1 Phosphoinositide 3-kinase family in channel catfish and their
2 regulated expression after bacterial infection

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ABSTRACT

The phosphoinositide-3-kinase (PI3Ks) family of lipid kinases is widely conserved from yeast to mammals. In this work, we identified a total of 14 members of the PI3Ks from the channel catfish genome and transcriptome and conducted phylogenetic and syntenic analyses of these genes. The expression profiles after infection with *Edwardsiella ictaluri* and *Flavobacterium columnare* were examined to determine the involvement of PI3Ks in immune responses after bacterial infection in catfish. The results indicated that PI3Ks genes including all of the catalytic subunit and several regulatory subunits genes were widely regulated after bacterial infection. The expression patterns were quite different when challenged with different bacteria. The PI3Ks were up-regulated rapidly at the early stage after ESC infection, but their induced expression was much slower, at the middle stage after columnaris infection. RNA-Seq datasets indicated that PI3K genes may be expressed at different levels in different catfish differing in their resistance levels against columnaris. Future studies are required to confirm and validate these observations. Taken together, this study indicated that PI3K genes may be involved as a part of the defense responses of catfish after infections, and they could be one of the determinants for disease resistance.

Key words:

Fish; PI3K; Phosphoinositol kinase; Genome; Gene expression; Immune response

1. Introduction

Phosphoinositol 3-kinases (PI3Ks) belong to a family of intracellular lipid kinases that phosphorylate the 3'-hydroxyl group of phosphatidylinositol (also known as PI or *PtdIns*) and phosphoinositides in cellular membranes either acting constitutively or in response to extracellular stimuli such as growth factors and hormones. The lipid products of PI3Ks serve multiple functions in the cell by regulating cellular membrane trafficking and acting as second messengers. Cytosolic PH-domain containing signaling proteins recruited to the membrane could activate diverse signal transduction pathways which in turn act synergistically to mediate a number of cell behaviors and properties in both normal and pathological conditions, including cell growth, proliferation, differentiation, survival, metabolism, vesicular trafficking, degranulation, cytoskeletal rearrangement, and motility [1-3]. PI3Ks are an evolutionarily conserved family of enzymes; based on their structural features, *in vitro* lipid substrate specificity, tissue distribution, mechanism of activation, and function, they were classified into three distinct classes, Class I to III [4]. Their structural components and domains were well depicted in the review by Vanhaesebroeck et al. [4].

Class I enzymes exist as a heterodimer consisting of one catalytic subunit and one regulatory subunit. Notably, members of this class are activated by various cell surface receptors, leading to further subdivision of this class into subfamilies IA and IB. Members of the subclass IA are activated by receptor tyrosine kinase, whereas

those of subclass IB are activated by G-protein coupled receptors (GPCRs) [1, 3, 4]. Class IA PI3Ks enzymes include p110 α (PI3KCA), p110 β (PI3KCB), and p110 δ (PI3KCD), which can pair with one of the five regulatory subunits p85 α , p55 α , p50 α (alternatively spliced from PIK3R1), p85 β (PIK3R2), and p55 γ (PIK3R3) [5-7]. In contrast, Class IB PI3K enzymes consist of the p110 γ (PI3KCG) catalytic subunit which forms heterodimers with either p101 (PIK3R5) or p87 (PIK3R6) [2, 8, 9]. The regulatory subunits seemed to be primarily responsible for spatiotemporal control of PI3Ks activation [10]. For example, the regulatory subunits p85 of class IA PI3Ks acts to localize the catalytic subunit p110 to the plasma membrane in response to growth factor stimulation and activation of growth factor receptors such as the platelet derived growth factor receptor (PDGFR) [3]. All Class I PI3K enzymes have been shown to phosphorylate phosphatidylinositol (PI), PI(4)P, and PI(4,5)P₂ to produce the lipid products PI3P, PI(3,4)P₂, and PI(3,4,5)P₃, respectively *in vitro*. However, phosphorylation by class I PI3Ks was observed *in vivo* only with PI(4,5)P₂ to produce PI(3,4,5)P₃ [6, 11, 12].

