International Journal of Agricultural Technology 2020 Vol. 16(3): 749-760
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Species diversity and existence of virulence genes in clinical *Aeromonas* spp. causing motile *Aeromonas septicemia* (MAS) isolated from cultured Nile tilapia (*Oreochromis niloticus*)

U-taynapun, K., Nganwisuthiphan, T. and Chirapongsatonkul, N.*

Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat, Thailand.

U-taynapun, K., Nganwisuthiphan, T. and Chirapongsatonkul, N. (2020). Species diversity and existence of virulence genes in clinical *Aeromonas* spp. causing motile *Aeromonas septicemia* (MAS) isolated from cultured Nile tilapia (*Oreochromis niloticus*). International Journal of Agricultural Technology 16(3): 749-760.

Abstract Motile *Aeromonas septicemia* (MAS) is an emerging major disease in Nile tilapia (*Oreochromis niloticus*) caused by virulent clonal isolates of *Aeromonas* spp. including *A. hydrophila*, *A. veronii*, *A. veronii* biovar *sobria* and *A. jandaei*, aquaculture in Thailand. In this study, we investigated the species diversity and existence of virulence genes of *Aeromonas* spp. by analyzing 5 MAS incidences which occurred in the different areas (10 *Aeromonas* isolates from each case). According to the partial *16S rRNA* sequence and phylogenetic analysis, 50 tested isolates were assigned to 4 different species with *A. veronii* or *A. veronii* biovar *veronii* as a dominant species (39 isolates, 78%), followed by *A. hydrophila* (6 isolates, 12%), *A. veronii* biovar *sobria* (3 isolates, 6%) and *A. jandaei* (2 isolates, 4%) respectively. Among the 39 confirmed that *A. veronii* isolates were the most prevalently virulence genes that were *elastase* and *enolase* (100%), and followed by *alt* (32 isolates, 82.05%), *hly/aerA* (24 isolates, 61.54%) and *lipase* (18 isolates, 46.15%). This study demonstrated the currently 4 major pathogenic species of *Aeromonas* in Nile tilapia reared in Thailand. Moreover, it suggested that *A. veronii* is the major pathogen that causes MAS and is widely distributed in this area.

Keywords: *Aeromonas veronii*, Motile *Aeromonas septicemia*, Nile tilapia, Species diversity, Virulence genes

Introduction

Nile tilapia (*Oreochromis niloticus*) and other related cultured tilapia such as red hybrid tilapia/tubtim and *Oreochromis* spp. are the most important freshwater fishes in Thailand and worldwide due to its high production and consumer demands. Recently, the production of Nile tilapia in Thailand has increased with approximately 200,000 tons per year (Srisapoome and Areechon, 2017). The production volume made Thailand to be one of the top producers worldwide in 2017. However, behind the rapid development of tilapia farming, high mortality due to disease outbreaks has become a serious issue. *Aeromonas*

***Corresponding Author:** Chirapongsatonkul, N.; **Email:** nim_nion@hotmail.com, nion.c@rmutsv.ac.th

is an important bacterial pathogen in Nile tilapia culture especially for nursery and grow-out fish. The genus *Aeromonas* is a member of the Gammaproteobacteria class along with *Vibrio*, *Pseudomonas* and *Plesiomonas* that cause diseases in aquatic animals (Smith *et al*., 2012). *Aeromonas* bacterium is Gram-negative, oxidase-positive, facultatively anaerobic and short-rod which maybe non-motile, motile, mesophilic and psychrophilic (Dallaire-Dufresne *et al*., 2014). Motile *Aeromonas septicemia* (MAS) is an acute or chronic infectious disease of Nile tilapia caused by *Aeromonas* spp. The causative species mostly are *A. hydrophila*, *A. veronii*, *A. veronii* biovar *sobria*, *A. jandaei* and *A. shuberti* (Dong *et al*., 2017a; Chirapongsatonkul *et al*., 2018; 2019; Liu *et al*., 2018; U-taynapun *et al*., 2018). Fish farmers and published knowledge mostly revealed the association of MAS and *A. hydrophila* infection. However, this disease is highly diverse among disease causing species and is different among niches of tilapia culture. A significant species of *Aeromonas* associated with MAS in tilapia has taxonomically identified but the proof of prevalent *Aeromonas* species is still scarce.

