Invitrogen).

ECP preparation

Approximately 2×10^8 colony forming units (CFU) of ML10-51K in 100 µL stock culture thawed from −80 °C were inoculated in 100 mL tryptic soy broth (TSB; BD, Sparks, MD) and propagated at 28 °C with constant shaking at 200 rpm for 18–20 h. The culture was then subjected to centrifugation at 5000×*g* for 30 min. Resulting supernatant was filtered through a 0.22-µm filter (PES membrane) unit (Millex-GP, Merck Millipore Ltd, Tullagreen, IRL) and concentrated to a volume of approximately 500 µL using a Pierce Concentrator (PES, 20k MWCO; Thermo Scientific, Rockford, IL). The concentrated preparation was hereafter referred to ECP, the protein amount of which was approximately 3.5 µg μL^{-1} estimated using Coomassie Protein Assay Reagent (Thermo Scientific) with bovine serum albumin (BSA) as standards.

Identification of chitin-binding proteins in ECP

Colloidal chitin was used in this assay and prepared as described previously (Zhang et al. 2015). Briefly, coarse shrimp chitin flakes (Sigma) were dissolved in 12 N HCl and then mixed with cold (4 °C) ethanol and 0.1 M sodium acetate (NaAc, pH 6.2). The resulting chitin suspension was centrifuged; and the precipitate was washed four times with 0.1 M NaAc (pH 6.2). The colloidal chitin produced was suspended in 0.1 M NaAc (pH 6.2) with concentration of approximately 100 mg mL^{-1} (W/V). An aliquot of 1 mL of colloidal chitin suspension was subjected to centrifugation again and the pellet was washed three times with 0.1 M NaAc (pH 7.4) and then mixed into 400 µL of ECP described above. The mixture was incubated at 28 °C with constant shaking at 100 rpm. Following 1 h incubation, the colloidal chitin was pelleted by centrifugation and washed three times with 1 mL 0.1 M NaAc (pH 7.4). The chitin pellet was then resuspended in 1×NuPAGE LDS sample buffer (Life Technologies) and heated at 75 °C for 10 min. Following centrifugation, aliquots of the supernatant (containing chitin-binding proteins) were subjected to SDS-PAGE (see description below), along with ECP samples before and after chitin binding. Chitin-binding proteins revealed following SDS-PAGE were excised from the gel and analyzed by Tandem Mass Spectrometry (MS/MS).

Protein electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was conducted using NuPAGE 4–12% Bis–Tris precast gel and MES/SDS running buffer (Invitrogen). Following electrophoresis, the gel was stained using SimplyBlue SafeStain (Life Technologies).

Assessment of chitinolytic activity of ECP

The chitinolytic activity of ECP was assessed in following two ways: A) Whole ECP reaction—approximately 10 mg colloidal chitin was mixed with 100 µL ECP and 400 µL 0.1 M NaAc (pH 7.4); the mixture was incubated at 28 °C with shaking at 100 rpm for 24 h. B) Partial ECP reaction—the same mixture as A was prepared and incubated at 28 °C with shaking for 1 h and then was subjected to centrifugation. The supernatant labeled as unbound ECP solution was removed, and the pellet was washed three times with 1 mL 0.1 M NaAc (pH 7.4). Following washing, the pellet was resuspended in 250 µL 0.1 M NaAc (pH 7.4) and incubated at 28 °C with shaking for another 24 h. The same composite mixture without ECP served as the control. Samples of individual reactions were analyzed using TLC as described below.