Class II PI3Ks have been identified based on structural homology with Class I PI3Ks but have received relatively little attention compared with their class I counterparts. Unlike class I PI3Ks, class II PI3Ks are monomers with only a single catalytic domain without regulatory subunit, and are distinguished by a carboxy-terminal C2 domain [13, 14]. There are three isoforms of class II PI3K, named C2 α , C2 β , and C2 γ [5, 11]. Class II PI3Ks have well-recognized *in vitro*

substrates, but their *in vivo* targets are still being identified. PI(4)P is the substrate for the generation of PI(3,4)P₂, and PI is the substrate for the generation of PI(3)P [9].

Class III PI3Ks has a single member, the vacuolar protein-sorting 34 (VPS34, also called PIK3C3) that was first identified in mammals in 1995 [15]. It is the oldest PI3K and is the only one found in yeast and plants as well as in metazoans and is highly conserved among yeast, plants, and mammals [3]. Class III PI3K enzymes are structurally more similar to class I PI3Ks than the class II PI3Ks, since they consist of a catalytic (VPS34) and regulatory subunit p150 (also called VSP15 or PIK3R4). VPS34 enzymes are unique among PI3Ks in that they only use phosphatidylinositol as the substrate. VPS34 could therefore share protein effectors with the class II PI3Ks, but it is not clear whether the functions of class II and class III PI3Ks overlap [16]. The most recognized function of class III PI3Ks is the regulation of vesicular trafficking in the endosomal/lysosomal system [17-21]. Class III PI3Ks also activate additional mechanisms in mammalian cells, such as endocytosis and phagocytosis. Additionally, VPS34 kinase may also play a role in autophagy and protein synthesis through an mTOR-dependent mechanism [22]. As such, class III PI3Ks may be highly relevant to disease defense responses.

Catfish industry, the most prominent aquaculture industry in the United States, has encountered great challenges including devastating diseases which cause large economic losses. In particular, bacterial diseases, enteric septicemia of catfish (ESC) and columnaris disease, cause huge losses to the catfish industry [23, 24]. Recently, a

third bacterial disease, caused by *Aeromonas hydrophila*, emerges to be another devastating disease to the catfish industry [25]. With columnaris, the most frequently occurring bacterial disease, a recent QTL study indicated that the PI3K pathway is highly related to the host resistance against this disease [26]. As the first step to understand the mechanism of resistance against columnaris disease, the goal for this study is to identify and annotate PI3Ks genes in channel catfish, and determine their expression after infection with *E. ictaluri* and *F. columnare*.

2. Materials and methods

2.1. Gene identification and sequence analysis

To identify the PI3K genes, the channel catfish transcriptome database [27-29] and the whole genome database of channel catfish (unpublished data) were searched using available PI3Ks amino acid sequences from teleost fish, including zebrafish (*Danio rerio*), stickleback (*Gasterosteus aculeatus*), medaka (*Oryzias latipes*), tilapia (*Oreochromis niloticus*), fugu (*Takifugu rubripes*), turtle (*Pelodiscus sinensis*), lizard (*Anolis carolinensis*), chicken (*Gallus gallus*), mouse (*Mus musculus*), and human (*Homo sapiens*)) as query sequences. TBLASTN was performed by searching against the channel catfish transcriptome database [29]. The e-value was set at an intermediately stringent level of e-10 for collecting potential PI3Ks.

The ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) was used to predict the open reading frames of retrieved sequences. The predicted ORFs were

verified by BLASTP against NCBI non-redundant protein database. Simple Modular Architecture Research Tool (SMART <http://smart.embl-heidelberg.de>) was used to predict the conserved domains based on sequence homology. To determine copy numbers of PI3Ks genes in channel catfish genome, BLASTN was used to search against channel catfish draft whole genome assembly database. FGENESH [30] were used to predict amino acid sequence from genomic sequences.

2.2. Phylogenetic analysis

The full-length amino acid sequences of PI3Ks from several representative vertebrates including those from human, mouse, chicken, lizard, and several fish species such as zebrafish, tilapia, medaka and stickleback were retrieved from NCBI and Ensembl databases for phylogenetic analysis. Multiple protein sequences alignments were conducted using the Clustal W2 program [31]. The maximum likelihood method was used to conduct phylogenetic analysis using MEGA 5.2 [32]. Jones–Taylor–Thornton (JTT) and gamma distributed rate within variant sites (G + I) model were chosen based on the alignment result [33]. Gaps were removed by pair-wise deletion and 1000 bootstrap replicates were performed in phylogenetic analysis.