Disease severity has been reported to be associated with virulence factors of pathogens including those related to the replication, invasion and evasion mechanisms. The pathogenicity of *Aeromonas* spp. infection is multifactorial involving several virulence factors and toxins produced by these bacterial pathogens. Virulence factors are asscosiated with primary and secondary metabolites including antigen, membrane protein (S-layer), enterotoxins, cytotoxins, exoenzymes, and type III secretion system which are able to adhere to and invade host cell (Oliverira *et al*., 2012). Li *et al*. (2011) have reported that genes encoding for aerolysin (*aerA*), cytotoxic enterotoxin (*alt*) and serine protease (*ahp*) are more frequent virulence genotype in *A. hydrophila* isolates obtained from clinical diseased specimens than those from healthy fish. The occurrence of *aerA*, *alt* and *ahp* can be used for virulence typing of *Aeromonas* isolates. Enolase is a glycolytic enzyme which involved in catalyzation of the reversible conversion of 2-phosphoglycerate (2-PGE) to phosphoenolpyruvate (PEP). Enolase has been implicated for pathophysiological processes by modulating the heat shock protein gene transcription. Moreover, its involvement in microbial diseases contributes by interaction with plasminogen in host plasma and extracellular fluid (Sha *et al*., 2009).

The objective was to investigate the *Aeromonas* spp. diversity and occurrence of virulence genes pointed by aforementioned literatures (*lipase*, *elastase*, *enolase*, *hly/aerA*, and *alt*) in *Aeromonas* isolates obtained from MAS diseased Nile tilapia cultured in Thailand.

Materials and Methods

Fish samples and bacterial isolation

MAS-exhibiting moribund Nile tilapia were collected from 5 tilapia rearing areas in Thailand including Suphanburi Province (SP), Phetchaburi Province (PC), Chachoengsao Province (CC), Nakhon Si Thammarat Province (NK) and Surat Thani Province (ST) (Figure 1) between 2018 and 2019. Gross pathology was considered according to Hassan *et al*. (2017). For bacterial pathogen isolation, sample surface was disinfected with 70% (v/v) ethanol and aseptically dissected. *Aeromonas* isolation was conducted using *Aeromonas* selective medium (BSIBG agar, Himedia). Bacterial specimens were collected from the brain, liver and kidney of the diseased fish. Representative of selected *Aeromonas* colonies were purified by cross streak on Tryptic Soy Agar (TSA, Difco). All pure bacterial isolates were stocked in Tryptic Soy Broth (TSB, Difco) containing 15% (v/v) glycerol and kept at -80°C until use.

Figure 1. Sampling locations of MAS-expressing tilapia in Thailand

Bacterial strains and characterization

Fifty pure bacterial isolates were biochemically identified as members of the genus *Aeromonas* by randomly selected from a large collection of 265

Aeromonas isolates from all incidences. Ten isolates were obtained from each area.

Each isolate of the randomly selected *Aeromonas* was cultured on TSA at 35°C for 24 h. Bacterial morphology was classified by direct examination under a light microscope after staining with standard Gram stain method. The oxidase test was determined by tetramethyl-*p*-phenylenediamine dihydrochloride following the method of Choopun *et al*. (2002). Other phenotypic tests were performed using API 20 E kit (BioMérieux) following the manufacturer's instruction.

