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RESEARCH ARTICLE

GENOME COMPARATIVE OF *EDWARDSIELLA* REVEALS POTENTIAL DEVELOPMENT OF SUSTAINABLE PROPHYLAXES

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ABSTRACT

Aquatic diseases caused by the massive wealth of pathogenic bacteria pose major challenges to the development of a sustainable bio-control method, such as antimicrobial measures and vaccine strategies. Recent advances in genome sequencing technology have revolutionized the field of pathogenic pan-genomics and have also influenced disease management in aqua farms. In this study, *Edwardsiella* strains were differentially classified into four species by a phylogenomics construction based on the pan-genome analysis. *Edwardsiella* species were correctly classified by pan-genome analysis (core gene, dispensable gene, singleton gene) of 15 complete genomes. Based on the presence of the gene repertoires, gene encoding extracellular protein, outer membrane protein, adhesion ability and antigenic sites, 9 genes (*E. ictaluri*), 13 genes (*E. anguilarium*), 9 genes (*E. piscicida*), 12 genes (*E. tadar*), and 14 genes (all species) screened from core-gene of 2686, 2673, 2877, 2920, and 1957 gene, respectively have potential in developing reverse vaccinology strategy to the prevention of *Edwarsiellosis*. The *in-silico* analysis will also help to optimize the time and improve the cross-serotype reaction of vaccines in farmed fish. The RV research implementing pan-genome analysis will be a strategy that is applicable to pathogens in both aquatic and terrestrial animals.

KEYWORDS

Edwarsiella, Phylogenomics, pan-genome, Phylogenetic marker, Reversed vaccine.

1. INTRODUCTION

The sustainability of aquaculture industry is critical both for global food security and economic welfare. However, the massive wealth of pathogenic bacteria is posing a key challenge to the development of a sustainable bio-control method. Challenges of aquaculture disease management are of the biological diversity of pathogens, host-pathogen interactions (e.g. different modes of adaptation and transmission), and shifting environmental pressures, in particular climate change. Hence, comparative genome analyses including the pan-genome analysis are to compare the genomes of different bacterial strains in a species (intra-species) or genus (inter-species) (Snipen et al., 2009). Studies on genomics significantly reveal insights into the understanding of bacterial evolution, proper adaptation, population structure and host interaction, but also can be applied to search such potential development of a sustainable prophylaxes as highly informative identified genetic targets for a rapid diagnostic PCR assay or vaccine candidates and drug design (Chaplin et al., 2015).

Pangasius, *Pangasianodon hypophthalmus,* is the flagship species of South Vietnam's aquaculture industry. It is the world's largest producer and exporter of the species, with production in 2018 of 1,292,100 tonnes, worth about US$ 2,26 billion – or 32.21 per cent of the Vietnam's total aquaculture value. However, the infection of bacterial pathogens, especially caused by *Edwardsiella ictaluri*, has been regarded as a systemic disease associated with severe economic losses due to mass mortality, reduced fish production, and increased treatment costs (Fergusin et al., 2001). For fry, mortality can be as high as 100%, and from 30-50% for meat fish (Dung et al., 2010). Isolation of species *Edwardsiella* spp. from different sources is likely that they are adaptable to changing environmental conditions (e.g. temperature, antibiotics, and hosts) which pose a challenge to developing *Edwardsiellosis* prevention methods. To understand the ecological characteristics of *Edwardsiella* spp., the genome comparative of *Edwardsiella* strains was analyzed to extract genes shared among species that could server for adaptability and species specificity and thus can be used to develop early diagnostic methods by Pan-PCR for clinical samples. In addition, *in-silico* screening of genes that code for cell surface proteins (SEP), including the outer membrane protein and extracellular protein can also be considered as candidates for the producing reverse vaccines in a further fish model. This prophylaxis is believed to be safe and able to cross-react.

2. MATERIALS AND METHODS

2.1 Retrieval of *Edwardsiella* genome sequence from data bank

In this study, a total of 15 complete genomes of the *Edwardsiella* strain isolated from different sources were obtained from NCBI bacterial genome database (ftp://ftp.ncbi.nih.gov/genomes/).

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2.2 Analysis of *Edwardsiella* pan-genome

Comparative pan-genome analysis of hitherto-sequenced *Edwardsiella* strains was performed with EDGAR v2.2 (Blom et al., 2016). Accordingly, genomic subsets, including the number of core genome and singletons (strain-specific) in the gene pool, were extracted to understand the estimation of tracing horizontal gene-flux across strains and obtain insights into their evolution.