2.3. Syntenic analysis

The homologies of the PI3Ks, and their neighboring genes in channel catfish were examined through comparing those among human, zebrafish, and tilapia, to provide additional evidence for gene identification and orthology. The genome

information for each species was retrieved from Ensembl and Genomicus. The upstream and downstream genes surrounding the putative PI3Ks genes were identified from the channel catfish scaffolds by FGENESH program [30]. BLASTP was used to annotate these neighboring genes by searching against NCBI database. The catfish PI3Ks genes were named following the Zebrafish Nomenclature Guidelines [34].

2.4. Expression analysis using available RNA-Seq datasets

The Illumina-based RNA-Seq reads were retrieved from bacterial challenge experiments in catfish: intestine samples challenged with *Edwardsiella ictaluri* (SRA Accession SRP009069) [35], and gill samples challenged with *Flavobacterium Columnare* (SRA Accession SRP012586, SRP017689.) [36, 37]. For ESC studies, fish were challenged with a concentration of 4×10^8 CFU/ml in 30 L aquaria for 2 h by immersion exposure. At 3 h, 24 h and 3 d after challenge, 30 fish were collected from each of the appropriate control and treatment aquaria at each timepoint and euthanized with MS-222 (300 mg/L). The entire intestinal tracts from 10 fish were dissected, bisected and gently washed and pooled together for RNA extraction [35]. For columnaris, Challenge experiments were conducted by immersion exposure for 2 h at a final concentration of the bacteria at 3×10^6 CFU/mL. At 4 h, 24 h, and 48 h after challenge, 18 fish from both control and treatment were randomly selected and divided into 3 replicate pools (6 fish each) respectively. Gill tissues in the 3 replicates were placed into 5 ml RNA later™ (Ambion, Austin, TX, USA) for RNA extraction [36]. The gill samples of resistant and susceptible families differing in their

susceptibility to *F. columnare* were collected at 0-h, 1-h, 2-h and 8-h after *F. columnare* infection, as previously reported [37]. Trimmed high quality reads were mapped onto the catfish PI3Ks genes using CLC Genomics Workbench software (version 5.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as $\geq 95\%$ of the reads in perfect alignment and ≤ 2 mismatches.

The total mapped reads number for each transcript was then determined and RPK (Reads Per Kilobase of exon model) was calculated. Beta actin gene (*actb*) was used as internal control for mapping along with PI3Ks transcripts and calculating relative expression value. The expression fold-change of each PI3Ks gene was determined based on the ratio of its RPK to that of *actb* in the same sample. Transcripts with absolute expression fold change value ≥ 1.5 and total gene reads ≥ 5 were included in the analyses as significantly differentially expressed genes.

3. Results

3.1. Identification of PIK3 genes in channel catfish

A total of 14 PI3Ks genes were identified in channel catfish, including catalytic unit genes PIK3CAa, PIK3CAb, PIK3CB, PIK3CD, PIK3CG, PIK3C2A, PIK3C2B, PIK3C3 and regulatory unit genes PIK3R1, PIK3R3b, PIK3R4, PIK3R5, PIK3R6a and PIK3R6b. Characteristics of the PIK3 genes, including the sizes of the transcripts, lengths of open reading frames, their GenBank accession numbers and the linkage group location of the genes, were summarized in Table 1. PIK3R2 and PIK3C2G

were not found in the catfish genome, and two genes were present in the catfish genome for PIK3CA and PIK3R6.

3.2 Phylogenetic analysis of channel catfish PI3K genes

Phylogenetic analysis was conducted to annotate the catfish PI3K genes. Because of the many genes, phylogenetic analysis was conducted separately for the catalytic PI3K and the regulatory PI3K genes. The eight catalytic PI3K genes in catfish fell into seven distinct clades, well positioned in the clades compatible with phylogenetic expectations as they were most closely related with the zebrafish genes. Two PIK3CA genes fell into a single clade, with one of the two most similar to zebrafish PIK3CA gene (Fig. 1). However, phylogenetic analysis placed the other catfish gene within this clade furthest from the fish PI3K genes, suggesting that phylogenetic analysis alone may not be capable of annotating these two genes properly.