For bacterial identification based on the molecular aspect, all test *Aeromonas* strains were cultured and extracted for DNA using the Bacteria DNA Isolation Kit (Geneaid) according to the manufacturer's instruction. The concentration of DNA was checked by measuring the absorbance at 260 nm. The guanidine contaminant was determined by calculating the ratio of absorbance at 260 nm and at 230 nm (260/230). The contaminated protein in the extracted DNA was determined by calculating the ratio of absorbance at 260 nm and at 280 nm (260/280). The guanidine contaminant and DNA purity ratio of 1.8−2.0 is accepted as good DNA quality. DNA products were stored at -80°C until use. The obtained DNAs were used as the template for *16S rRNA* amplification performed under the manufacturer's instruction of EmeraldAmp® GT PCR master mix (Takara). The PCR condition was set as follows: 95°C for 5 min, 35 cycles of 95°C for 45 s, 60°C for 30 s, 72°C for 1 min and final extension at 72°C for 5 min. The detail of primers used for *16S rRNA* amplification are shown in Table 1. PCR products were purified by using Gel/PCR DNA Fragments Kit (Geneaid) following the manufacturer's protocol. The purified DNA fragment of each isolated was cloned into pGEM-T® Easy Vector (Promega). All *16S rRNA* gene of the test *Aeromonas* spp**.** were analyzed compared to those of other *Aeromonas* spp**.** available in the GenBank using nucleotide BLAST tool (Altschul *et al*., 1990). The maximum likelihood phylogenetic trees were reconstructed using MEGA X **(**Kumar *et al*., 2018) with Kimura 2-parameter model. Maximum likelihood (ML) method was conducted to infer phylogenetic relationships among the *Aeromonas* spp. and its sister group. The bootstrap analysis was perfromed with 1,000 replicates and values below 50% were excluded as non-significance. The *16S rRNA* of *Bacillus subtilis* (NR_112116.2) and *B. licheniformis* (EF113327.1) were used as an out-group species in phylogenetic analysis.

Determination of virulence factors

The DNA of all 50 *Aeromonas* isolates were examined for the existence of virulence genes, *elastase, enolase, alt, hly/aerA*, and *lipase* using PCR

technique through specific primers shown in Table 1. PCR reactions for all test virulence genes were performed following the manufacturer's protocol (AccuStart II PCR ToughMix, Quantabio). The thermocycling condition of PCR was 95°C for 3 min followed by 35 cycles of 95°C for 45 s, annealing at the optimum temperature for the test genes (60°C for *elastase, enolase* and *alt*; 58°C for *lipase*; 55°C for *hly/aerA*) for 30 s, 72°C for 30 s and final extension at 72°C for 3 min. The PCR products were analyzed on 2% (w/v) agarose gel electrophoresis stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen) and visualized through a LED transilluminator.

Table 1. Primer used in this study

Gene	Primer name	Sequence	Product size (bp)	References
16S rRNA	20F	5´-AGAGTTTGATCATGGCTCAG-3´	~1,500	Weisburg *et al.* (1991)
	1500R	5´-CGGTTACCTTGTTACGACTT-3´		
elastase	Elas-F	5´-CGACATGTACCGCAACTGGTA-3´	466	Chirapongsatonkul *et al.* (2018)
	Elas-R	5´-GGTGTTGGCCAGCAGGTA-3´		
enolase	Enol-F	5´-CGACGGTACCGAGAACAAA-3´	212	Chirapongsatonkul *et al.* (2018)
	Enol-R	5´-CTTGGATGTCGACGTTGTTG-3´		
alt	Alt-F1	5´-AGGATGCCCTCAACACCATC-3´	272	Chirapongsatonkul *et al.* (2018)
	Alt-R1	5´-GCTCTGTTTCAGGTTGTCGC-3´		
hly/aerA	hly/aerA-F	5´-GAAGGTGACCACCAAGAACAA-3´	409	This study
	hly/aerA-R	5´-CCAGTCCCACCACTTCACTT-3´		
lipase	Lip-F	5´-GACTCCCTCAAGGACAGCAG-3´	594	Chirapongsatonkul *et al.* (2018)
	Lip-R	5´-AGAGGCTTTCAGGGCATTG-3´		

Results

Bacterial isolation

In the present study, samples were collected from the 13 cases of MAS in Nile tilapia that occurred between 2018 and 2019**.** The clinical signs of infected fish included external signs of eroded fins, epidermal ulcers and hemorrhagic ulcers**.** Pale and hemorrhagic liver and abdominal swelling with visceral liquid was observed as internal signs after necropsy**.** *Aeromonas* colonies isolated from the brain, kidney and liver of infected tilapia grew well in *Aeromonas* selective medium after incubation 24 h at 35°C and showed green translucent colony. The morphological characterization based on Gram-stain of all bacteria growing after subculture showed that they are Gram-negative with shot-rod shape. Fifty isolates (10 from each incidence area) were randomly selected and subjected to further phenotypic and phylogenetic analysis. The number of MAS cases in each location as well as number of *Aeromonas* spp. is present in Table 2.