Analysis of chitinolytic products

Thin-layer chromatography (TLC) was performed to analyze ECP-mediated chitinolytic products using a modified version of the method published by Bond et al. (1999). Briefly, an aliquot of 2–6 µL of samples was spotted on a TLC plate (silica gel 60; Sigma). Chromatography was developed in composite solvent (*n*-butanol:acetic acid:water = 9.0:5.5:5.5 by volume). The plate, after drying, was stained by spraying with anisaldehyde reagent (ethanol:acetic acid:sulfuric acid:anisaldehyde = 9.0:0.1:0.5:0.5 by volume) and heated at 120 °C for 5 min. A mixture of following amino sugars was used as reference standards for TLC analysis: GlcNAc (Sigma), diacetyl chitobiose [$(GlcNAc)_2$; Megazyme, Chicago, IL], triacetyl chitotriose [$(GlcNAc)_3$; Megazyme] and tetraacetyl chitotetraose [$(GlcNAc)_4$; Megazyme].

Use of GlcNAc as the sole carbon source by ML10-51K to grow

The ability of ML10-51K to use GlcNAc as the sole carbon source to grow was assessed using a minimal base medium (1×M9 minimal salt (Sigma) solution plus 2 mM $MgSO_4$ and 0.1 mM $CaCl_2$) supplemented with 0.4% GlcNAc. Approximately $5.0 \pm 0.1 \times 10^7$ CFU of ML10-51K, washed with the base medium, were inoculated to 50 mL medium plus GlcNAc, resulting in the start cell density of 1×10^6 CFU mL^{-1}. The culture in a 250-mL flask was kept at 28 °C with constant shaking at 200 rpm. An aliquot of 100 µL of the culture was sampled at 8-, 16- or 24-h intervals, and numbers of CFU were determined by conventional tenfold dilution and plating methods using tryptic soy agar (TSA) as growth medium. There were three replicate flasks for the CFU determination. Base medium supplemented with 0.4% glucose was included in this assay as positive control, while base medium without addition of a carbon source served as negative control.

Use of colloidal chitin or coarse chitin flakes as the sole carbon source

This assay was conducted using 50 mL base medium supplemented with 0.4% colloidal chitin or coarse shrimp chitin flasks (autoclaved separately). Methods of inoculation, culture and sampling were the same as described above.

Results

Chitin and GlcNAc utilization subsystem in ML10-51K

There were 29 proteins predicted to be encoded by ML10-51K genome that were associated with GlcNAc and chitin utilization as revealed by RAST analysis. The identification numbers (Chi01–Chi29), function, enzyme classification (EC) numbers and GenBank accession numbers are shown in Supplemental Table 1. Those predicted gene products associated with chitinolytic activity include: 1) four chitinases (Chi01–Chi04; EC 3.2.1.14), all of which have signal peptide cleavage sites at N-terminus predicted by SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP). Chitinases Chi01 and Chi04 are affiliated with the glycosyl hydrolase (GH) family 18, and chitinases Chi02 and Chi03 are with GH19. The amino acid lengths of Chi01–Chi04 are 1021, 869, 482 and 640, respectively, with less than 45% sequence similarities. 2) Two β-hexosaminidases, or chitobiases (Chi05 and Chi06; EC 3.2.1.52), with amino acid lengths of 887 and 622, respectively. They share about 31% sequence similarity. Both of these chitobiases are affiliated with GH20 but only Chi05 has predicted signal peptide sequence. And 3) one chitin-binding protein (Chi07). The protein consists of 474 amino acids and has a predicted signal peptide sequence.

Other proteins listed in Supplemental Table 1 contain those necessary for amino sugar metabolism, including GlcNAc-regulated methyl-accepting chemotaxis, deacetylase, deaminase, phosphatase, kinase and transporter components.