Similarly, the PI3K regulatory unit genes were grouped into six clades as expected from the phylogenetic relationship with various organisms (Fig. 2). However, the phylogenetic analysis placed PIK3R3b into the clade containing other PIK3R3b genes from other organisms, but its position within the clade is not compatible with expected relationship with genes from zebrafish and other fish species (Fig. 2). In addition, there are two genes for PIK3R6, the orthologies of these genes need to be analyzed for their proper annotation.

203

204 3.3 Syntenic analysis

205 Phylogenetic analysis has provided strong support for the identities of most of
206 the PI3K genes except for the PIK3R3 gene, and a couple of cases where duplications
207 existed such as the two PIK3CA genes and the two PIK3R6 genes. Syntenic analysis
208 was essential to provide additional evidence for the annotation of these five genes.
209 When the genomic neighborhood regions surrounding the PI3K genes are displayed, it
210 is apparent that the two PIK3CA genes are paralogs. They both share the same
211 orthology with the human PIK3CA genes. Apparently, there is only one PIK3CA
212 gene from zebrafish, but it is duplicated in tilapia and catfish. For catfish, the two
213 genes were found on two different linkage groups, linkage group 15 and 20 (Fig.3).
214 Similarly, syntenic analysis revealed that the two catfish PIK3R6 genes are paralogs
215 from whole genome duplication because the conserved syntenies shared a high level
216 of similarity on catfish linkage group 3 and 12 (Fig. 4).

217 For catfish PIK3R3 gene, the conserved syntenic blocks indicated that it is most
218 similar to the zebrafish PIK3R3b gene (Fig. 5), and therefore, we named this gene in
219 catfish as PIK3R3b.

220

221 3.4. Gene copy numbers of PI3Ks

222 Copy number comparisons of PI3K genes among various species are
223 summarized in Table 2. Overall, the total number of PI3K genes varied between 10 to

21 genes, with the general pattern of more PI3K genes in teleost genomes. In all cases except PIK3R2 where two genes existed in the human genome, all other PI3K genes exist as single copy genes in mammals, birds, amphibians, and reptiles. However, duplicated PI3K genes existed in teleosts, derived from whole genome duplications (Table 2), as supported by both phylogenetic and syntenic analyses (see above).

3.5 Expression of PI3K genes after bacterial infections

To determine the involvement of PI3K genes in disease defense responses, expression profiles of catfish PI3Ks genes were determined after bacterial infections with two pathogens, *E. ictaluri* and *F. columnare* by meta-analysis of RNA-Seq datasets. Of the 14 PI3Ks genes, 12 genes were determined to be differentially expressed after bacterial infections, with 8 genes differentially expressed after ESC infection (Fig. 6) and 9 genes differentially expressed after columnaris disease infection (Fig. 7). Of the eight genes differentially expressed after ESC infection, six were catalytic unit genes including PIK3CAa, PIK3CAb, PIK3CB, PIK3CG, PIK3C2A, and PIK3C2B. The other two were regulatory unit genes PIK3R1 and PIK3R3b. Interestingly, most of the PI3K catalytic unit genes were up-regulated, but only two regulatory unit genes were up-regulated. The up-regulation was at early stages after infection, detected as early as 3 h after infection with exception of PIK3CAb and PIK3C2A that were induced at 24 h after infection. Of these up-regulated genes, some are relatively transient with up-regulation detected only at

one or two time points after infection, such as PIK3CAb and PIK3C2A while several genes were more stably up-regulated to last during 3h-72h after infection such as PIK3CAa, PIK3CB, PIK3C2B and PIK3R3b (Fig. 6). Overall, however, it appears that ESC induced PI3K genes expression was both systematic and specific. For instance, of the six regulatory unit genes, only two were specifically induced, suggesting that PIK3R1 and PIK3R3b may be more important in ESC disease defense responses than other regulatory unit genes.

The expression patterns of PI3K genes after columnaris infection (Fig. 7) were drastically different from those after ESC infection. First of all, almost all catalytic unit genes except PIK3CAa were all up-regulated after columnaris infection, and two regulatory unit PI3K genes, PIK3R4 and PIK3R6b, were upregulated. Secondly, the induced expression of PI3K genes after columnaris infection started relatively later than after ESC infection, mostly 24h after infection, as compared to 3h after infection with ESC infection. Thirdly, all induced expression was highly transient being detected only at one time point except for PIK3CAb. Although most of the catalytic genes were up-regulated, the two up-regulated regulatory genes are different from those after ESC infection (PIK3R1 and PIK3R3b). In all cases, however, the extent of induced expression was only modest, being induced only 2-3 fold after infection (Fig. 7). Once again, these results suggested that PI3K genes are involved in defense responses after bacterial infection, although the involved genes may be specific with various infectious agents.

