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OmpR Is Essential for Growth and Expression of Virulence-related Genes in the Fish Pathogen *Edwardsiella piscicida*

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Edwardsiella piscicida is a significant cause of hemorrhagic septicemia in fish and gastrointestinal infections in humans. Survival bacteria require specialized mechanisms to adapt to environmental fluctuations. Hence, to understand the mechanism through which *E. piscicida* senses and responds to environmental osmolarity changes, we determined the protein expression profile and physiological properties under various salinity conditions in this study. The OmpR protein is a part of the EnvZ-OmpR two-component system that has been implicated in sensing salt stress in bacteria. However, the physiological role played by this protein in *E. piscicida* remains to be elucidated. Therefore, in this work, the function of the OmpR protein in response to salt stress was investigated. Phenotypic analysis revealed that, in the mutant, three of the biochemical phenotypes were different from the wild type, including, citrate utilization, hydrogen sulfide, and indole production. Introduction of the plasmid containing the entire *ompR* gene to the mutant strain returned it to its parental phenotype. The retarded growth rate also partially recovered. Furthermore, in our studies, OmpR was not found to be related to cell motility. Taken together, our results from the mutational analysis, the growth assay, MALDI-TOF MS, qRT-PCR, and the phenotype studies suggest that the OmpR of *E. piscicida* is implicated in osmoregulation, growth, expression of porins (ETAE_1826), virulence-related genes (*EseC*, *EseD* and *EvpC*), and certain genes of unknown function (ETAE_1540 and ETAE_2706).

Key words : *Edwardsiella piscicida*, ompR regulator, salinity

Introduction

An organism's ability to survive in a particular environment depends on its capacity to sense and respond to variabilities in its surroundings [2]. Specifically, bacterial pathogens, throughout their infection cycle must acclimatize themselves to a wide variety of changing environmental signals, like alterations in nutrient availability, temperature, osmotic pressure and salinity fluctuations etc. [3, 8, 10]. Of these, osmotic stress prompted by variations in ecological osmotic strength is of great physiological significance for the survival of the microorganism [29]. Generally, microorganisms acclimatize to fluctuations in medium osmolarity via two common approaches. First, one includes re-establishment of osmotic balance by accumulation, through import from the medium or by synthesis, of intracellular osmoprotectants identified as compatible solutes [26]. The second strategy involves varying membrane composition to cope better with the altered turgor pressure. A proper understanding of the interactions between these various regulatory systems is required to get insights into the overall adaptability of bacteria in certain environments [18].

The EnvZ-OmpR system, which responds to changes in environmental osmolarities, is one of the most important and well-characterized two-component signal transduction systems (TCS) in many gram-negative bacteria [23, 29]. EnvZ is a pleiotropic response regulator that controls the phosphorylation of OmpR by acting as a sensory histidine kinase [24, 29]. OmpR (osmolarity response regulator) has been recognized as a global transcriptional regulator that coordinates the expression of not only the outer-membrane porin genes, but also genes involved in different cellular processes like regulation of virulence factors, chemotaxis, flagella formation, motility, acid resistance, survival under nutrient limitation, adaptation to high osmolarity, oxidative stress and low pH [1, 23].

The genus *Edwardsiella*, is a causative agent of edwardsiel-

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losis, a septicemia that affects a wide range of marine and freshwater fishes, including Japanese flounder, turbot, tilapia, trout and so on [14, 16, 17]. Marine and freshwater ecosystems are very different and complex [17]. Remarkably, *E. piscicida* can survive under an extensive range of salinity such as fresh water (FW, 0.05%), brackish water (BW, 1.7%) and seawater (SW, 3.5%). Microbes, like *E. piscicida*, persisting in such diverse milieus, offer a valuable model for exploring how bacteria respond to and persist under different ecological stresses [2].

This study deals with the effects of variation in salinity on the growth and survival of *E. piscicida*. In order to better comprehend the mechanism through which *E. piscicida* senses and reacts to environmental osmolarity changes, we prepared an *ompR* mutant strain. Using this strain, we demonstrated that the latter not only regulates porin genes in response to osmolarity changes but also virulence-related secretory proteins and also has a role in cell growth.