Colloidal chitin-binding proteins in ECP

Following incubation with colloidal chitin, some proteins were apparently depleted from ECP (Lanes 1 and 2, Fig. 1a); they were released from chitin particles and present in 1×NuPAGE LDS sample buffer (Lane 3, Fig. 1a). Mass spectrometric analysis revealed that four of the protein bands shown in Lane 3 of Fig. 1a had unique peptide

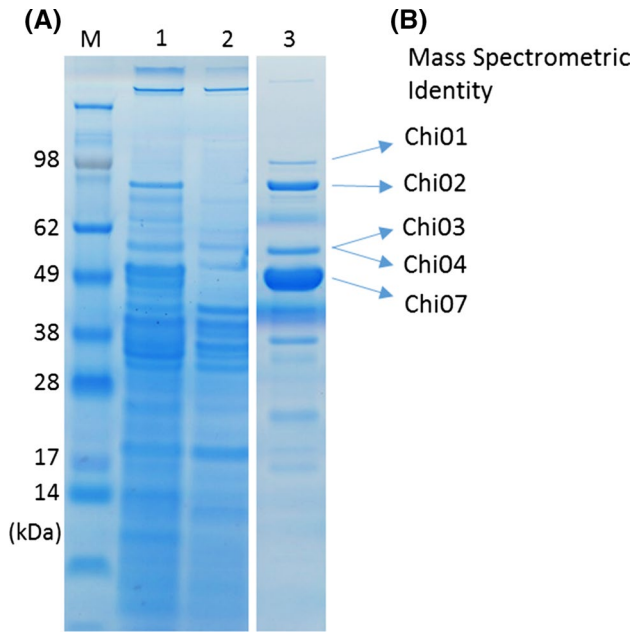

Fig. 1 SDS-PAGE and MS analyses of extracellular proteins (ECP) of ML10-51K and colloidal chitin proteins in ECP. **a** Lane 1: total ECP; Lane 2: ECP after chitin binding; Lane 3: proteins bound in chitin; and Lane M: pre-stained protein marker (Invitrogen). **b** Identity of proteins bound in chitin, revealed by mass spectrometry (details in Table 1)

sequences that matched four chitinases and one chitin-binding protein, corresponding to Chi01, Chi02, Chi03, Chi04 and Chi07 (Fig. 1b; Columns 1–4, Table 1). All of these proteins contain putative conserved domains, and all chitinases have at least one chitin-binding domain at N- or C-terminus based on NCBI's BLASTp analysis (Columns 5–7, Table 1).

Chitinolytic activity of ECP

The cell-free ECP was observed to degrade colloidal chitin and generate mostly GlcNAc and $(GlcNAc)_2$ and all amino sugars showed a characteristic orange color upon anisaldehyde staining (Fig. 2). Using whole ECP in the reaction, the major chitinolytic product was GlcNAc though some minor product was observed with Rf (retention factor) between $(GlcNAc)_2$ and $(GlcNAc)_3$ (Lane 2). When most unbound proteins in ECP were washed away following a 1-h incubation, the proteins that remained bound to chitin produced $(GlcNAc)_2$ predominantly (Lane 3). The unbound ECP solution after 1-h incubation contained some GlcNAc (Lane 4). No degradation product was seen in colloidal chitin without addition of ECP (Lane 5).

Growth of ML10-51K using GlcNAc as a sole carbon source

ML10-51K was able to rapidly proliferate in a M9 minimal salt medium supplemented with GlcNAc, showing a growth rate similar to that using glucose as the sole carbon source (Supplemental Fig. 1). At 16-h post-inoculation (HPI), the bacterium in GlcNAc medium grew as well as in glucose medium with CFU increasing from 1×10^6 to $0.71 \pm 0.05 \times 10^9$ mL^{-1}. The numbers of cells reached to a plateau at around 48 HPI in both media, with CFU of $2.22 \pm 0.14 \times 10^9$ mL^{-1}. At 96 HPI, numbers of CFU in both media leveled to approximately

Table 1 Identification, function and protein domains of chitin-binding proteins in extracellular proteins (ECP) of virulent *Aeromonas hydrophila* strain ML10-51K