266

267 3.6 Expression of PI3K genes in resistant and susceptible channel catfish

268 The expression of PI3K genes in channel catfish differing in their susceptibility
269 to *F. columnare* was evaluated by meta-analysis of RNA-Seq datasets [37]. Before
270 infection, the RPKM values were generally higher with susceptible catfish than with
271 resistant fish, but the difference was modest, less than 1.5 fold. Three PI3K genes,
272 PIK3CAa, PIK3CB and PIK3C2B, were expressed at higher levels in resistant catfish
273 2 h after infection than in susceptible fish (Fig. 8). PIK3C2B and PIK3R6b were
274 expressed at higher levels in susceptible channel catfish than in resistant catfish at 1 h
275 and 8 h after infection, respectively (Fig. 8).

276

277 4. Discussion

278 Phosphoinositide-3-kinase are important in signal transductions involved in
279 various cellular processes and immune responses [3]. Because of their importance,
280 they have been extensively studied with mammalian species. However, these genes
281 have not been studied from teleost fish species. In this work we identified and
282 characterized a whole set of 14 PI3K genes from channel catfish, representing the first
283 analysis of PI3K genes among teleosts. The gene copy numbers of these genes were
284 comparatively determined among various species including several teleost fish
285 species whose whole genome sequences are available. Along with phylogenetic

analysis, syntenic analysis of conserved syntenic blocks allowed proper annotation of all these 14 catfish genes.

Of the 14 distinct PI3K genes existing in mammalian species, two, PIK3C2G and PIK3R2, were not found from the catfish genome. It is unknown at present if these two genes were truly missing from channel catfish or they have not yet been found. A total of 14 individual genes were found from channel catfish. Annotation of these genes was relatively straightforward through phylogenetic analysis. However, there are two genes for PIK3CA and PIK3R6. In addition, PIK3R3 gene was not placed properly in the clade containing PIK3R3 genes from other species. Therefore, we retrieved genomic regions containing the relevant genes and conducted syntenic analysis. It is clear from the conserved syntenies that the duplicated genes of PIK3CA and PIK3R6 were derived from teleost-specific whole genome duplications (Fig. 3-4). This is supported by two lines of evidence: 1) With the exception of PIK3R2 in humans, no duplications of PI3K genes were observed in mammals, birds, amphibians, or reptiles; and 2) syntenic analysis of duplicated PI3K genes in teleost fish species clearly indicated the presence of duplicated genomic regions on different chromosomes (Figs. 3-5).

The annotation was relatively easy with phylogenetic and syntenic analyses. However, nomenclature of duplicated genes in teleost fish has always been somewhat complicated. In most cases, the nomenclature of zebrafish has been followed, i.e., when the two duplicated genes were derived from whole genome duplication, they

were named a and b following the gene names, for instance, PIK3R3a and PIK3R3b. However, there are currently inconsistencies in the GenBank as well as in literature in such a pattern of nomenclature. We feel it is crucially important that such nomenclature is strictly followed. In cases where there is only one copy of the zebrafish, but two copies of the catfish genes, we named the catfish gene with the highest similarity (being clustered with the zebrafish in phylogenetic analysis) as the a gene, and the other copy as the b gene.

PI3K genes have been demonstrated as partners to regulate mammalian immune cell signaling and functions [38-41]. Most studies have focused on the possible functions of PI3K genes as an essential intra-cellular signaling pathway in NK cells [41-44]. For instance, expression of PI3K genes in NK cells is required for lymphocyte function-associated antigen-1 (LFA-1) adherence to intercellular adhesion molecule-1 (ICAM-1)-expressing cells and thus, important for formation of the NK immune synapses [38] and for facilitating signaling through various NK activating receptors [45]. In addition to the identification, annotation, and characterization of PI3K genes in catfish, one of our goals was to determine if PI3K genes were involved in disease defense responses in catfish, as demonstrated in mammals. In this study, meta-analysis of RNA-Seq datasets was conducted after disease challenges. The results showed that 12 of 14 PI3K genes were up-regulated after bacterial infection, suggesting their involvement in disease responses. In both of the bacterial infections, most of the catalytic subunits of PI3K were regulated after

disease challenges. However, the regulatory subunit genes were quite specific to bacterial pathogens. With ESC infection, the PIK3R1 and PIK3R3b were up-regulated, while with columnaris infection, PIK3R4 and PIK3R6b were up-regulated. Although details need to be learned in future studies, it is apparent that PI3K genes are involved in the disease defense responses in catfish, in a pathogen-specific manner. Although the level of induced expression was modest in most cases, the elevated expression can still be extremely important considering the signal transduction functions of PI3Ks.

