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Literature study

New Development of *Edwarsiella Tarda* Infection

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Abstract:

Edwardsiella tarda is a pathogen in aquatic animals and as an opportunistic pathogen for humans. This bacteria is known to cause gastroenteritis and wound infections. There is evidence to suggest that these microorganisms can be a public health problem, as well as a threat to other animals such as pigs and cows. There is a cathepsin H (CsCatH) homology of C. semilaevis, in the healthy palm of the tongue, transcriptional expression of CsCatH was detected in nine different tissues. Knockdown of CsCatH and CsCatB significantly increased *Edwardsiella tarda*-induced replication and reduced *Edwardsiella tarda*-induced apoptosis. These findings reveal the importance of CsCatH and CsCatB in the anti-bacterial immunity of the tongue. Subsequent studies suggest that the flagellin secreted from *Edwardsiella tarda* may be a responsible factor for macrophage stimulation activity. The virulence factors of these bacteria are the secretion system type III, type VI and other proteins. *Edwardsiella tarda* can be identified and characterized by agglutination test methods, Enzyme-Linked Immunosorbent Assay (ELISA), and fluorescent antibody techniques, real-time PCR, loop mediated isothermal amplification (LAMP), and multiplex nested PCR.

Keywords: *E. tarda*, CsCatH, CsCatB, flagellin

1. Introduction

Edwardsiella is the bacteria that causes the systemic bacterial disease Enteric Septicemia of Catfish (ESC). *Edwardsiella tarda* is a disease-causing agent that is often found to cause failure in catfish cultivation and various other types of farmed fish in Indonesia. *Edwardsiella tarda* is a facultative anaerobic bacterium that is also pathogenic in humans but rarely, but in patients with certain risk factors, can cause severe and widespread infections. In humans, it is inoculated through the digestive tract when consuming undercooked or raw seafood or through skin penetration. Edwardsiella tarda has been isolated from the marine environment, including lakes, rivers, wells, and wastewater (Murray, et. al., 2020, Pandey, et. al., 2021).

Although the bacteria have not been isolated directly from seawater, they have been bred from animals that inhabit seawater environments. Complications from these bacteria usually arise in patients with liver disease, excess iron, or cirrhosis or in those who are immunocompromised or immunosuppressive therapy. Histopathological observations are carried out to provide an overview of changes in the tissues infected with the disease. Diagnosis of disease infection is the first step that needs to be implemented, in determining the disease. In the process of diagnosing disease infections, there are several things that need to be considered, namely clinical signs which include behavior, external and internal characteristics, and pathological changes (CABI, 2022).
Because of the importance of this problem, this paper discusses the problem, epidemiology, morphology, characteristics, virulence factors, pathogenesis, and the latest research on the bacteria that causes unusual infectious diseases, namely *Edwardsiella tarda.*

2. Problems and Epidemiology

Edwardsiella is a genus of gram-negative and fermentative bacteria. Members of the Enterobacteriaceae based on biochemical and physiological characteristics. *Edwardsiella* is a pathogen for aquatic animals and occasionally an opportunistic pathogen for humans. They are known to cause gastroenteritis and wound infections. The most relevant species of the genus *Edwardsiella* is *Edwardsiella tarda*. The other two species, *Edwardsiella hoshinae*, and *Edwardsiella ictaluri*, are less clinically relevant. Biochemically similar to *E. coli* with the exception that *Edwardsiella tarda* produces hydrogen sulfide. This species is associated with freshwater ecosystems (ITIS, 2023)

Edwardsiella was first discovered in snakes in 1962. The source of *Edwardsiella tarda* may be the intestinal contents of carrier animals, but it may be a common inhabitant of aquatic environments. *Edwardsiella tarda* is a ubiquitous organism, isolated from animals or the environment on most continents and found in freshwater and marine waters. The list of countries where *Edwardsiella tarda* is found is very long, but most include the United States, Japan, Taiwan, Thailand, Israel, and many developing countries. *Edwardsiella tarda* poses a health threat to other animals, including humans (Tingting & Xiao, 2014).

In fact, some of the first isolates of *Edwardsiella tarda* came from human waste. In humans, bacteria usually cause diarrhea and gastroenteritis, while extraintestinal infections can cause diseases such as typhus, peritonitis. with sepsis and cellulitis. Occasionally, an abscess induced by *Edwardsiella tarda* is seen in the liver. Several other clinical conditions in humans have been associated with *Edwardsiella tarda*, including meningitis. In 1988 by Funada, it was discovered that *Edwardsiella tarda* septicemia was a complication in acute leukemia patients in Japan, while in 1971 by Gilman, it was argued that the organism was involved in forest diarrhea and may be related to *Entamoeba histolytica* (protozoa) infection in Thailand. In 1980, Van Damme and Vandepitte reported that sporadic cases of tropical diarrhoea in humans with *Edwardsiella tarda* were traced to freshwater fish consumption in Zaïre. Serious and potentially life-threatening *Edwardsiella tarda* infection of the muscles, resulting from wounds received during fishing or puncture wounds caused by catfish spines, has been described in humans (Park, et.al., 2012).