Code name	MS/MS identification		Identity (#amino acids)	Protein domains (specific hits)[a]		
	Unique peptides	Sequence coverage (%)		N-terminus	Center	C-terminus
Chi01	53	66	Chitinase (1021 aa)		GH18 chitinase	ChiC/ChtBD
Chi02	75	86	Chitinase (869 aa)	ChitinaseA_N/E_set_Chitinase/PDK	GH18 chitinase	ChiC_BD/ChtBD3/CBM_5_12/PDK
Chi03	35	61	Chitinase (482 aa)		GH19 chitinase	ChiC_BD/ChtBD3/CBM_5_12
Chi04	2	16	Chitinase (640 aa)	CBM_5_12/ChiC_BD/ChtBD3	GH19 chitinase	ChiA1_BD/ChtBD3
Chi07	30	88	Chitin-binding protein (474 aa)		Chitin_bind_3	

[a] Putative conserved domains recognized by NCBI's BLASTp program

GH18 glycosyl hydrolase family 18, *GH19* glycosyl hydrolase family 19, *ChiC* C-terminus of Chitinase C, *ChitinaseA_N* chitinase A N-terminal domain, *E_set_Chitinase* N-terminal early set domain associated with the catalytic domain of chitinase, *PDK* repeats in polycystic kidney disease 1, *CBM_5_12* carbohydrate-binding domain, *ChiC_BD* chitin-binding domain (ChiC), *ChtBD3* chitin-binding domain (type 3), *ChiA1_BD* chitin-binding domain (Chi A1-like), *Chitin_bind_3* chitin-binding domain

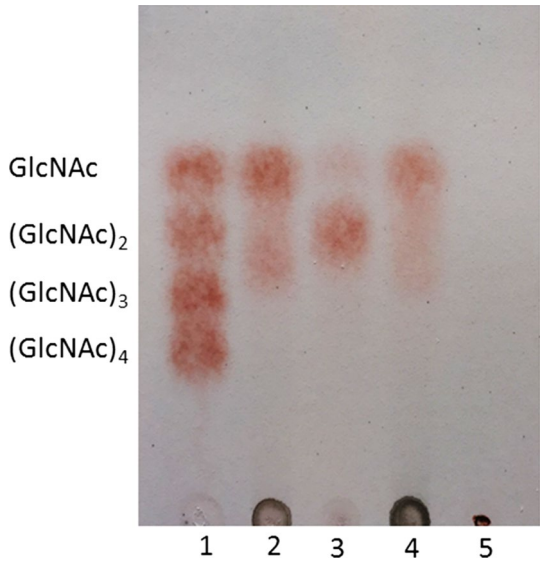

Fig. 2 Thin-layer chromatograph (TLC) of chitin degradation products catalyzed by ECP of *Aeromonas hydrophila* strain ML10-51K. Lane 1: standards including *N*-acetylglucosamine (GlcNAc), diacetyl chitobiose [$(GlcNAc)_2$], triacetyl chitotriose [$(GlcNAc)_3$] and tetraacetyl chitotetraose [$(GlcNAc)_4$]; Lane 2: chitinolytic products of whole ECP; Lane 3: chitinolytic products of chitin-binding proteins; Lane 4: chitinolytic products of partial whole ECP; and Lane 5: colloidal chitin without ECP

$2.17 \pm 0.11 \times 10^9$ mL^{-1}. Without addition of a carbon source, ML10-51K in minimal salt medium showed limited growth in first 24 h with CFU increasing to 2×10^6 mL^{-1} from 1×10^6 and no further increase was observed in next 3 days.

Growth of ML10-51K using colloidal chitin and chitin flakes as sole carbon sources

With colloidal chitin and chitin flakes as the sole carbon sources, ML10-51K grew to highest cell densities of approximately $2.22 \pm 0.08 \times 10^9$ and $2.34 \pm 0.05 \times 10^9$ CFU mL^{-1}, respectively, within 48 HPI although there was a lag in growth in chitin flake medium in the first 16 h (Supplemental Fig. 2). After 48 h, the number of CFU in colloidal chitin medium declined to $1.95 \pm 0.03 \times 10^9$ mL^{-1} in the next 24 h and then maintained at the same level through 96 HPI. In chitin flake medium, the highest CFU remained from 48 to 96 HPI.