2.3 Selection of antigenic genes

For antigenic analyses, genomic subsets of core genes were first subjected to the tools such as PSORTb3.0, CELLO, and SOSUI-GramN to analyze cellular components (subcellular localization), SPAAN software to analyze adhesin probability, and VaxiJen server to predict genes with high antigen. VaxiJen is based on auto cross covariance (ACC) and has a threshold of 0.5 in the antigeniity value (Doytchinova and) [9]. For prediction of B-cell Linear Epitope and Cytotoxic T-cell (CTL) epitopes, the screening genes were further investigated by available B-cell Linear Epitope at IEDB sever (http://tools.iedb.org/) including calculation of flexibility, hydrophilicity and surface accessibility of the predicted epitope with default settings, while CTL Epitopes that can bind MHC class I and class II molecules were predicted by CTLPred sever (http://crdd.osdd.net/) (Momtaz et al., 2019).

2.3 Vaccine candidate selection

The vaccine candidates were selected using all the results obtained. The candidates possess the best values of the parameters calculated and exert diverse functions that render them useful as different targets in the microorganism.

3. Result and Discussion

3.1 Pan-Genomic Analysis of *Edwarsiella*

As shown in the pan-genome graph (Fig. 1A), the size of pan-genome inferred from genome sequences of 15 *Edwardsiella* strains was constituted by 6,733 orthologous groups. The core-gene consists only 29.07% of which (1,957) were core genes, and the remaining 70.93% were dispensable and singleton genes. A pan development plot analysis shows an open pan-genome model with the value from the Heap's Law function ranging between 0 and 1 (0.302, Fig. 1A), indicating that *Edwardsiella* spp. can adapt to a variety of environments (Tettelin et al., 2005).

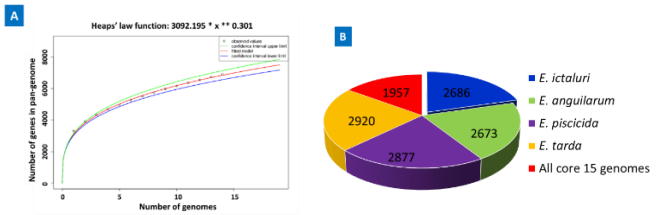

Figure 1: Pan-genome analyses of 15 *Edwardsiella* strains. (A) Pan-genome development extrapolation of 15 strains indicated an open pan-genome according to Heap's Law model. (B) Calculated core gene sets to each species group. These were extracted using EDGAR. Each species is grouped by polymorphism of dispensable genes among *Edwardsiella* strains indicated in Fig. 2.

Polymorphism of dispensable genes among *Edwardsiella* strains showed that 15 *Edwardsiella* strains were divided into 5 categories as follows: *E. ictaluri* including strains 93-146, RUSVM-1 and MS-17-156; *E. anguilarum* including ET080813, EA181011 and LADL05-105; *E. piscicida* includes C07-087, S11-285, ETW41, ET-1, EIB202 and FL6-60 (with ET-1, EIB202, FL6-60 previously identified as *E. tadar*); *E. tadar* include FL95-01 and KC-Pc-HB1; *E. hoshinae* ATCC_35051. The core genes of 4 species of *E. ictaluri, E. anguilarum, E. piscicida, E. tadar* and total 15 strains were extracted (Fig 1B) in order to screen vaccine candidate genes. For *E. hoshinae*, the NCBI data showed that only one complete genome was published at the time of analysis, so they were not selected to isolate the core gene in this study. However, *E. hoshinae* is also considered to be a pathogen (Janda et al., 1991), so they were analyzed with all the remaining strains to find the genes involved in development. multidrug vaccine. In particular, this polymorphism analysis would provide very valuable information on species-specific control measures against *Edwardsiellosis*. For instance, the gene presence/absence (white box in Fig 2) may indicate good markers for discriminatory molecular techniques, an unending search to accurately identify *Edwardsiella* isolates, especially when differentiating new species from *E. tarda* (Bujan et al., 2018; Fogelson et al., 2016).