In 1979, according to researcher Wyan, he was unable to correlate or prove *Edwardsiella tarda* in aquatic animals with human infection. However, there is enough evidence to suggest that the organism can be a public health problem, as well as a threat to other animals such as pigs and cows. Then, in 2002, according to Nucci, *Edwardsiella tarda* isolate from fish is similar to that of human origin, therefore fish isolate is a potential human pathogen. Because *Edwardsiella tarda* has extensive host vulnerability and environmental adaptation that can enter the human food chain through fish processing plants and/or poor sanitation (Wang, et.al., 2023).

3. Classification Morphology and Characteristics

The species *Edwardsiella* is an oxidase-negative, catalase-positive, Gram-negative bacillus that is motile through peritrichous flagella. These bacteria ferment D-glucose as an energy source. The genus is said to be a "strict phenotype", showing little biochemical variability in individual reactions. *Edwardsiella tarda*, the human pathogen of this group, is most biochemically similar to the Salmonella species, a fact that sometimes leads to misidentification. More than 60 somatic antigens (0) and 45 flagellates (H) are currently recognized by the international typing scheme developed for this species. Its most prominent phenotypic feature, however, is its ability to produce hydrogen sulfide in a number of common laboratory media, including triple sugars and Kliger iron agar. The nickname tarda, which means inactive, refers to the inability of this species to ferment most carbohydrates (Murray, et. al., 2020, Pandey, et.al., 2021).

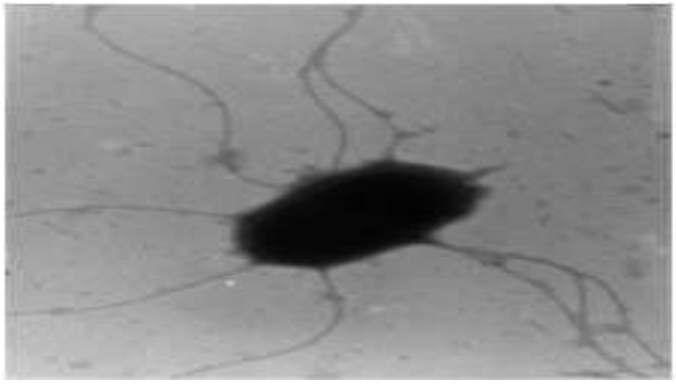

Figure 1. *Edwardsiella tarda* H1 with peritrichous flagella (Tingting & Xiao, 2014).

The following is the taxonomic hierarchy or classification of Edwardsiella tarda according to the Integrated Taxonomic Information System (ITIS) Report (2023) :

Kingdom	: Bacteria
Subkingdom	: Negibacteria
Filum	: Proteobacteria
Kelas	: Gammaproteobacteria
Ordo	: Enterobacteriales
Famili	: Enterobacteriaceae
Genus	: Edwardsiella
Spesies	: *Edwardsiella tarda*

Two *Edwardsiella tarda* biotypes exist in nature, the most commonly known as the wild type or the classic biotype. This group strain can be distinguished from the biogroup 1 strain based on the ability of the wild-type strain to produce hydrogen sulfide and reduce tetrathionate and its inability to ferment some of the fermented sugars of the biogroup 1 strain. Wild-type strains are usually involved in infections in humans and animals, while biogroup 1 isolates have been found only from snakes and water. On rare occasions, wild-type strains with a single atypical reaction have been isolated from the infectious process in humans (Sun, et. al., 2023)

Due to the unique biochemical characteristics of wild-type strains (sucrose and lactose-negative, H2S-positive), mostly selective and differential agar not only favors the growth of *Edwardsiella tarda* but also produces suspected colonies that must be screened for potential gastrointestinal pathogens in fecal examination. Selenite and GN broths are excellent for recovering *Edwardsiella tarda* from the gastrointestinal contents of people with subacute or chronic diarrhea or with career status. Selective media has also been developed recently for the recovery of *Edwardsiella tarda* from environmental samples (Healey, et.al., 2021).

4. Pathogenesis and Virulence Factors

The study concluded that the LD value of 50 Edwardsiella tarda for grass carp was 1.3 × 10 9 CFU ml−1 and 60% of the fish died within 24 hours of infection after receiving the bacterial inoculum 10 10 CFU ml−1. Histopathological changes that occur in post-challenge goldfish include edema and secondary lamellar fusion as well as basal epithelial layer hyperplasia between secondary lamellas in the gills (Murray, et. al., 2020)

Furthermore, submucosal to mucosal and finally muscular disorders in the intestines, hepatocyte necrosis and infiltration of red blood cells in the liver, tubular disintegration in the kidneys and loss of the red pulp capsule boundary in the spleen (Pandey, et. al., 2021)