Materials and Methods

Bacterial strains, media, and growth conditions

All *Edwardsiella piscicida* strains (CK108: CK41 derivative, pCK41 cured; CK284: CK108 derivative, *ompR* gene mutant) [28] were cultured in Tryptic Soy Broth (TSB) (Difco, USA) with shaking at 200 rpm, or were grown on TSB agar at 28℃. *Escherichia coli* strains (DH5α: transformation host; χ7213: DH5α derivative (△*asd*), Km^r, DAP required) [20] were grown at 37℃ in Luria-Bertani (LB) medium. 2,6-Diaminopimelic acid (DAP) (Sigma-Aldrich, USA) 50 µg/ml was added for the growth of Δ*asd* strains. When necessary, antibiotics were added to culture media at the following concentrations: 50 µg/ml kanamycin; 15 µg/ml Tetracycline; 100 µg/ml for ampicillin.

DNA/RNA manipulations and analysis

In this study isolation of plasmid and chromosomal DNA from *E. coli* and *E. piscicida,* and standard molecular cloning procedures, were followed according to Sambrook [21]. Oligonucleotides used in this study are listed in Table 1 and were purchased from Cosmogenetech Co. Korea. PCR products were purified using the PCR purification kit from Qiagen (Hilden, Germany) as recommended by the manufacturer. Total RNA samples were prepared using the TRIzol reagent (Invitrogen) as per manufacturer's instructions. DNase treatment was done with 1 µg of RNA, 1 U of RNase-free DNase I (Takara Mini BEST Universal RNA extraction kit). Reverse transcription of 1 µg of total RNA was performed using Primescript cDNA synthesis kit (Takara Tokyo, Japan).

Construction of *ompR* deletion mutant

The *ompR* (ETAE_3279) gene deletion in *E. piscicida* wild-type was introduced by means of the double selection strategy of allelic exchange mutagenesis [5]. Briefly, a 778-base pair (bp) fragment containing the upstream region and an 895-bp fragment containing the downstream region of *ompR* gene were obtained by PCR using the primers given in Table 1. The two fragments were annealed together yielding a DNA fragment having a deletion of 522 bp in the coding region of *ompR*. The construct was ligated into pDMS197 (a suicide vector, R6K *ori*, *SacB*, Tet^r) [9] to obtain pBP1090 (recombinant suicide plasmid for Δ*ompR*, Tet^r). After introduction of the recombinant suicide plasmid pBP1090 to *E. piscicida*, double crossover mutation was achieved by plating the bacterial cells on TSA plates with 10% sucrose, thus obtaining the *ompR* mutant, CK284. Disruption of gene was confirmed by PCR and precise deletion was verified by DNA sequencing.

Phenotypic characterization

Growth characteristics at 28℃ were monitored at different NaCl concentrations (0.05, 1.7 and 3.5%). Utilization of carbon substrates and other energy sources were determined using the analytical profile index system, API-20E (bioMerieux) following the manufacturer's directions. Examination of the strips was done after 24 hr of incubation. Haemolytic activity was assessed following overnight culture on blood agar plates (Asan Pharmaceutical Co., South Korea). Hemolysis was visualized by the development of clear hemolytic zones around colonies after incubation for 48 hr at 28℃. Swimming motility was measured on TSB agar plates containing 0.3% agar. Bacterial zones formed by the growing cells were then measured.

Outer Membrane Protein (OMP) and Extracellular Protein (ECP) Isolation

Pure cultures of the *E. piscicida* (CK108) and mutant samples were grown overnight at 28℃ in TSB medium under constant shaking at 200 rpm. Then, the cells were harvested by centrifugation at 8,000 rpm. The supernatant was preserved for ECP isolation and the precipitate was suspended