Among the catalytic subunit genes, PIK3CA genes were the most highly regulated under both bacterial infection situations. This is consistent with the findings of PI3K involvement in immune functions in other species. P110 α (PIK3CA) gene encode for one of Class IA PI3Ks, found in many cell types but not in leukocytes, is required for the upregulation of the NKG2D ligand RAE-1 following murine cytomegalovirus (MCMV) infection [46]. p110 β acts in the immune system by cooperating with PI3K δ in the generation of reactive oxygen species (ROS) in neutrophils in response to fungal infection or immune complexes [47, 48]. PI3K δ is highly abundant in cells of the hematopoietic lineage and plays important, non-redundant roles in the development and function of T cells and in mast cell activation [49, 50]. Class IB PI3Ks (including p110 γ only) is required for NK cell cytokine production [51, 52]. PI3K γ has been reported to plays an important role in the signaling pathways that link Gi-coupled GPCRs for soluble, proinflammatory stimuli, including interleukin-8 (IL-8), N-formyl-Met-Leu-Phe (fMLP), fragment of

complementary protein C5 (C5a), and leukotriene B4 (LTB4), to the regulation of neutrophil chemotaxis, extravasation, and the production of ROS [39, 53]. Different p110s preferentially interact with different signaling components, for example, distinct isoforms of the Ras GTPase [54]. Thus, PI3Ks may regulate NK cell behavior not only in a cell intrinsic manner, but also via regulation of activating ligands expressed by target cells [41].

Less is known about the cellular functions of class II and class III PI3Ks. It was reported that knockout of PI3KC2A caused chronic renal failure and exhibited a range of kidney lesions, including glomerular crescent formation and renal tubule defects in early disease, which progressed to diffuse mesangial sclerosis, with reduced podocytes, widespread effacement of foot processes, and modest proteinuria, suggesting its roles in renal homeostasis [55, 56]. Several studies showed that Class III PI3Ks are involved in vesicle trafficking through production of PI3P [15, 57]. Here in this study, Class I PI3K genes are more involved in responses after ESC infection, but Class IB and Class III PI3K genes are up-regulated after columnaris infection, suggesting that different PI3K genes may be involved in responses to different pathological processes. One interesting aspect of the findings was the more rapid and more persistent induction of PI3K gene expression after ESC infection than after columnaris infection. These findings coincide with the development of the diseases. ESC pathogen, *Edwardsiella ictaluri*, is an intracellular pathogen and the disease

369 progression is more rapid than columnaris. In contrast, *Flavobacterium columnare* is
370 a facultative pathogenic bacteria and its pathology is much slower than ESC disease.

371 One important question is how the expression level of PI3K genes is related to
372 the resistance against columnaris disease. Our recent QTL analysis using high density
373 SNP arrays mapped the resistance genes to linkage groups 7, 12, and 14. The
374 interesting point was that genes included in LG7, 12, and 14 all included a similar sets
375 of genes involved in PI3K gene pathways. Our analysis of PI3K gene expression
376 between the resistant line and the susceptible line of catfish against columnaris
377 disease indicated that basal expression levels of PI3K genes were generally higher in
378 susceptible catfish than in resistant catfish. After infection, there appears to be
379 induction of PIK3CAa, PIK3CB and PIK3C2B in resistant catfish 2 h after infection,
380 suggesting that some members of Class IA and Class II PI3K genes may be involved
381 in the disease responses. However, due to the lack of biological replicates, statistical
382 analysis cannot be conducted. Future experimentation is warranted to confirm and
383 validate these preliminary observations. It was reported that PI3K is required for
384 modifying the cytoskeleton dynamics, regulating membrane traffic, coordinating
385 exocytic membrane insertion and pseudopod extension, which could be utilized by
386 some pathogenic bacteria for entry into host cell [58-62].