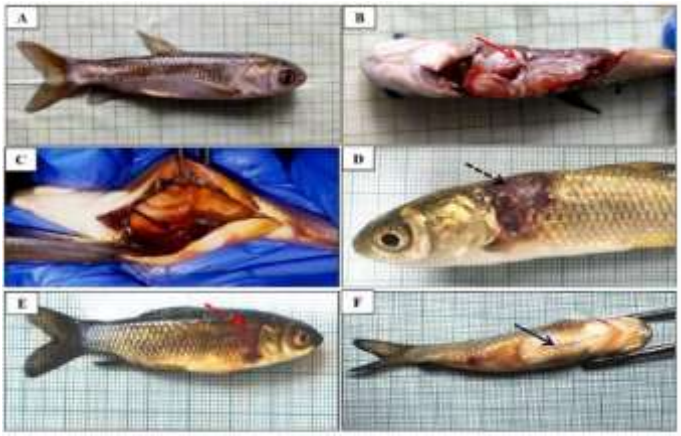

Figure 2. Pathological symptoms of *Edwardsiella tarda* infection in goldfish test samples. Grass (C-E) and Control group (A and B) (Pandey, et. al., 2021)

In figure 2 above, it is determined that part A is a healthy fish from the control group. Part B is the internal organs of the healthy fish of the control group (indicated by the red arrow). Part C is the accumulation of abdominal fluid and organ damage (indicated by a black arrow). Lesion D on the posterior operculum (indicated by a black dotted arrow). Part E is a sign of bleeding (indicated by a red dotted arrow). Part F represents internal bleeding and abdominal edema (indicated by the blue arrow) (Tingting & Xiao, 2014).

Edwardsiella tarda survives in its host by utilizing several important substances and abilities that function as virulence factors in the host. A study using green fluorescent protein (GFP) showed that both avirulent and virulent Edwardsiella tarda were able to attach, invade, and replicate in carper epithelial papilloma (EPC) cell lines using host microfilaments and tyrosine kinase proteins. Histopathology and infection kinetics studies using GFP revealed that the gills, gastrointestinal tract, and body surface of the blue gourami (Trichogaster trichopterus) are the entry sites of virulent strains (Park, et.al., 2012).

Table 1. Virulence factors of *Edwardsiella tarda* (Park, et.al., 2012)

Abbreviation	Name	Accession number	Function
Type III secretion systems			
EsaB	putative TTSS apparatus protein B	AAV69410	apparatus
EsaC	putative TTSS apparatus protein C	AAV69411	apparatus
EsaD	putative TTSS apparatus protein D	AAV69412	apparatus
EsaG	putative TTSS apparatus protein G	AAV69415	apparatus
EsaH	putative TTSS apparatus protein H	AAV69416	apparatus
EsaI	putative TTSS apparatus protein I	AAV69417	apparatus
EsaJ	putative TTSS apparatus protein J	AAX76915	apparatus
EsaK	putative TTSS apparatus protein K	AAX76913	apparatus
EsaL	putative TTSS apparatus protein L	AAV69401	apparatus
EsaM	putative TTSS apparatus protein M	AAX76922	apparatus
EsaN	putative TTSS apparatus protein N	AAX76920	apparatus
EsaQ	putative TTSS apparatus protein Q	AAV69420	apparatus
EsaR	putative TTSS apparatus protein R	AAX76923	apparatus
EsaS	putative TTSS apparatus protein S	AAV69419	apparatus
EsaT	putative TTSS apparatus protein T	AAX76924	apparatus
EsaU	putative TTSS apparatus protein U	AAV69421	apparatus
EsaV	putative TTSS apparatus protein V	AAX76921	apparatus
EscA	putative TTSS chaperone protein A	AAV69403	chaperone
EscB	putative TTSS chaperone protein B	AAX76917	chaperone
EscC	putative TTSS chaperone protein C	AAV69402	chaperone
EseB	putative TTSS effector protein B	AAX76903	effector
EseC	putative TTSS effector protein C	AAV69404	effector
EseD	putative TTSS effector protein D	AAV69405	effector
EseE	putative TTSS effector protein E	AAV69406	effector
EseG	putative TTSS effector protein G	AAX76916	effector
EsrA	TTSS regulator protein A	AAV69423	regulator
EsrB	TTSS regulator protein B	AAX76904	regulator
EsrC	TTSS regulator protein C	AAV69414	regulator
Type VI secretion systems			
EvpA	*E. tarda* virulent protein A	AAR83927	apparatus
EvpB	*E. tarda* virulent protein B	AAR83928	apparatus
EvpC	*E. tarda* virulent protein C	AAR83929	extracellular apparatus
EvpD	*E. tarda* virulent protein D	AAR83930	apparatus
EvpE	*E. tarda* virulent protein E	AAS58123	apparatus
EvpF	*E. tarda* virulent protein F	AAS58124	apparatus
EvpG	*E. tarda* virulent protein G	AAS58125	apparatus
EvpH	*E. tarda* virulent protein H	AAS58126	apparatus
EvpI	*E. tarda* virulent protein I	ABW69081	extracellular apparatus
EvpJ	*E. tarda* virulent protein J	ABW69082	apparatus
EvpK	*E. tarda* virulent protein K	ABW69083	apparatus
EvpL	*E. tarda* virulent protein L	ABW69084	apparatus
EvpM	*E. tarda* virulent protein M	ABW69085	apparatus
EvpN	*E. tarda* virulent protein N	ABW69086	apparatus