Table 1. Oligonucleotides used in this study

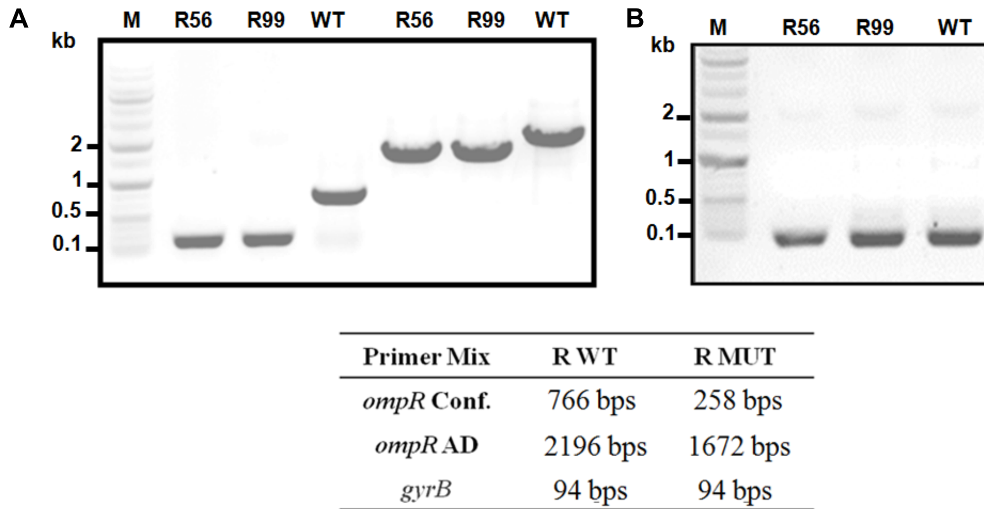
Primer	Nucleotide sequence*
ompR B- *Eco*RI	CTA GAATTC GCACCTGGAATCCCTGTTCA
ompR C+ *Eco*RI	ATC GAATTC CGTCTGCGTCGAATGGTAGA
ompR D- *Sac*I	CTA GAGCTC GATAGGCATCCTGCGGACTC
ompN A+ *Kpn*I	ATC GGTACC GCAAATAAACCCGCTACGGC
ompN B- *Eco*RI	CTA GAATTC ACGACTGCCAGCAGATTACG
ompN C+ *Eco*RI	ATC GAATTC ACATCGTTGCTCTGGGTCTG
ompN D- *Sac*I	CTA GAGCTC CGCTTGGGTGAGCTTTAGCA
ompR Conf F+	CCGCTATACAGATAAGCACCG
ompR Conf R-	TCATGCTTTACTGCCGTCCG
ompN Conf F+	CTCATACCTGACCGCACCG
ompN Conf R-	GCCGCGTTAAGCCGAACTC
EP_gyrB_F	CTTTGATCATGGTTGCGGAA
EP_gyrB_R	CGGCGTTTTCTTTTCTCG
OmpN_3_F	GGTTCTATCGACTACGGCCG
OmpN_3_R	GTTGTCAGTCTGGGCAGAGG
TRX_1_F	CTGTTTATTCCCGCCCTGCT
TRX_1_R	GTAGACGTCAACCAGCACCG
ABC_T_1_F	TCGACATCAAACCCGACCAG
ABC_T_1_R	GAGTAGCGACGAGTTAGCGG
EseD_1_F	CGTTGCTAATGGTATGCGCC
EseD_1_R	AGACTTTCAGGCCAAGCAGG
EvpC_F	GGGCTGAATACCGGTAAGGC
EvpC_R	TCCAGCGTGGGAAAGTTCTG
eseB-qfor	CCAACGGCTACGACAACAAC
eseB-qrev	ATTAGCCACCTGCTGGGAGT
eseC_F	TGCCGATATCCTCTTTGGCG
eseC_R	ACCAGGGCGTTATCACCAAG
ompF 1_F	CCGGTCATGAAGTTGTCGGT
ompF 1_R	TCTCTGGACTACGGCCGTAA
ompRp_F	CTTCTCGGGTAATCAGCGGG
ompR_R	TCATGCTTTACTGCCGTCCG

*The corresponding restriction sites (*Kpn*I, *Eco*RI or *Sac*I) are underline and enzymes indicated in parentheses of the primers.

in 4 ml of 10 mM Tris-HCl. The cells were lysed and centrifuged at 8,000 rpm for 15 minutes at 4℃. A total of 400-700 μl of 1% Lauroylsarcosine sodium (Sigma, Germany) solution was added to the supernatant. The mixture was centrifuged at 14,000 rpm for 1 hr at 4℃. The precipitate was dissolved in 1 ml of 10 mM Tris-HCl and stored at -20℃. The preserved supernatant samples were centrifuge further at 12,000 rpm 10 min. To the supernatant one volume of TCA, solution (500 g TCA in 350 ml double distilled water) to four volumes of protein sample and incubated for 30-35 min at 4℃. After centrifugation at 12,000 rpm, for 10 min supernatant was removed. The pellet was washed twice with 300 μl cold acetone and dried to remove acetone. The protein concentrations of the prepared ECPs and OMPs were measured by the method of Bradford. After SDS-PAGE, protein bands were detected after staining with 0.25% Coomassie brilliant blue R250 (Sigma, Germany) or by silver staining.