Discussion

This study revealed that *Aeromonas hydrophila* strain ML10-51K is equipped with a suite of proteins (enzymes) that enable the utilization of chitin as a growth substrate. This capability would make it possible for the bacterium to maintain high population densities when abundant chitin is available in aquatic ecosystems. The ability to use chitin could promote the persistence and growth of vAh and may also be important in their production of virulence factors which could enhance their pathogenesis in fish.

The efficiency in chitin utilization could be due to multiple genes coding for chitinases in the vAh genome. As predicted by the signal peptide sequence, all four chitinases produced were secreted in ECP and participated in chitin binding, which is an indication of synergistic enzyme interaction for efficient chitin use (Svitil et al. 1997; Beier and Bertilsson 2013). The four chitinases (Chi01–Chio4) of ML10-51K possess different functional domains, suggesting that they have differential action sites against insoluble, densely packed chitin. According to Vaaje-Kolstad et al. (2013), three chitinases attack chitin differentially in chitinolytic system of *Serratia marcescens*: ChiA degrades chitin chain from the reducing end and ChiB from the non-reducing end, while ChiC makes random cuts in the more amorphous regions and leaves openings for ChiA and ChiB. In addition, a chitin-binding protein, CBP21, makes oxidative cuts in the most crystalline regions and forms aldonic acid ends. For the four chitinases of ML10-51K, it is currently unknown how each individual enzyme degrades chitin due to lacking of crystallographic data, but the end products catalyzed by these chitinases are similar to those produced by chitinases of *S. marcescens*, i.e., mostly $(GlcNAc)_2$. This was demonstrated in TLC analysis of products generated by proteins bound to colloidal chitin.

$(GlcNAC)_2$ generated by chitinases are converted to GlcNAc by β-hexosaminidase or chitobiase. In ML10-51K, there are two chitobiases with one being a secrete form and the other non-secrete form as predicted by signal peptide sequence. Since predominant product in the whole ECP reaction was GlcNAc, there must be a chitobiase secreted in the ECP and participated in chitinolysis. The chitobiase was apparently not bound in the chitin since it was not found in proteins bound to chitin (SDS-PAGE gel) and its activity was only present in the whole ECP reaction and unbound ECP solution. The non-secreted chitobiase is most likely present in the periplasmic space where it processes $(GlcNAc)_2$ taken by the bacterium from extracellular activity since several $(GlcNAc)_2$ ABC (ATP-binding cassette) transporters were predicted to be encoded by ML10-51K. Additionally, the chitin-binding protein (Chi07) found in ECP may have the same function as CBP21 (Vaaje-Kolstad et al. 2013) mentioned above. The product observed in the whole ECP reaction with Rf between $(GlcNAc)_2$ and $(GlcNAc)_3$ could be an oxidized form of $(GlcNAc)_2$ with an aldonic acid function group, which resulted in the small Rf observed by TLC.

With its array of chitinolytic enzymes, ML10-51K was shown to be able to use the chitin degradation product,

GlcNAc, as the sole carbon source for growth. The overall growth rate in GlcNAc medium was similar to that in glucose medium. The cell density increased more than 3 orders of magnitude within 24 h. Furthermore, the bacterium grew fast on insoluble chitin as well. The cells proliferated by approximately 2.8 and 2.1 orders of magnitude in 24 h in colloidal chitin and chitin flake media, respectively. The lower counts of CFU between 16 and 24 h in chitin flake medium were probably because most inoculated bacteria were absorbed/attached on chunks of chitin flakes (most surface area ≥ 5 mm^3). As in GlcNAc medium, the highest numbers of CFU counts were observed at 48 HPI in both colloidal chitin and chitin flake media. Following slightly decline, cell densities kept as high as $2.17 \pm 0.13 \times 10^9$ CFU mL^{-1} in the next 48 h. Approximately more than 95% chitin flakes were visibly diminished at day 9, and particles of colloidal chitin were not visible at 48 HPI (data not shown).