EvpO	*E. tarda* virulent protein O	ABW69087	apparatus
EvpP	*E. tarda* virulent protein P	ABW69080	extracellular apparatus
The other proteins			
AidA	putative autotransporter protein AidA	BAH03175	autotransporter adhesin
HhaEt	α-hemolysin-modulator like protein	YP_003295064	nucleoid-associated proteins
EthA	*E. tarda* hemolysin A	BAA21097	hemolysin
EthB	*E. tarda* hemolysin B	BAA21096	hemolysin activation/secretion
QseB	DNA-binding transcriptional regulator QseB	ADO13165	Quorum sensing (QS) system
QseC	sensor protein QseC	ADO24152	Quorum sensing (QS) system
PhoP	two-component regulator protein PhoP	ADB28435	DNA-binding transcriptional regulator
PhoQ	two-component sensor protein PhoQ	ADB28436	sensor

The III-type secretory system (T3SS) and the VI-type secretory system (T6SS) play an important role in the adherence, penetration, survival, and replication of *Edwardsiella tarda* in epithelial and phagocyte cells (Table 1). The T6SS of Edwardsiella tarda consists of 16 genes, and 13 of the encoded proteins are involved in the secretion of EvpP (*Edwardsiella tarda* virulence protein). Three proteins (EvpP, EvpI and EvpC) are secreted into the extracellular environment, and the secretion of EvpC and EvpI is required for EvpP secretion. The ATPase is suspected, EvpO, contains the motive of Walker A, which may interact with EvpA, EvpL, and EvpN. T3SS is a multi-protein complex that is important for host-pathogen interactions. The main component of T3SS is the needle complex, which is structurally similar to the bacterial flagella, which span the inner and outer membranes of bacteria. These needles can be connected to the host cell membrane through the end complex via a translocon, which allows the delivery of bacterial effector proteins from an ATPase-dependent way. In Edwardsiella tarda, the T3SS protein includes the *Edwardsiella tarda* protein. equipment of tarda secretion systems (EsaB and EsaN), effectors (EseB, EseC and EseD), companions (EscA, EscB and EscC), and regulators (EsrA, EsrB and EsrC). Proteomic studies have revealed that EseB, EseC and EseD are the main ECPs, and mutations of these genes in *Edwardsiella tarda* reduce virulence compared to parent Edwardsiella tarda (Park, et.al., 2012).

Some reports have found that motility-related proteins, such as flagellin and autotransport adhesin AIDA, a fimbrial adhesin-like protein, are important for attachment and penetration into host epithelial cells. The *Edwardsiella tarda* mutant containing the ethA gene deletion (the hemolysin gene locus of Edwardsiella tarda), regulated by the two-component system EsrA-EsrB and the HhaEt nucleoid protein, shows a decrease in capacity to internalize into EPC cells (Park, et.al., 2012). Interestingly, a recent study showed that the two-component qseB and qseC systems of *Edwardsiella tarda* inhibit flagella biosynthesis and motility, and induce T3SS expression after invasion into host cells. These findings show that *Edwardsiella tarda* is able to modulate the expression of genes involved in adaptation to environmental changes, such as adaptation to intracellular life (Park, et.al., 2012).

Edwardsiella tarda is able to survive and adapt to a variety of host environmental conditions, including changes in host hormones, temperature, pH, salinity, and variations in several important nutrient elements, such as iron, phosphate, and Mg2+. The two-component system qseB and qseC, important virulence regulators that contribute to intracellular replication and systemic infection, is capable of regulating flagella motility and expression of the intracellular T3SS elements of EseB and EsaC in response to eukaryotic hormone-like signals, such as epinephrine and norepinephrine. *Edwardsiella tarda's* two-component PhoP-PhoQ system is able to sense changes in temperature and concentration of Mg2+ and control T3SS and T6SS through esrB activation. The study showed that changes in PhoQ conformation in the temperature range of 23-37°C and at low Mg2+ concentrations led to PhoQ autophosphorylation and subsequent activation of PhoP, which promotes esrB expression and causes the secretion of virulent proteins, while below 20°C or above 37°C, there is no conformational change in PhoQ and the production of virulence proteins decreases (ITIS, 2023). Similarly, based on observations of mutants containing the insertion of the pstSCAB-phoU operon, which is part of the phosphate regulator, Srinivasa Rao et al. suggested that the natural condition of low inorganic phosphate in phagocyte and epithelial cells may stimulate virulen genes to improve survival and replication in the host. In another study, high concentrations of NaCl (3%) were shown to induce hemagglutination activity, which correlated with the expression of the fimbrial major subunit (FimA), a 19.3 kDa protein; In addition, Edwardsiella tarda enriched with fimbrial protein showed higher virulence in challenge trials compared to Edwardsiella tarda raised in 0% NaCl broth (ITIS, 2023).