Quantitative real-time polymerase chain reaction (RT-qPCR)

To detect the mRNA expression levels of selected genes, RT-qPCR assays were performed using the Step One Real-Time PCR system (Applied Biosystems, USA). The qPCR was performed with the first-strand cDNA mixture, gene-specific primers and Topreal qPCR 2× PreMix with SYBR green (Enzynomics, South Korea), with three technical replicates. The PCR conditions used were set as follows: initial incubation steps for 4 minutes at 50℃, followed by, 14 minutes at 95℃, 30 cycles of 10 seconds at 95℃, 30 seconds at 57℃, 30 seconds at 72℃, and finally 15 seconds at 95℃, 40 seconds at 60℃, and 15 seconds at 95℃. Expression was assessed by evaluating threshold cycle (CT) values. The rela-



Primer Mix	R WT	R MUT
ompR Conf.	766 bps	258 bps
ompR AD	2196 bps	1672 bps
gyrB	94 bps	94 bps

Fig. 1. Genetic confirmation of the *ompR* gene deletion. A. The genes of *ompR* were PCR amplified using different primer combinations *ompR* Conf. and *ompR* A^+D^- primer sets (Table 1); B. *gyrB* (Table 1) primer combinations. DNA fragments were separated on a 0.8% agarose gel. PCR fragment lengths of the Wild-type (WT) and mutated (MUT) are shown underneath the figures. The Numbers show the different mutant colonies analysed.

tive expression level of tested genes was normalized to the 16s RNA gene and calculated using the $2^{-\Delta\Delta}$CT method.

Results

The genomic context of the *ompR* gene

ompR and *envZ* are a part of the *ompB* operon and are co-transcribed together as a polycistronic mRNA from a promoter region, which is located towards the 5′ of the *ompR* gene [4, 15]. To characterize the physiological role of the *ompR* gene in *E. piscicida*, an *ompR* mutant strain was constructed by an allelic exchange procedure [5]. The deletion in the resultant strain was confirmed by PCR amplification of *ompR* and *gyrB* genes, using different primer combinations (Table 1). The *gyrB* gene is known to be an *E. piscicida* specific gene [12]. The *gyrB* gene amplification in the *ompR* deletion mutant, verified that the mutant was an *E. piscicida* strain (Fig. 1). The mutant was further confirmed by sequencing.

Characterization of a strain deleting *ompR*

Subsequently, the growth rate of the *ompR* mutant strain, CK284 in TSB under a diverse array of salinity was compared with that of the wild-type strain (WT). This assay revealed that the growth patterns of the high salinity group showed the slowest growth rate. The growth rate of the strains decreased with the increase in medium osmolarity. The overall growth of CK284 was slower when compared to the wild-type (Fig. 2). In the complement mutant, final growth rate was partially recovered (data not shown). This data indicated that the OmpR protein is very important in cell survival and growth. To observe the physiological changes resulting from the lack of OmpR protein, biochemical characteristics of the mutant were analyzed using the API-20E strip. Remarkably, the mutant exhibited three different phenotypic properties from the wild-type strain.