Table 2. Number of cases and *Aeromonas* spp. collected from 5 sampling locations

Sampling locations	Number of cases	Number of infected Nile tilapia	Total *Aeromonas* collection	Selected *Aeromonas* isolation
Suphanburi	2	20	41	10
Chachoengsao	3	28	46	10
Phetchaburi	2	18	38	10
Surat Thani	3	31	63	10
Nakhon Si Thammarat	3	35	68	10
Total	13	130	256	50

Biochemical characteristics of Aeromonas spp. from infected tilapia

API 20 E was designed for classification of a member of the family Enterobacteriaceae. The characterization of all 50 *Aeromonas* isolates in this study and reference strains based on biochemical characteritics and the results obtained from the API 20 E are presented in Table 3. There were 3 groups of the test *Aeromonas* divided according to the biochemical characteristics. Six isolates (12%) of *Aeromonas* biogroup I (Bio-Gr. I) showed positive fermentation of AMY and ARA; however, it showed negative fermentation in biogroup II and III (Bio-Gr. II and Bio-Gr. III). Based on API 20 E, *Aeromonas* isolates in Bio-Gr. I were classified in a group of *Aeromonas hydrophila/caviae/sobria* 1 with 99.7%. Negative of lysine decarcoxylase and positive of inositol fermentation were different result between Bio-Gr. II and III. The majority of the test *Aeromonas* in this study, 42 from 50 isolates or 84%, were Bio-Gr. II. The members of Bio-Gr. II were classified as *Aeromonas hydrophila/caviae/sobria* 2 with 89.6% . Only 2 isolates (4%) were grouped as Bio-Gr. III that was classified in a group of *Aeromonas hydrophila/caviae/sobria* 1 with 88.6% .

Phylogenetic analysis

Due to the doubtful species classification using biochemical descriptions, phylogenetic study of the tested *Aeromonas* strains documented in this study were reconstructed via *16S rRNA* gene. Sequence alignment of *16S rRNA* gene of 50 test *Aeromonas* indicated that there were 4 groups of genetic sequence pattern; Bio-Gr. I, Bio-Gr. II-I, Bio-Gr. II-II, and Bio-Gr. III (Figure 2). Within the major group Bio-Gr. II, it could be divided into 2 sub-groups, Bio-Gr. II-I and Bio-Gr. II-II with a member of 3 and 39, respectively.

Table 3. Biochemical characteristics of the test *Aeromonas* spp. (50 isolates) using API 20 E

Biochemical characteristics	**Isolated bacteria**			**Reference strains**		
	***Aeromonas* Bio-Gr. I**	***Aeromonas* Bio-Gr. II**	***Aeromonas* Bio-Gr. III**	***A. hydrophila*[1]**	***A. veronii* biovar *sobria*[2]**	***A. jandaei*[3]**
Gram	Negative	Negative	Negative	Negative	Negative	Negative
Bacteria morphology	Shot-rod	Shot-rod	Shot-rod	Shot-rod	Shot-rod	Shot-rod
ONPG	+	+	+	+	+	+
ADH	-/+	-/+	+	+	+	+
LDC	+	+	-	+	+	+
ODC	-	-	-	-	-	-
Cirate utilization	-/+	+	-	+	+	-
H_2S production	-	-	-	-	+	+
Urease	-	-	-	-	-	-
TDA	-	-/+	-	+	-	-
Indole production	+	+	+	+	+	+
VP	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+
Glucose fermentation	+	+	+	+	+	+
Mannital fermentation	+	+	+	+	+	+
Inositol fermentation	-	-	+	-	-	-
Sorbital fermentation	-	-	-	-	-	-
Rhamnose fermentation	-/+	-	-	-	-	-
Sucrose fermentation	+	+	+	+	+	-
Melibiose fermentation	-	-	-	+	-	-
Amygdaline fermentation	+	-	-	+	-	+
Arabinose fermentation	+	-	-	+	-	+
Oxidase	+	+	+	+	+	+

Note: (+) Positive reaction, (-) Negative reaction Abbreviations**:** ONPG, *o*-nitrophenyl-b-D-galactopyranoside; ADH, arginine dihydrolase; LDC, lysine decarboxylase; ODC, ornithine decarboxylase; TDA, tryptophan deaminase; VP, Voges-Proskauer

1/: A reference *A. hydrophila* is described by Nahar *et al*. (2016)