The ability of bacteria to acquire iron acquisition using bacterial iron chelators, siderophores, is essential for bacterial survival and replication. Natural mutants with lower siderophore production and mutants with the coding of the aryl sulfate sulfotransferase gene that produce fewer siderophores showed significantly reduced virulence in the *Edwardsiella tarda* challenge experiment. Recently, a mutant of the *Edwardsiella tarda* declement that lacks the T6SS evpP component, which encodes the ferric uptake regulator (Fur) consensus box, exhibits low virulence in vivo and in vitro. These findings may suggest that the EvpP protein in T6SS plays an important role in the invasion mechanism and thus may be a critical virulence factor (Wang, et.al., 2023).

It has been reported that *Edwardsiella tarda* produces two types of hemolysine; one is a related cell, iron-regulated hemolysine, encoded by ethA and ethB, which is secreted as an extracellular protein (ECP) under iron-regulated conditions, and the other is extracellular hole-forming hemolysine that is different from EthA and EthB that is not regulated by iron. A recent functional study showed that EthA is essential for in vivo and in vitro invasion, and is regulated by the two-component system EsrA-

EsrB and the nuclear protein HhaEt. Other enzymes, including catalase, chondroitinase, dermatotoxins, proteases, and collagenases, are also important for the pathogenesis of *Edwardsiella tarda* (Zulhan et.al., 2023).

Some studies show that *Edwardsiella tarda* is able to survive and replicate in phagocytes, causing systemic infections. Virulent *Edwardsiella tarda* opsonized with blue gourami serum can replicate within phagocytes and fail to induce oxidative explosions, allowing for a mechanism to avoid phagocyte-mediated killing. A subsequent study revealed that the expression of the catalase gene (KatB) Edwardsiella tarda was responsible for resistance to H2O2 and phagocyte-mediated killing. Similarly, a comparison of the peritoneal macrophage response of olive flounder to the high and low virulence of *Edwardsiella tarda* showed that only highly virulent strains were able to resist the reactive oxygen species produced by macrophages, and survive and replicate within macrophages. In the next report, the authors of this latest study expanded on their results, showing that virulent *Edwardsiella tarda* produced significantly greater nitric oxide induction and tumor necrosis factor (TNF)-α by macrophages, an action that could explain the pathogenicity of *Edwardsiella tarda* infection. In addition, a study on Edwardsiella tarda septicemia revealed that *Edwardsiella tarda* induces systemic immunosuppression through lymphocyte apoptosis, which suppresses the systemic immune response during the early stages of septicemia (Sun, et. al., 2023).

Understanding the virulence factors of *Edwardsiella tarda* can inform the development of protection strategies against edwardsiellosis in fish. Recent advances in analytical methods, such as genomics and proteomics, have revealed important virulence factors, including T3SS, T6SS, and two-component systems. Verjan et al. demonstrated seven antigenic proteins, identified as lipoproteins, periplasmic proteins, exported, and secreted proteins. Additional proteomic studies on outer membrane proteins (OMPs), ECPs, and outer membrane vesicles (OMVs) may also contribute to the development of effective protection strategies against edwardsiellosis. In addition, knowing the complete genome sequence of *Edwardsiella tarda* will improve our understanding of the relationship between *Edwardsiella tarda* and the host, and further develop novel prophylactic and therapeutic strategies for managing edwardsiellosis in fish (Healey, et.al., 2021).

Laboratory Examination

Edwardsiella tarda is usually identified based on its unique biochemical characteristics after being isolated on a brain-heart infusion agar or soy tryptone agar from infected fish. Several studies have shown serological techniques are useful for the diagnosis of Edwardsiella tarda infection, including agglutination tests, Enzyme-Linked Immunosorbent Assay (ELISA), and fluorescent antibody techniques. Currently, PCR-based methods have been reported for accurate, sensitive, and differential diagnosis. Real-time PCR has been used to analyze the blood of infected oyster toadfish (Opsanus tau) (Azaldin, et.al., 2020).

The loop mediated isothermal amplification (LAMP) method is also able to detect infected tissue samples and pond water. Researchers have also. developed a multiplex nested PCR for four important fish pathogens in subtropical Asia that can simultaneously detect A. hydrophila, Edwardsiella tarda, Photobacterium damselae and Streptococcus iniae from pure colonies and tissue homogenates. In addition, a set of primers, evaluated using 53 strains of Edwardsiella tarda isolated from various sources and 18 representative strains of related and unrelated bacterial species, was shown to be able to detect 2 cells from pure cultures and 3 × 102 cells in superior turbot tissues (Sun, et.al., 2023).

New developments in research results on *Edwardsiella tarda*

There was a study on cathepsin H and cathepsin B of Cynoglossus semilaevis involved in anti-bacterial immunity against Edwardsiella tarda. Cathepsin H and B are two lysosomal cysteine proteases that participate in various physiological processes including immune responses. In fish, the functional roles of Cathepsin H and Cathepsin B during bacterial infection are poorly understood. In previous work, researchers characterized the homologous cathepsin B (CsCatB) of the palm of the tongue (Cynoglossus semilaevis), a species of fish of economic value in China (Wang. et.al., 2023).