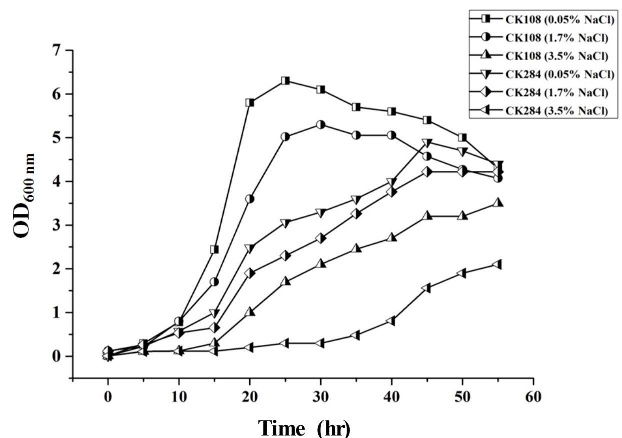


Fig. 2. The Growth of *Edwardsiella piscicida* (CK108) and *ompR* mutant CK284 in TSB media at 28℃, under different concentrations of NaCl (0.05%, 1.7% or 3.5%). Briefly, *E. Piscicida* cells were grown overnight in tryptic soy broth (TSB) at 28℃ with shaking and then diluted to 0.1% into fresh TSB containing an appropriate concentration of NaCl and grown with shaking (200 rpm) at 28℃. Optical densities at 600 nm wavelength were measured every 5 hr.

Citrate utilization, hydrogen sulfide (H_2S) production, and indole production were absent in the mutant strain. Whereas the complemented strain regained the wild-type phenotype. Deletion of the *ompR* gene had no effect on motility of the cell. Haemolytic activity was also not disrupted in the CK284 (data not shown).

Protein profile analyses

To determine the effect of *ompR* deletion on the expression of extracellular and outer membrane proteins, the *E. piscicida* WT and *ompR* mutant's ECPs and OMPs were isolated and analyzed via 12-15% SDS-PAGE. Analysis of the results showed that the CK284 and WT shared a similar background band profiles. The ECP profile, of the mutant, showed significant overexpression of five of the major bands, approximately at 42, 24, 20 and 18-kDa range, which were repressed in *E. piscicida* WT strain (Fig. 3). Similarly, OMP profile of the *ompR* mutant showed major differences in protein expression patterns. Specifically, it was noticed that a protein band between 35 kDa and 42 kDa in the OMP of the wild-type but not in that of the CK284. The overall protein banding pattern also varied with the increase in salinity in the medium.

Protein bands of interest were excised from the SDS-PAGE gel and subjected to MALDI-TOF-MS. The identified proteins are labelled in Fig. 3 and listed in Table 2. The amino acid sequence of the OMP band 1 was identical to the sequence of a putative outer membrane protein (porins), showing 100% sequence similarity with the outer membrane protein N (*E. piscicida* C07-087). It shows 89% sequence similarity with the OmpN2 of *E. ictaluri* [27]; because this protein from *E. ictaluri* was named OmpN2, the protein product of ETAE_1826 was thus named as OmpN2. Band 5 was identified as opacity type porin (*E. piscicida* C07-087). This protein is similar to opacity proteins from *Neisseria meningitidis*, where it is suspected to mediate various pathogen/host cell interactions [7] (Fig. 3A, Table 2).

The amino acid sequence of the ECP band 3 and 4 were similar to the sequences of the type III secretion system (T3SS) proteins C and D (EseC and EseD), respectively. The sequence of the band 6 protein matched with the sequence of type VI secretion system (T6SS) protein EvpC. The sequence of band 7 and 8 corresponded with the amino acid ABC transporter substrate-binding protein and thioredoxin (H-type, TRX-H) proteins respectively (Fig. 3B and C, Table 2). Taken together, these results suggest that OmpR protein is necessary for the accurate functioning of the secretion systems in *E. piscicida.*