2/: A reference *A. veronii* biovar *sobria* is described by Awan *et al*. (2005)

3/: A reference *A. jandaei* is described by Awan *et al*. (2005)

The DNA fragments of approximately 1,450 bp in length of the studied *Aeromonas* isolates were compared with other strains that are deeply embedded in *Aeromonas* spp**.** group and other species in Gammaproteobacteria**.** Phylogenetic analysis conducted using the ML algorithm resulted in a single most parsimonious tree (Figure 2). The clades of *Aeromonas* species were divided into 3 main clades that were strongly supported by bootstrap values. Phylogenetic topology analysis tree demonstrated that the Bio-Gr**.** I is placed in the clade of *A***.** *hydrophila*. Bio-Gr**.** II-I and Bio-Gr**.** II-II are shown in the clade

of *A. veronii* biovar *sobria* and *A. veronii* biovar *veronii*, respectively. Bio-Gr. III is associated with the clade of *A. jandaei*. Phylogram was separated into a distinct clade strongly supported by high bootstrap values. Species classification of *Aeromonas* spp. collected from 5 tilapia culture areas is summarized in Table 4.

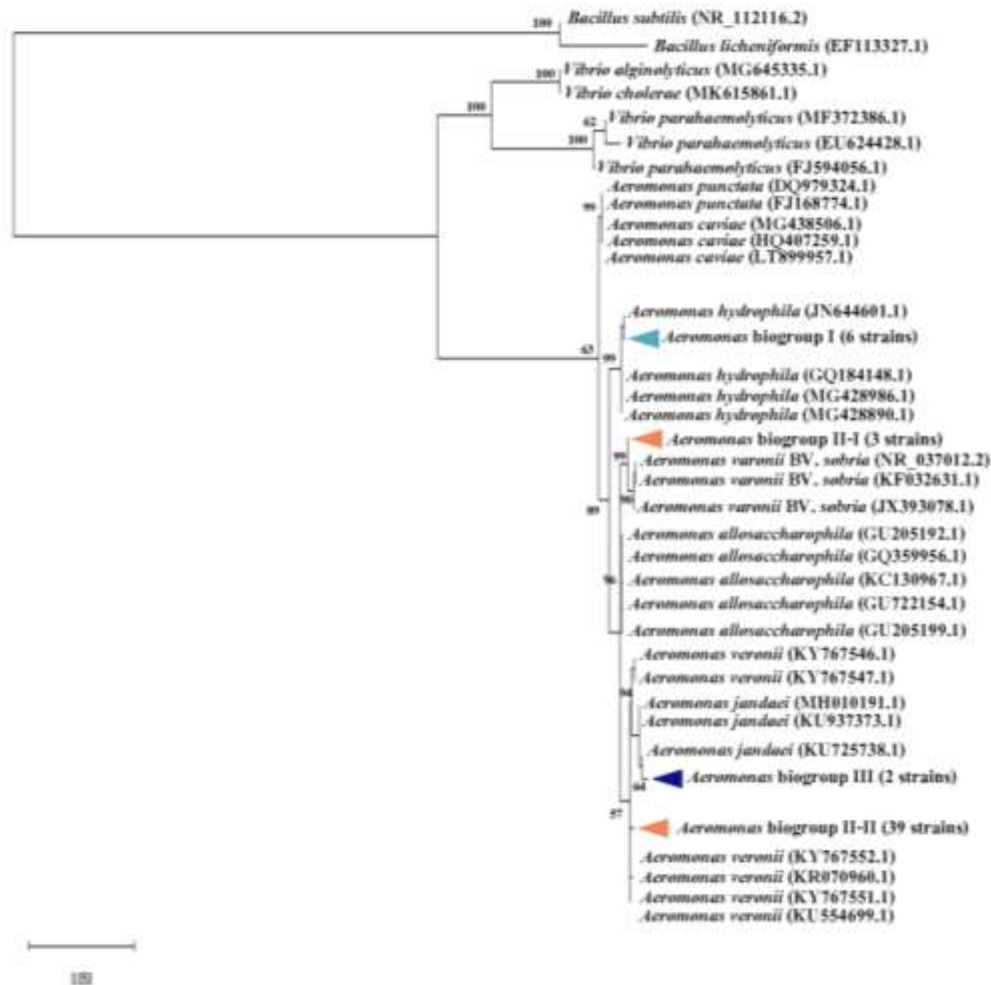

Figure 2. Phylogenetic analysis by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-4,728.58) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any

position. There were a total of 1,389 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018).