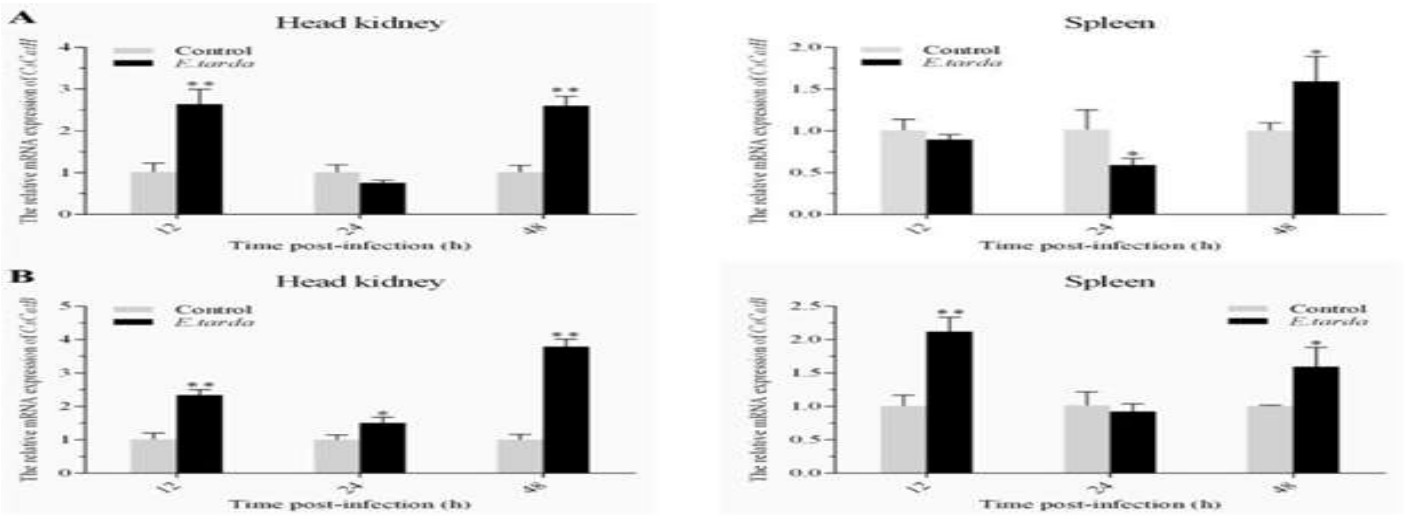

Figure 3. Effect of *Edwardsiella tarda* infection on CsCatH atau CsCatB expression (Wang. et.al., 2023)