Transcriptional analysis of differentially expressed genes

To further confirm the results of the protein profile data

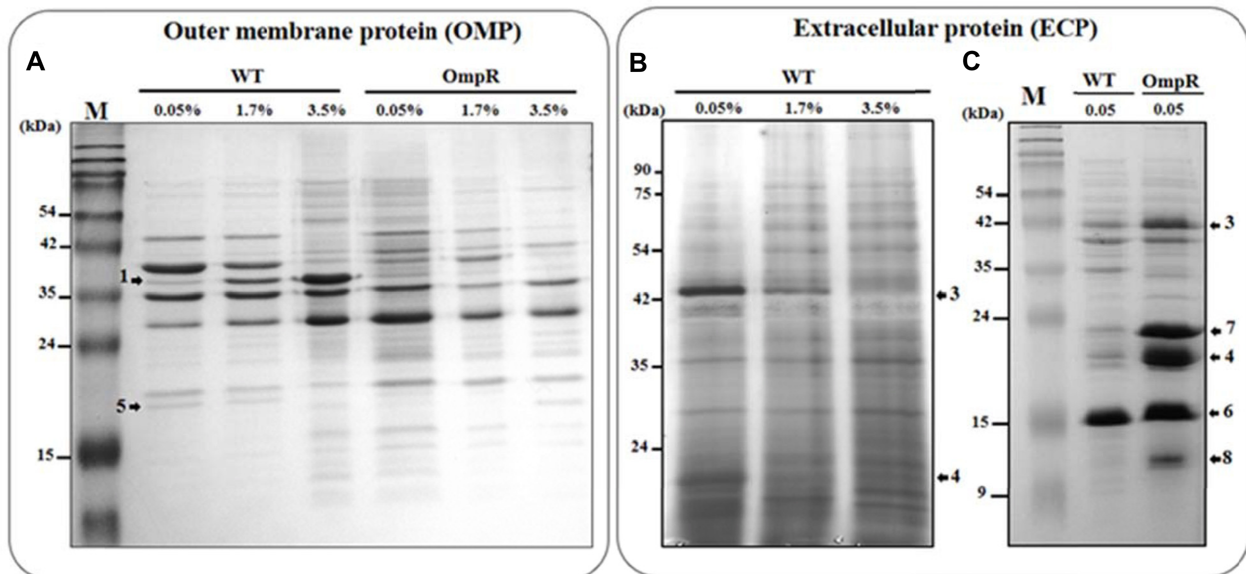


Fig. 3. SDS-PAGE analysis of Outer membrane (A) and Extracellular protein (B and C). Protein expressed under different levels of NaCl concentrations, (0.05%, 1.7% or 3.5%). M, protein marker; WT, wild-type; OmpR, *ompR* mutant CK284. Gels were stained with CBB Staining or silver staining. Black arrows indicate the difference in protein expression patterns between samples. The protein bands marked 1-8 were excised and identified using MALDI-TOF-MS (for gene IDs see Table 2).

Table 2. MALDI-TOF MS information of secreted protein sample (1-8) peptide fragments digested by trypsin were used for data mining from *E. Piscicida* database

Protein Band No. /name	Accession No.	Expect	Species identified	Mol wt. (kda)
1/OmpN	gil502611679	2.5e - 13	Porin [*Edwardsiella*]	42.35
2/OmpF	gil505274147	6.2e - 10	Porin [*Edwardsiella*]	39.9
3/EseC	gil502610733	3.1e - 12	Multi species: type III secretion protein [*Edwardsiella*]	50.7
4/EseD	gil73532652	0.017	Type III secretion system effector protein D [*Edwardsiella tarda*]	21.1
5/ETAE_0245	ACY83092.1	0.0011	MULTISPECIES: membrane protein [*Edwardsiella]*	22.4
6/EvpC	ACY85266.1	7.4e-06	Type VI secretion system protein EvpC [*Edwardsiella tarda EIB202*]	17.88
7/ETAE_1540	ACY84381.1	257 aa	MULTISPECIES: amino acid ABC transporter substrate-binding protein [Edwardsiella]	27.45
8/ETAE_2706	ACY85021.1	6.1e-05	EDWTE Thioredoxin (H-type,TRX-H) OS=*Edwardsiella tarda* (strain EIB202)	13.86

and to assess whether the variations perceived in protein expression were happening at the transcriptional level, RT-qPCR with total RNA prepared from the wild-type and *ompR* mutant, in presence and absence of 3.5% NaCl was carried out. Some of the important genes selected from ECP group for this analysis were *eseB, eseC, eseD, evpC,* ETAE_1540 and ETAE_2706 genes. From the OMP group *ompN2* was chosen. We also analyzed the expression of *ompF* gene, an important member of OmpR regulon and its expression is known to be stimulated at low osmolarity [22]. Complementary to the results obtained at the protein level, in wild-type grown in medium containing 0.05% NaCl, the transcript levels of *ompF, eseB, eseC, eseD, evpC,* ETAE_1540 and ETAE_2706 were significantly higher, when compared to wild-type grown in presence of 3.5% NaCl. As expected *ompF* was four folds down regulated in high osmolarity and contrary to it, OmpN2 was over-expressed (3.2 folds) in high-osmolarity. The *ompR* mutant strain displayed a marked decrease in *ompN2* (two folds) and *ompF* porin (-3.5 folds) gene expressions. In agreement with the SDS-PAGE gel data, qPCR also showed an enhanced transcription of the genes from ECP group, consisting of *eseB* (three folds), *eseC* (two folds), *eseD* (2.8 folds), *evpC* (1.85 folds), ETAE_1540 (three folds) and ETAE_2706 genes (three folds). In addition to porins, *OmpR* is also known to affect the transcription of flagellar genes [24]. Therefore, we analysed the *fliC*1 and *fliC*2 gene expressions in *E. piscicida.* To our surprise, the *ompR* mutation did not affect the two flagella genes. However, the effects of varying salt concentration on the expression of flagellin genes was clearly seen, i.e., in presence of high salt, expression of these genes decreased dramatically (Fig. 4).