Results of this study indicate that ML10-51K, a strain of new virulent pathotype of *A. hydrophila*, is a highly chitinolytic bacterium. It is unknown but worth considering whether the persistent outbreaks of MAS disease in catfish aquaculture is due to the pathogen's survivability on the readily available chitin substrate in aquatic ecosystems, especially with the organic load characteristic of farmed fish ponds. Hence, chitin utilization could maintain vAh at high population levels and promote the development of MAS outbreaks. Additionally, some bacterial chitinases were reportedly involved in virulence and pathogenesis through modulation of host innate immunity (Chaudhuri et al. 2013; Mondal et al. 2014) and other bacterial chitinases and chitin-binding proteins may play a role in cell adhesion to the host mucosal tissues (Tran et al. 2011). Further research will investigate the contribution of these chitinolytic proteins to vAh pathogenesis.

In conclusion, virulent *A. hydrophila* ML10-51K was demonstrated to be highly chitinolytic. Apart from intracellular chitin metabolic enzymes, there are four chitinases, one chitobiase and one chitin-binding protein that were found to be secreted extracellularly and to be participated in chitin degradation. The pathogen can not only use GlcNAc and colloidal chitin but also crystalline chitin flakes as the sole carbon source for growth. Whether this survival trait is associated with persistent outbreaks of MAS in fish aquaculture will be investigated to provide new routes for the disease prevention.

Acknowledgement Dr. Junqiang Qiu was a visiting scientist, sponsored by special funds for the Development of Science & Technology of Shanghai Ocean University, to USDA-ARS Aquatic Animal Health Research Unit. This research was supported by USDA-ARS CRIS Project #6010-32000-026-00D. All authors certify that there is no conflict of interest with any financial/research/academic organization.

References

Bai Y, Eijsink VGH, Kielak AM, van Veen JA, de Boer W (2016) Genomic comparison of chitinolytic enzyme systems from terrestrial and aquatic bacteria. Environ Microbiol 18:38–49

Bebak J, Wagnerb B, Burnesc B, Hanson T (2015) Farm size, seining practices, and salt use: risk factors for *Aeromonas hydrophila* outbreaks in farm-raised catfish, Alabama, USA. Prev Vet Med 118:161–168

Beier S, Bertilsson S (2011) Uncoupling of chitinase activity and uptake of hydrolysis products in freshwater bacterioplankton. Limnol Oceanogr 56:1179–1188

Beier S, Bertilsson S (2013) Bacterial chitin degradation—mechanisms and ecophysiological strategies. Front Microbiol 4:149. doi:10.3389/Fmicb.2013.00149

Bond DR, Tsai BM, Russell JB (1999) Physiological characterization of *Streptococcus bovis* mutants that can resist 2-deoxyglucose-induced lysis. Microbiology 145:2977–2985

Chaudhuri S, Gabter BN, Ye RD, Cianciotto NP, Freitag NE (2013) The *Listeria monocytogenes* ChiA chitinase enhances virulence through suppression of host innate immunity. mBio 4(2):e00617-12. Doi:10.1128/mBio.00617-12

Chen JP, Nagayama F, Chang MC (1991) Cloning and expression of an *Aeromonas hydrophila* chitinase gene in *Escherichia coli*. Appl Environ Microbiol 57:2426–2428

Frederiksen RF, Paspaliari DK, Larsen T, Storgaard BG, Larsen MH, Ingmer H, Palcic MM, Leisner JJ (2013) Bacterial chitinases and chitin-binding proteins as virulence factors. Microbiology 159:833–847

Griffin MJ, Goodwin AE, Merry GE, Liles MR, Williams MA, Ware C, Waldbieser GC (2013) Rapid quantitative detection of *Aeromonas hydrophila* strains associated with disease outbreaks in catfish aquaculture. J Vet Diagn Invest 25:473–481