Table 4. Species classification of the isolated *Aeromonas* spp. from 5 tilapia culture areas

Species	Nile tilapia culture area				
	Suphanburi (n=10)	**Phetchaburi (n=10)**	**Chachoeng sao (n=10)**	**Nakhon Si Thammarat (n=10)**	**Surat Thani (n=10)**
Bio-Gr. I (*A. hydrophila*)	2 (20%)	2 (20%)	1 (10%)	1 (10%)	-
Bio-Gr. II-I (*A. veronii* biovar *sobria*)	1(10%)	1 (10%)	-	-	1 (10%)
Bio-Gr. II-II (*A. veronii* biovar *veronii*)	7 (70%)	7 (70%)	8 (80%)	8 (80%)	9 (90%)
Bio-Gr. III (*A. jandaei*)	-	-	1 (10%)	1 (10%)	-

Determination of virulence factors

Several reports have suggested that *Aeromonas* species could carry either one or various virulence genes that were involved in pathogenecity. This study, PCR screening of 5 virulence genes of *Aeromonas* isolated from MAS-exhibiting tilapia including *elastase*, *enolase*, *alt*, *hly/aerA*, and *lipase* was reported (Table 5). All virulence genes were present in 33.33−100% of the test *Aeromonas* strains. The *elastase* gene was the most frequently found virulence gene since it was detected in all test *Aeromonas* isolates. The *enolase* gene was found in every strains of *A. veronii* biovar *veronii*, *A. veronii* biovar *sobria* and *A. jandaei* while only 66.66% in *A. hydrophila.* The *alt* gene was highly detected in *A. veronii* biovar *veronii* and *A. jandaei* while *hly/aerA* was detected in 83.33% of *A. hydrophila* and absent in *A. jandaei*. All collected *A. varonii* biovar *sobria* carried lipase while this gene was observed at 50, 46.15 and 33.33% in *A. jandaei*, *A. veronii* biovar *veronii* and *A. hydrophila*, respectively.

Table 5. Genetic detection of 5 virulence genes in *Aeromonas* spp. isolated from MAS Nile tilapia

Virulence genes	***Aeromonas* species**			
	***A. hydrophila* (n=6)**	***A. veronii* biovar *sobria* (n=3)**	***A. veronii* biovar *veronii* (n=39)**	***A. jandaei* (n=2)**
elastase	6 (100%)	3 (100%)	39 (100%)	2 (100%)
enolase	4 (66.66%)	3 (100%)	39 (100%)	2 (100%)
alt	2 (33.33%)	1 (33.33%)	32 (82.05%)	2 (100%)
hly/aerA	5 (83.33%)	1 (33.33%)	24 (61.54%)	-
lipase	2 (33.33%)	3 (100%)	18 (46.15%)	1 (50%)

Discussion

Over the past dacades, the epidemiology of *Aeromonas* infection has shown to increase in freshwater fish. MAS is one of the most important diseases of tilapia culture. The mostly associated pathogen causing MAS was *A. hydrophila.* In fact, the causative pathogen of MAS is diverse. Not only *A. hydrophila* but also other *Aeromonas* species have been reported as the MAS causing agents (Dong *et al*., 2017b). This is in agreement with the misleading attributes of this genus on the basis of phenotypic identification. In this study, 78% of the isolated *Aeromonas* species from MAS-expressing tilapia were classified as *A. veronii* biovar *veronii* while *A. hydrophila*, *A. veronii* biovar *sobria* and *A. jandaei* were also detected. However, the other species of *Aeromonas* were less than 15% compared to that of the classified *A. veronii* biovar *veronii.* This result indicated that *A. veronii* biovar *veronii* may contribute as an important causative agent of MAS diseased tipalia in Thailand. Similarly, in channel catfish in Vietnam, *A. veronii* was reported as a major pathogen that caused MAS disease (Hoai *et al*., 2019). So far, a relatively high number of freshwater economic fish including Chinese longsnout catfish (*Leiocassis longirostris*) (Cai *et al*., 2012), African catfish (*Clarias gariepinus*), rui (*Labeo rohita*) (Rahman *et al*., 2002), and tilapia (Hassan *et al*., 2017) have been reported to be infected by *A. veronii* in various aquaculture areas of the world. The findings suggested that *A. veronii* could pose a risk to fish health and should be studied more detail regarding pathogenicity and control strategy. Pathogenic bacteria causing disease in an infected fish requires a set of features that called bacterial virulence. Bacteria can infect the host successfully whenever their virulence factors can overcome the defense mechanisms of the host (Dallaire-Dufresne *et al*., 2014). In this study, the occurence of important virulence factor genes: *elastase*, *enolase*, *alt*, *hly/aerA*, and *lipase* was evaluated through PCR in the pathogenic *Aeromonas* species isolated from MAS-exhibiting tilapia. *Elastase* gene was prevalent (100%) in all test *Aeromonas* isolates which was different from the previous works studied in different areas. The reports of *A. hydrophila* in China and *A. veronii* in USA demonstrated that aerolysin is the major contributor to the virulence of the pathogenic *Aeromonas* strains (Nawaz *et al*., 2010; Hu *et al*., 2012; Zheng *et al*., 2012).