387 Although the extent of up-regulation is only modest, 2-4 fold overall, PI3Ks are
388 important signal transduction genes, and any subtle change in their expression
389 upstream could have a profound downstream effect through a series of amplification

of the signals. They act as intermediate signaling molecules and are most well known for their roles in the PI3K/AKT/mTOR signaling pathway [63, 64]. PI3Ks transmit signals from the cell surface to the cytoplasm by generating second messengers – phosphorylated phosphatidylinositols - which in turn activate multiple effector kinase pathways, including BTK, AKT, PKC, NF-kappa-B, and JNK/SAPK pathways, and ultimately result in survival and growth of normal cells [2, 63-66]. The downstream products of PI3K signaling cascade participate in various signal transduction pathways.

5. Conclusions

In this work, a complete set of 14 PI3K genes in channel catfish were identified, annotated and characterized, and their expression profiles were determined after infections with *F. columnare* and *E. ictaluri*. Of the 14 PI3Ks genes, 12 genes and 8 genes were determined to be differentially expressed after ESC and columnaris infection, respectively. The response patterns of these genes to the two bacteria were quite different, with rapid and more persistent induced expression with ESC infection and slow and only transient induced expression with columnaris, suggesting pathogen-specific defense responses of these genes. As a first step of attempts to understand the involvement of PI3K genes in disease responses in catfish, this project demonstrated that PI3K is an important part of the disease defense response after bacterial infection. Future studies are warranted to understand the detailed

mechanisms of how the expression of PI3K genes are associated with disease resistance and host defense responses.

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Table 1. Characteristics of phosphoinositol 3-kinase (PI3K) genes in channel catfish.
Asterisk (*) indicated partial coding sequences.

Gene name		mRNA (bp)	CDS (aa)	Accession no.	Location
Class IA	PIK3CAa	7417	1069	JT455679	LG20
	PIK3CAb	3876	1072	JT476055	LG15
	PIK3CB	5745	1065	JT418313	LG2
	PIK3CD	5755	1044	JT407151	LG15
	PIK3R1	2412	590*	JT439101	LG14
	PIK3R3b	2351	465	JT415898	LG14
Class IB	PIK3CG	6640	1105	JT412435	LG23
	PIK3R5	3586	863	JT339861	LG12
	PIK3R6a	2615	742	JT425488	LG3
	PIK3R6b	4094	731	JT414239	LG12
Class II	PIK3C2A	6178	1673	JT409423	LG4
	PIK3C2B	7781	1583	JT406086	LG21
Class III	PIK3C3	3552	877	JT410651	LG8
	PIK3R4	4812	1381	JT407421	LG1

Table 2. Copy numbers of PI3Ks genes in various selected vertebrate genomes.
Species names were abbreviated: Hum: Human; Mmu: Mouse; Chk: Chicken; Lzd: Lizard; Trtl: Turtle; Skb: Stickleback; Mdk: Medaka; Til: Tilapia; Zbf: Zebrafish; Chc: Channel Catfish.

Genes	Hum	Mou	Chk	Lzd	Trtl	Frog	Skb	Fugu	Mdk	Til	Zbf	Chc
Class IA												
PIK3CA	1	1	1	1	1	1	2	2	2	2	1	2
PIK3CB	1	1	1	1	1	1	1	1	1	1	1	1
PIK3CD	1	1	1	1	1	0	1	1	1	1	1	1
PIK3R1	1	1	1	1	1	1	2	2	2	2	1	1
PIK3R2	2	1	1	1	0	1	1	1	0	1	1	0
PIK3R3	1	1	1	0	1	1	2	2	2	2	2	1
Class IB												
PIK3CG	1	1	1	1	1	1	1	2	1	1	1	1
PIK3R5	1	1	1	1	1	1	2	1	2	2	1	1
PIK3R6	1	1	1	1	1	1	3	1	1	2	2	2
Class II												
PIK3C2A	1	1	1	1	1	1	1	1	1	1	1	1
PIK3C2B	1	1	1	1	1	0	2	0	1	1	1	1
PIK3C2G	1	1	1	0	1	0	1	1	1	1	1	0
Class III												
PIK3C3	1	1	1	1	1	1	1	1	1	1	1	1
PIK3R4	1	1	1	1	1	1	1	1	2	1	1	1
Total	15	14	14	13	13	10	21	17	18	19	16	14

Fig. 1. Phylogenetic analysis of channel catfish PI3K catalytic unit genes. The phylogenetic tree was constructed by using MEGA 5.2 software with the maximum likelihood method. Bootstrap values were indicated by numbers at the nodes. For species other than channel catfish, duplicated genes were not annotated, but just indicated with a number following the gene names, separated with an underscore. The accession numbers of genes from other species were provided in Appendix: Supplementary File S1.