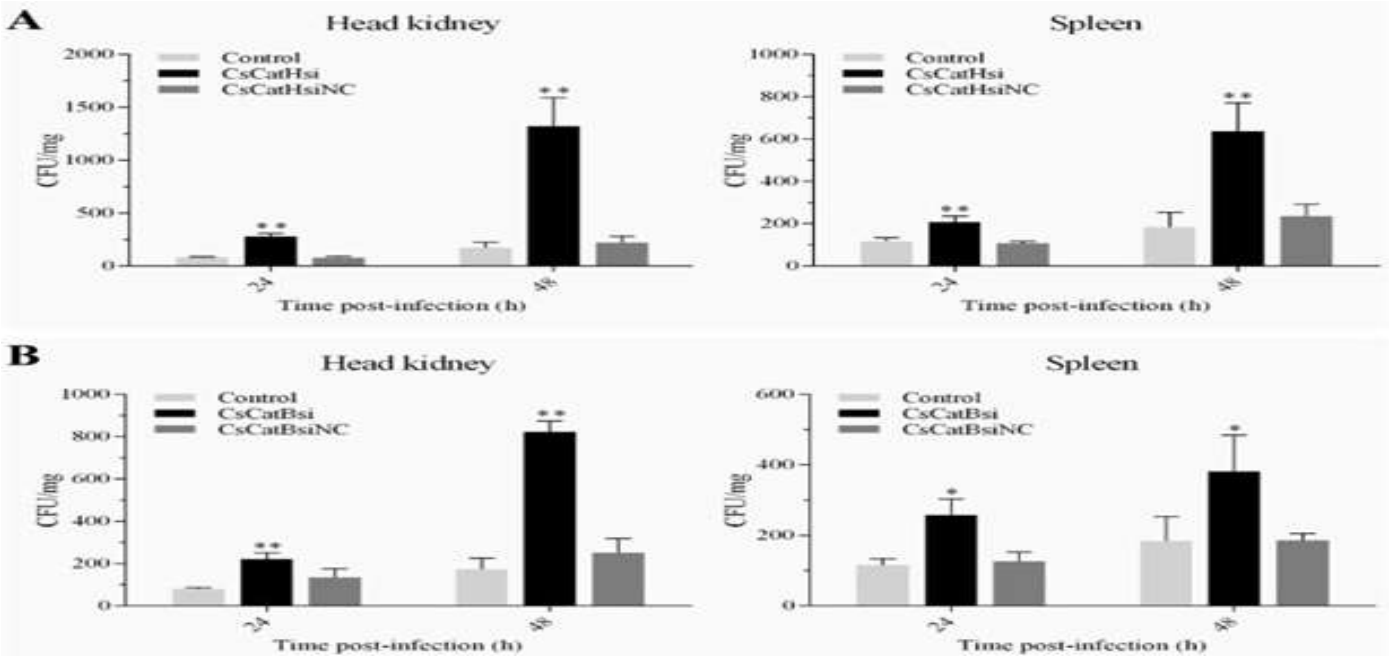

Figure 4. Effect of CsCatH/CsCatB knockdown on *Edwardsiella tarda* infection (Wang. et.al., 2023)

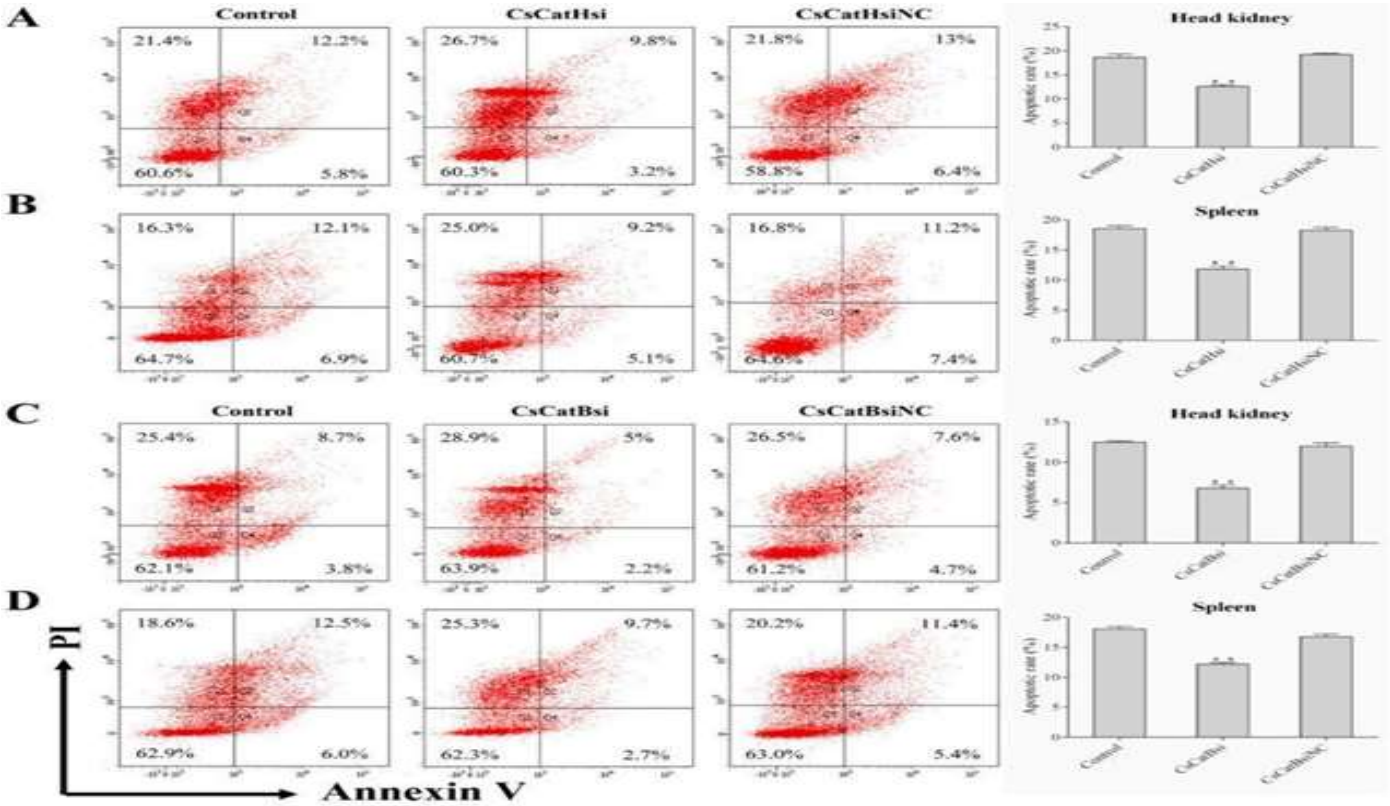

Figure 5. Effect *Edwardsiella tarda* infection on CsCatH atau CsCatB expression (Wang. et.al., 2023)

In this report, researchers identified the homologous cathepsin H (CsCatH) from *C. semilaevis*. In healthy tongue palms, CsCatH transcriptional expression was detected in nine different tissues. Confocal microscopic analysis of laser scanning showed that ectopic expressed CsCatH and CsCatB were localized together with lysosomes. After being infected by *Edwardsiella tarda*, a significant fish pathogen that causes a severe fish disease called edwardsiellosis, the expression of CsCatH and CsCatB is highly regulated. Knockdown of CsCatH and CsCatB significantly increased *Edwardsiella tarda*-induced replication and reduced *Edwardsiella tarda*-induced apoptosis in single tongue tissue. These findings reveal the importance of CsCatH and CsCatB in the anti-bacterial immunity of the tongue (Sun. et.al., 2023).