In conclusion, *A. veronii* biovar *veronii*, *A. veronii* biovar *sobria*, *A. hydrophila,* and *A. jandaei* were the causative bacteria of outbreaks in Nile tilapia farms in Thailand. The presence of virulence genes contributes to *Aeromonas* pathogenicity were *elastase*, *enolase*, *alt*, *hly/aerA*, and *lipase.* Our findings provide the information that may be required for further disease control management strategy.

Acknowledgement

This research was a part of the research program No. 2919 and the project No. 23288 granted by the National Research Council of Thailand (NRCT) and Rajamangala University of Technology Srivijaya.

References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tools. Journal of Molecular Biology, 215:403-410.

Awan, M. B., Bari, M. M. A. A. and Saad, A. M. (2005). Biochemical characterization of the Aeromonas species isolated from food and environment. Pakistan Journal of Physiology, 1:1-2.

Cai, S. H., Wu, Z. H., Jian, J. C., Lu, Y. S. and Tang, J. F. (2012). Characterization of pathogenic *Aeromonas veronii* bv. *veronii* associated with ulcerative syndrome from Chinese longsnout catfish (*Leiocassis longirostris* Günther). Brazilian Journal of Microbiology, 43:382-388.

Chirapongsatonkul, N., Srichanun, M. and U-taynapun, K. (2018). Virulence factor gene profiles of *Aeromonas veronii* isolated from diseased Nile tilapia (*Oreochromis niloticus*) in Nakhon Si Thammarat province and its expression towards diurnal water temperature changes. Journal of Agricultural Technology, 14:1115-1128.

Chirapongsatonkul, N., Mueangkan, N., Wattitum, S. and U-Taynapun, K. (2019). Comparative evaluation of the immune responses and disease resistance of Nile tilapia (*Oreochromis niloticus*) induced by yeast β-glucan and crudeglucan derived from mycelium in the spent mushroom substrate of *Schizophyllum commune*. Aquaculture Reports, 15:100205.

Choopun, N., Louis, V., Huq, A. and Colwell, R. R. (2002). Simple procedure for rapid identification of *Vibrio cholera* from the aquatic environment. Applied and Environmental Microbiology, 68:995-998.

Dallaire-Dufresne, S., Tanaka, K. H., Trudel, M. V., Lafaille, A. and Charette, S. J. (2014). Virulance, genomic features and plasticity of *Aeromonas salmonicida* aubsp. *salmonicida*, the causative agent of fish furunculosis. Veterinary Microbiology, 167:1-7.

Dong, H. T., Nguyen, V. V., Le, H. D., Sangsuriya, P., Litrakorn, S., Saksmerprome, V., Senapin, S. and Rodkhum, C. (2017a). Naturally concurrent infections of bacterial and viral pathogens in disease outbreaks in cultured Nile tilapia (*Oreochromis niloticus*) farms. Aquaculture, 488:427-435.

Dong, H. T., Techatanakitarnan, C., Jindakittikul, P., Thaiprayoon, A., Taengphu, S., Charoensapsri, W., Khunrae, P., Rattanarojpong, T. and Senapin, S. (2017b). *Aeromonas jandaei* and *Aeromonas veronii* caused disease and mortality in Nile tilapia, *Oreochromis niloticus* (L.). Journal of Fish Diseases, 40:1395-1403.