Discussion

Although the biological roles of OmpR have been studied vastly, very little is known about its regulatory functions in *E. piscicida*. The *ompR* mutant showed reduced rates of growth over extended incubation time in the presence of diverse array of salinity (Fig. 2).

The OMPs and ECPs of *E. piscicida* WT and *ompR* mutant expressed under varying salt concentrations were investigated via SDS-PAGE (Fig. 3). Searching for different bands in protein expression accordance with increasing range of salinity, we selected differentially expressed fractions of OMPs and ECPs in each salt concentration. These bands were further identified by MALDI-TOF-MS. Two OMPs and three ECPs were confirmed as porins (ETAE_1826 and ETAE_0245) and secretion system protein (from T3SS and T6SS), respectively. Secretion system protein identified by their mass spectra were putative effector proteins (EseC and EseD) that are homologous to SseC and SseD of TTSS in *Salmonella* species. Parallel to *Salmonella,* EseC and EseD along with EseB, assemble into a translocon complex, which transport effectors into host cells. The third one identified was EvpC, and showed sequence similarity with Eip18 from *E. ictaluri* [6, 25]. The T3SS and T6SS are conserved in many bacteria and are considered as vital in virulence mechanisms of many gram-negative pathogens [11]. Secretion system gene mutations can considerably attenuate bacterial virulence and can augmented the 50% lethal dose (LD_{50}) values [13, 25], suggesting the importance of these genes in arbitrating bacterial virulence. Deletion of another secretion system gene, *evpC* in *E. tarda* led to reduced virulence in blue gour-