Hemstreet W (2010) An update on *Aeromonas hydrophila* from a fish health specialist for summer 2010. Catfish J 24:4

Hossain MJ, Sun DW, Mcgarey DJ, Wrenn S, Alexander LM, Martino ME, Xing Y, Terhune JS, Liles MR (2014) An Asian origin of virulent *Aeromonas hydrophila* responsible for disease epidemics in United States-Farmed Catfish. MBio 5(3):e00848-14. doi:10.1128/mBio.00848-14

Jeney Z, Jeney G (1995) Recent achievements in studies on diseases of common carp (*Cyprinus carpio* L.). Aquaculture 129:397–420

Lan X, Zhang X, Hu J, Shimosaka M (2006) Cloning, expression, and characterization of a chitinase from the chitinolytic bacterium *Aeromonas hydrophila* strain SUWA-9. Biosci Biotechnol Biochem 70:2437–2442

Mondal M, Nag D, Koley H, Saha DR, Chatterjee NS (2014) The *Vibrio cholera* extracellular chitinase ChiA2 is important for survival and pathogenesis in the host intestine. PLoS ONE 9(9):e103119. doi:10.1371/journal.pone.0103119

Nielsen ME, Hoi L, Schmidt AS, Qian D, Shimada T, Shen JY, Larsen JL (2001) Is *Aeromonas hydrophila* the dominant motile *Aeromonas* species that causes disease outbreaks in aquaculture production in the Zhejiang province of China? Dis Aquat Org 46:23–29

Pang M, Jiang J, Xie X, Wu Y, Dong Y, Kwok AH, Zhang W, Yao H, Lu C, Leung FC, Liu Y (2015) Novel insights into the pathogenicity of epidemic *Aeromonas hydrophila* ST251 clones from comparative genomics. Sci Rep 5:9833. doi:10.1038/srep09833

Pemberton JM, Kidd SP, Schmidt R (1997) Secreted enzymes of *Aeromonas*. FEMS Microbiol Lett 152:1–10

Plumb JA, Hanson LA (2011) Health maintenance and principal microbial diseases of cultured fishes, 3rd edn. Wiley-Blackwell, Ames, IA, p 492

Rasmussen-Ivey CR, Hossain MJ, Odom SE, Terhune JS, Hemstreet WG, Shoemaker CA, Zhang D, Xu D-H, Griffin MJ, Liu Y-J, Figueras MJ, Santos SR, Newton JC, Liles MR (2016) Classification of a hypervirulent *Aeromonas hydrophila* pathotype responsible for epidemic outbreaks in warm-water fishes. Front Microbiol 7:1615. doi:10.3389/fmicb.2016.01615

Svitil AL, Chadhain SMN, Moore JA, Kirchman DL (1997) Chitin degradation proteins produced by the marine bacterium *Vibrio harveyi* growing on different forms of chitin. Appl Environ Microbiol 63:408–413

Tran HT, Barnich N, Mizoguchi E (2011) Potential role of chitinases and chitin-binding proteins in host-microbial interactions during the development of intestinal inflammation. Histol Histopathol 26:1453–1464

Vaaje-Kolstad G, Horn SJ, Sørlie M, Eijsink GH (2013) The chitinolytic machinery of *Serratia marcescenes* —a model system for enzymatic degradation of recalcitrant polysaccharides. FEBS J 280:3028–3049

Zhang D, Pridgeon JW, Klesius PH (2014) Vaccination of channel catfish with extracellular products of *Aeromonas hydrophila* provides protection against infection by the pathogen. Fish Shellfish Immunol 36:270–275

Zhang D, Bland JB, Xu D, Chung S (2015) Degradation of chitin and chitosan by recombinant chitinase derived from a virulent *Aeromonas hydrophila* isolated from diseased channel catfish. AiM 5:611–619. doi:10.4236/aim.2015.59064