Hassan, M., Noureldin, E. A., Mahmoud, M. A. and Fita, N. A. (2017). Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. The Egyptian Journal of Aquatic Research, 43:161-167.

Hoai, T. D., Trang, T. T., Tuyen, N. V., Giang, N. T. H. and Van, K. V. (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. Aquaculture, 513:734425.

Hu, M., Wang, N., Pan, Z. H., Lu, C. P. and Liu, Y. J. (2012). Identity and virulence properties of *Aeromonas* isolates from diseased fish, healthy controls and water environment in China. Letters in Applied Microbiology, 55:224-233.

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16:111-120.

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35:1547-1549.

Li, J., Ni, X. D., Liu, Y. J. and Lu, C. P. (2011). Detection of three virulence genes *aly*, *ahp*, and *aerA* in *Aeromonas hydrophila* and their relationship with actual virulence to zebrafish. Journal of Applied Microbiology, 110:823-830.

Liu, C., Chang, O. Q., Zhang, D. F., Li, K. B., Wang, F., Lin, M. H., Shi, C. B., Jing, L., Wang, Q. and Bergmann, S. M. (2018). *Aeromonas shuberti* as a couse of multi-organ necrosis in internal organs of Nile tilapia, *Oerochromis niloticus*. Journal of Fish Diseases, 41:1529-1538.

Nahar, S., Rahman, M. M., Ahmed, G. U. and Faruk, M. A. R. (2016). Isolation, identification and characterization of Aeromonas hydrophila from juvenile farmed pangasius (*Pangasianodon hypophthalmus)*. International Journal of Fisheries and Aquatic Studies, 4:52-60.

Nawaz, M., Khan, S. A., Khan, A. A., Sung, K., Tran, Q., Kerdahi, K. and Steele, R. (2010). Detection and characterization of virulence genes and integrons in *Aeromonas veronii* isolated from catfish. Food Microbiology, 27:327-331.

Oliverira, S. T. L., Veneroni-Gouveia, G. and Costa, M. M. (2012). Molecular characterization of virulence factors in *Aeromonas hydrophila* obtained from fish. Pesquisa Veterinária Brasileira, 32:701-706.

Rahman, M., Colque-Navarro, P., Kühn, I., Huys, G., Swing, J. and Möllby, R. (2002). Identification and characterization of pathogenic *Aeromonas veronii* biovar sobria associated with eizootic ulcerative syndrome in fish in Bangladesh. Applied Environmental Microbiology, 68:650-655.

Sha, J., Erova, T. E., Alyea, R. A., Wang, S., Olano, J. P., Pancholi, V. and Chopra, A. K. (2009). Surface-expressed enolase contributes to the pathogenesis of clinical isolate SSU of *Aeromonas hydrophila*. Journal of Bacteriology, 191:3095-3107.

Smith, K. F., Schmidt, V., Rosen, G. E. and Amaral-Zettler, L. (2012). Microbial diversity and potential pathogens in ornamental fish aquarium water. PLOS ONE, 7:e39971.

Srisapoome, S. and Areechon, N. (2017). Efficacy of viable *Bacillus pumilus* isolated from farmed fish on immune responses and increased disease resistance in Nile tilapia (*Oreochromis niloticus*): Laboratory and on-farm trials. Fish & Shellfish Immunology, 67:199-210.

U-taynapun, K., Mueangkan, N. and Chirapongsatonkul, N. (2018). Efficacy of herbal extracts to control multi-antibiotics resistant (MAR) *Aeromonas veronii* isolated from motile *Aeromonas* septicaemia (MAS)-exhibiting Nile tilapia (*Oreochromis niloticus*). International Journal of Agricultural Technology, 14:219-2206.

Weisburg, W. C., Barns, S. M., Pelletier, D. A. and Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology, 173:697-703.

Zheng, W., Cao, H. and Yang, X. (2012). Grass carp (*Ctenopharyngodon idellus*) infected with multiple strains of *Aeromonas hydrophila*. African Journal of Microbiology Research, 6:4512-4520.

(Received: 20 August 2019, accepted: 3 April 2020)