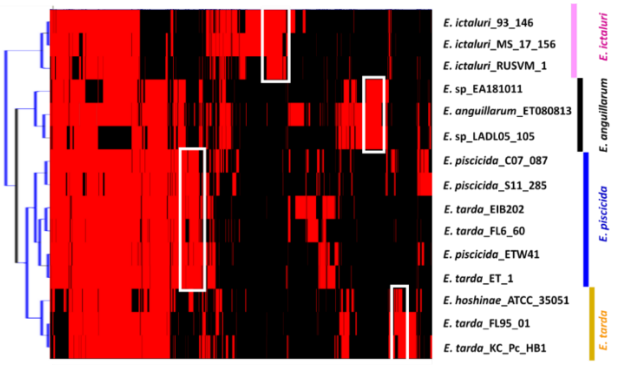

Figure 2: Polymorphism of *Edwardsiella* dispensable genes. The presence/absence of 2578 dispensable genes is shown in red/black.

3.3 Vaccine candidate selection from SEPs

As shown in Fig 3. genes coding for 73, 69, 72, 77 and 96 SEPs were respectively selected from 2686 core genes of *E. ictaluri*, 2673 core genes of *E. anguilarum*, 2877 core genes of *E. piscicida*, 2920 core genes of *E. tadar*, and 1957 core genes from total 15 *Edwardsiella* strains tested. These genes were further screened using the calculated characteristics of B-cell Linear Epitope and CTL Epitopes. The number of vaccine candidates was then reduced to 9, 13, 9, 12 and 14 genes representive for *E. ictaluri*, *E. anguilarum*, *E. piscicida, E. tadar* and total 15 *Edwardsiella* strains examined, respectively, with a stricter selection, as mentioned in the Methods section. These candidates are belived to be potential candidates for use in development of vaccine (Table 1).

In fact, the SEPs are considered potential candidates in the vaccine production model for animals and fish. For example, the expression of the *esa*1 gene from *E. tarda*, a surface antigen like D15, in the Japanese flounder model has resulted in the wide spectrum of genes expression that may be involved in both natural and specific immunity, as well as increase the survival rate of fish and the ability to produce specific serum antibodies (Sun et al., 2011). In previous, the researchers have been looked for gene function based on one genome sequence. In this study, pan-genome application combined with functional characteristics of SEPs analysis for *Edwardsiella* strain may initially help to reduce the time and the number of tested candidates. In particular, for disease caused by *E. ictaluri*, 9 candidates was found in this study will play a very important role in the screening and development of the experimental vaccine against *Edwarsiellosis* in Pangasius and other freshwater fishes in Vietnam. Moreover, the 14 gene candidates can be very important for further assay to enhance the effectiveness of a reverse vaccine to prevent *Edwarsiellosis* caused by multiple *Edwardsiella* species.

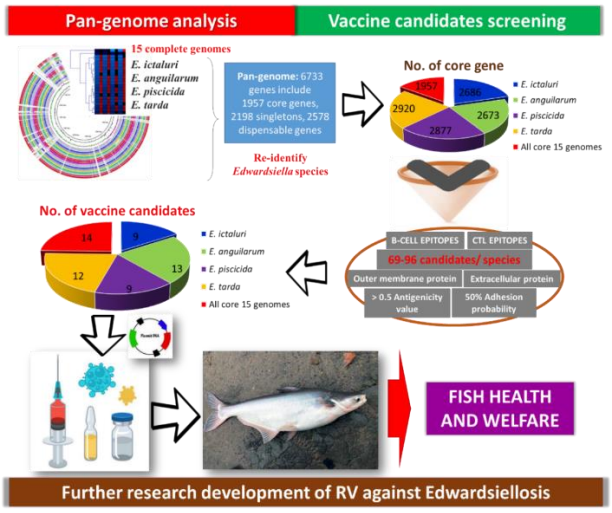

Figure 3: Diagram of strategy for development of reverse vaccine against *Edwardsiellosis* implementing with pan-genome analysis in research.

4. Conclusion

This results may contribute to the strategy for screening antigens that are consistent with the characteristic of reverse vaccine candidates and has the potential use for cross-react vaccine curing aquatic pathogenic *Edwarsiella* (Fig 3). In addition, the increase of aquatic pathogenic

bacterial genome sewuences will allow to develop a rapid assay to control disease outbreak, and also give valuable data for improved treatment results. A reversed vaccines to prevent diseases tend to change continuously in aquaculture. Thus, our screening strategy combined with pan-genome analysis data may become a routine tool in the lab that can distinguish all clinically relevant *Edwardsiella* strains and develop prophylaxis to prevent pathogens in both aquatic and terresteral animals.

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