Fig. 2. Phylogenetic analysis of channel catfish PI3K regulatory unit genes. The phylogenetic tree was constructed by MEGA 5.2 software with the maximum likelihood method. Bootstrap values were indicated by numbers at the nodes. For species other than channel catfish, duplicated genes were not annotated, but just indicated with a number following the gene names, separated with an underscore. The accession numbers of genes from other species were provided in Appendix: Supplementary File S1.

Fig. 3. Syntenic analysis of PIK3CAa and PIK3CAb genes. Sequences used in the analysis included genomic neighborhood containing PIK3CA related genes from human, zebrafish, tilapia, and channel catfish. Gene abbreviations are the following: FAM43A, family with sequence similarity 43, member A; KCNMB2, potassium large conductance calcium-activated channel, subfamily M, beta member 2; KCNMB2, potassium large conductance calcium-activated channel, subfamily M, beta member 2; LSG1, large subunit GTPase 1 homolog; PFKFB4, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha; STXBP3, syntaxin binding protein 3; TBL1XR1B, transducin (beta)-like 1 X-linked receptor 1b; XXYLT1, xyloside xylosyltransferase 1; ZMAT3, zinc finger, matrin-type 3. Lg: linkage group; Chr: chromosome.

Fig. 4. Syntenic analysis of PIK3R6a and PIK3R6b genes. Sequences used in the analysis included genomic neighborhood containing PIK3R6 related genes from human, zebrafish, tilapia, and channel catfish. Gene abbreviations are the following: BPTF, bromodomain PHD finger transcription factor; DDX5, DEAD (asp-glu-ala-asp) box polypeptide 5; GRB2B, growth factor receptor-bound protein 2b; HN1A, hematological and neurological expressed 1a; KPNA2, karyopherin alpha 2; NTN1, netrin 1; NTN1A, netrin 1a; NTN1B, netrin 1b; PIK3R5, Phosphoinositide-3-kinase regulatory subunit 5; SEPT9, septin 9; SMURF2, SMAD specific E3 ubiquitin protein ligase 2; STX8, syntaxin 8; USP43, ubiquitin specific peptidase 43; WDR16, WD repeat-containing protein 16. Lg: linkage group; Chr: chromosome.

Fig. 5. Syntenic analysis of PIK3R3b gene. Sequences used in the analysis included genomic neighborhood containing PIK3R3 related genes from human, zebrafish, tilapia, and channel catfish. Gene abbreviations are the following: ADCYAP1R1, adenylate cyclase activating polypeptide 1 (pituitary) receptor type I; IPP, intracisternal A particle-promoted polypeptide; MAST2, microtubule associated serine/threonine kinase 2; ORC1, origin recognition complex subunit 1; PIK3R3, Phosphoinositide-3-kinase regulatory subunit 3; POMGNT1, protein O-linked-mannose beta-1,2-N-acetylglucosaminyltransferase 1; PRPF38A, PRP38 pre-mRNA processing factor 38 (yeast) domain containing A; TMEM69, transmembrane protein 69. Lg: linkage group; Chr: chromosome.

Fig. 6. Expression of PI3K genes in the channel catfish intestine after infection with *Edwardsiella ictaluri*. Gene expression was presented as fold-change relative to control samples. Only those with absolute fold change value of ≥ 1.5 were shown.

Fig. 7. Expression of PI3K genes in the channel catfish gill after infection with *Flavobacterium columnare*. Gene expression was presented as fold-change relative to control samples. Only those with absolute fold change value of ≥ 1.5 were shown.

Fig.8 Relative expression levels of PI3K genes after infection in the gill of channel catfish with different susceptibility to *Flavobacterium columnare* Asterisk (*) indicated absolute fold change value of ≥ 1.5 .