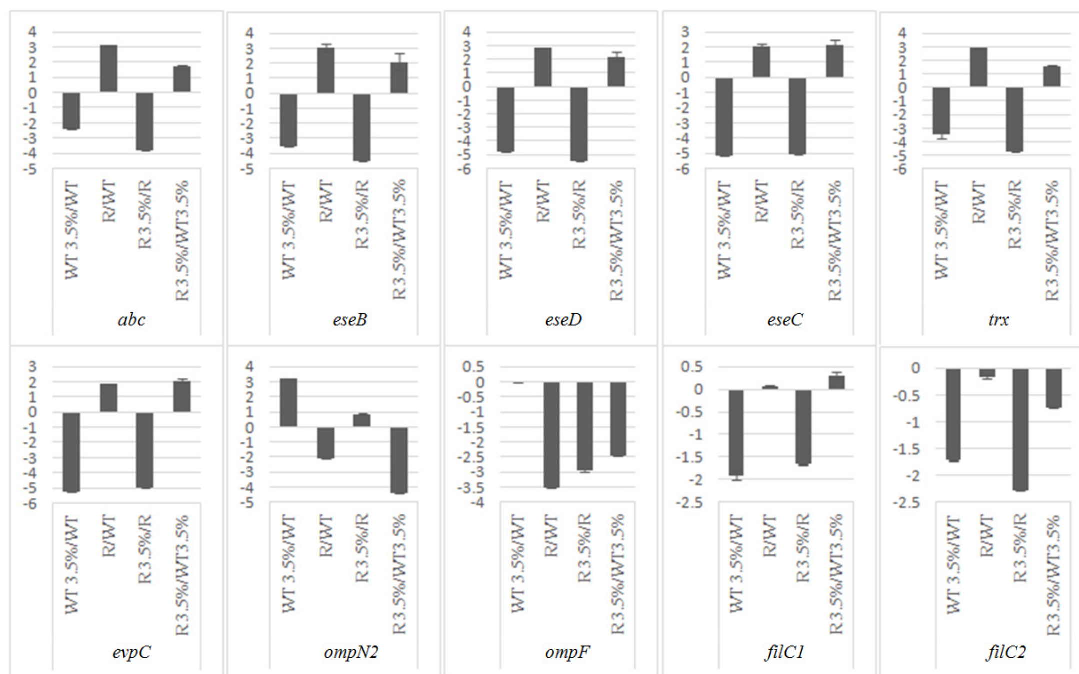


Fig. 4. Transcriptional analysis of differentially expressed genes detected by RT-qPCR in *ompR* deletion strain (R) compared to wild-type (WT). The mRNA ratios represent mean values of at least two RT-qPCR analyses starting from independent cultures. The strains were cultivated either in TSA medium with 0.05% NaCl or with 3.5% NaCl (WT 3.5% or R 3.5%). RNA was isolated in the early exponential growth phase. Where, gene abbreviations are as follows, *abc:* ETAE_1540, trx: ETAE_2706.

ami [13, 19]. Hence suggesting the importance of these proteins in pathogenesis in *E. piscicida.*

Analysis of the SDS-PAGE results showed that OMP profile of the *ompR* mutant showed major differences in protein expression patterns. Besides EseC, EseD, EvpC, two other protein were also up-regulated in the *ompR* mutant, they were identified to be amino acid ABC transporter substrate-binding protein and thioredoxin. Not much information is available in regards to these proteins in *E. piscicida* in the published literature. Further confirmation of differential transcription of selected target genes (like *eseC, evpC, ompN2,* etc.) were done by performing more sensitive RT-qPCR assays. In all the cases, RT-qPCR confirmed the data from the SDS-PAGE experiment (Fig. 4).

Taken together, these results suggest that protein product of *ompR* is necessary not only for the accurate growth and survival of *E. piscicida* cells but it also influences the proper functioning and secretion of the outer membrane and extracellular proteins, which in turn is necessary for enhancing the virulence of these cells. These findings provide new insights into the mechanism by which the EnvZ/OmpR system regulates the osmosensory pathway in *E. piscicida.*

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 어류 병원체 *Edwardsiella piscicida*의 OmpR은 생육과 병원성과 관련된 유전자의 발현에 필수적

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*Edwardsiella piscicida*는 어류의 출혈성 패혈증 및 사람의 위장 감염의 중요한 원인균이다. 세균이 생존을 하기 위해서는 환경변화에 적응하기 위한 특수한 메커니즘이 필요하다. 따라서 *E. piscicida*가 삼투압 변화 환경을 감지하고 이에 반응하는 메커니즘을 이해하기 위하여 본 연구에서는 다양한 염도 조건에서 단백질 발현 형태와 세균의 생리적 특성을 분석하였다. EnvZ - OmpR의 two-component 조절 시스템의 일부인 OmpR 단백질은 세균의 염분 스트레스 감지와 관련이 있다. 이 단백질이 *E. piscicida*에서 어떤 생리적 역할을 하는지는 밝혀지지 않고 있다. 이 연구에서는 염분 스트레스에 대한 OmpR 단백질의 기능을 조사 하였다. OmpR을 발현하지 못하는 돌연변이체를 분석한 결과 구연산염 이용, H2S 생성 및 인돌 생산의 능력이 야생형과 비교했을 때 차이가 나는 것으로 확인되었다. 전체 *ompR* 유전자를 가지는 플라스미드를 돌연변이 균주에 도입하여 분석한 결과 위의 세가지 표현형은 야생형과 같아졌다. 지연된 성장률도 부분적으로 회복되었음을 볼 수 있었다. 이 연구에서 OmpR이 세포운동성과의 관련성을 찾아볼 수 없었다. 이 연구의 결과들을 종합하면, 돌연변이 분석, 성장 분석, MALDI-TOF MS, qRT-PCR 및 표현형 연구 결과는 *E. piscicida*의 OmpR이 삼투압 조절, 생육, 포린 발현, 독성 관련 유전자 (*eseC*, *eseD* 및 *evpC*) (ETAE_1826) 및 기능을 알 수 없는 특정 유전자(ETAE_1540 및 ETAE_2706)와 관련이 있다고 사료된다.