Further research is on cloning, DNA sequencing, and flagellin expression of *Edwardsiella tarda* strains with high and low virulence and their macrophage stimulation activities. *Edwardsiella tarda* is the pathogen that causes edwardsiellosis in fish. Previous research studies on high (NUF251) and low (NUF194) strains of *Edwardsiella tarda* showed that the NUF251 strain induced significantly higher levels of NO and TNF-α from fish and mouse macrophages than the NUF194 strain. Subsequent studies suggest that proteins such as flagelin secreted from *Edwardsiella tarda* may be a factor responsible

for the stimulation activity of macrophages. To evaluate the flagellin activity of *Edwardsiella tarda*, in this study, the flagellin genes of the NUF251 and NUF194 strains were isolated by PCR and cloned to the pQE-30 and pCold I expression vectors, and then the recombinant flagellins of the two strains were overexpressed in E. coli on JM109 and pG-Tf/BL2 (Healey. et.al., 2021).

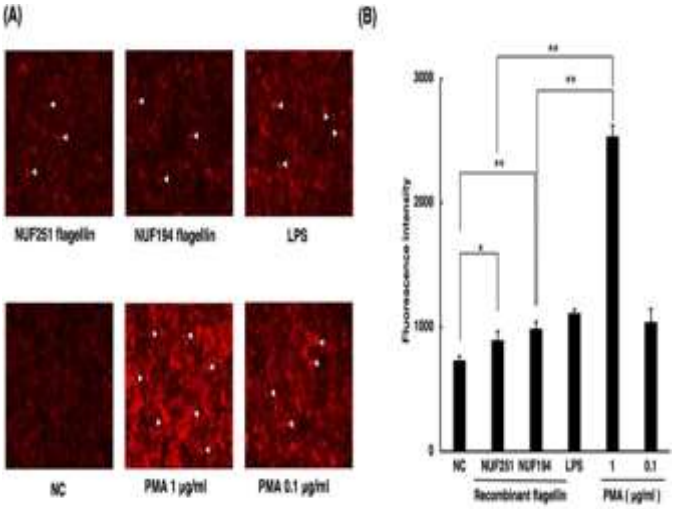

Figure 6. (A) Intracellular O2 levels in RAW264.7 cells were stimulated with recombinant Edwardsiella tarda flagellin analyzed with fluorescence microscopy. (B) After cell treatment, the fluorescence intensity of each well was measured with an excitation wavelength of 518 nm and an emission wavelength of 605 nm by a fluorescence micro plate reader (Elfrida. et.al., 2022).

Molecular weight of pure recombinant flagellin from NUF251 and NUF194 strains respectively estimated at 45 kDa and 37 kDa in the SDS-PAGE analysis. Referring to the three-dimensional structure of the Salmonella flagellin, which has been reported to have 4 domains (D0, D1, D2, and D3), the high-order homology between the two flagellins of Edwardsiella tarda was observed in the conservative domain (D0 and D1) regions, while the equivalent sequences with the D2 and D3 domains were different, and even equivalent to 57 amino acids removed in NUF194. Both recombinant flagellins induced NO production, mRNA expression levels from inducible NO synthase (iNOS), and intercellular ROS production within the RAW264.7 cell line mouse macrophages. Also, TNF-α secretion and its mRNA expression rate increased with the second treatment of recombinant flagellin (Rosidah, et.al., 2022).

These results suggest that recombinant flagellins from different strains of Edwardsiella tarda virulen can stimulate macrophages at almost the same rate as those assessed by the tested parameters. Although there are differences in structure and molecular weight, it suggests that the conservative D0 and D1 domains are quite structural. This recombinant flagellin element can induce a specific level of macrophage stimulation in vitro. Further research is needed to focus on the role of the D2 and D3 domain regions of recombinant flagellins as macrophage stimulating agents and their effects on the immune system of in vivo hosts (Eka, et.al., 2019).

Conclusion

Edwarsiella tarda has extensive host vulnerability and environmental adaptation can enter the human food chain through fish processing plants through sanitation that bad. Cathepsin H and cathepsin B of Cynoglossus semilaevis are involved in anti-bacterial immunity against *Edwardsiella tarda*. The virulence factors of *Edwardsiella tarda* are type III, type IV secretion system and other proteins. *Edwardsiella tarda* can be identified and characterized by agglutination test methods, Enzyme-Linked Immunosorbent Assay (ELISA), and fluorescent antibody techniques, real-time PCR, loop mediated isothermal amplification (LAMP), and multiplex nested PCR. Various basic to clinical and applied aspects that remain questions remain to be answered and further research efforts are still needed to study the pathogenesis of *Edwardsiella tarda*. Thus, it is hoped that it can continue to fight.

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