African Journal of Microbiology Research Vol. 6(20), pp. 4256-4260, 30 May, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.651
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Effect of Ultraviolet-C (UV-C) irradiation on the virulence genes expression in *Vibrio parahaemolyticus* and *Vibrio alginolyticus*

Rihab Lagha[1#], Fethi Ben Abdallah[1, 2*#], Ali Ellafi[1], Héla Kallel[2] and Amina Bakhrouf[1]

[1]Laboratoire d'Analyse, Traitement et Valorisation des Polluants de l'Environnement et des Produits, Faculté de Pharmacie Rue Avicenne. Monastir 5000, Tunisie.
[2]Unité de Fermentation et de Développement de Vaccins Virologiques, Institut Pasteur de Tunis.13 place Pasteur, 1002, Tunisie.

Accepted 27 December, 2011

In this study, *Vibrio parahaemolyticus* and *Vibrio alginolyticus,* marine foodborne pathogens, were treated with Ultraviolet-C (UV-C) irradiation (240 J.m^{-2}) to evaluate alterations in their virulence genes expression levels. Firstly, we searched for the presence of eight *Vibrio cholerae* virulence genes, *toxR*, *toxS*, *toxRS*, *ctxA*, *zot*, *ace*, *toxT*, and virulence pathogenicity island (VPI), in the genome of investigated strains. The expression of *toxR* and *toxS* genes in UVC-irradiated bacteria, studied by reverse transcriptase polymerase chain reaction, was found to be altered. These variations were manifested by an increase or a decrease in the expression level of tested virulence genes. Further, the mRNA quantities of VPI and *ace* genes remained stable after treatment.

Key words: *Vibrio*, Ultraviolet-C (UV-C), alteration, virulence genes expression, RT-PCR.

INTRODUCTION

Ultraviolet-C (UV-C) radiation has been suggested as one of the successful disinfection practices for water treatment. Therefore, UV-sterilization has become a practical solution for safe disinfection of water (Said et al., 2010). The effectiveness of UV light in biological inactivation arises primarily from the fact that Deoxyribonucleic acid (DNA) molecules absorb UV photons between 200 and 300 nm, with peak absorption at 254 nm (Jeffrey et al., 1990). This absorption creates damage in the DNA by altering the nucleotide base pairing, thereby creating new linkages between adjacent nucleotides on the same DNA strand. This damage occurs particularly between pyrimidine bases. Two types of mutagenic lesions in DNA were determined: cyclobutane pyrimidine dimers (CPD) formed between the C-4 and C-5 positions of adjacent thymidine or cytosine residues, and pyrimidine (6 to 4) pyrimidone (6 to 4) photoproducts formed between the C6 and C4 position of adjacent pyrimidine residues, most often between T-C and C-C residues (Zimmer and Slawson, 2002). UV radiation in the range of 250 to 260 nm is lethal to most micro-organisms, including bacteria, viruses, protozoa, mycelial fungi, yeasts and algae. Among the bacteria, *V. alginolyticus* and *V. parahaemolyticus*, marine foodborne pathogen, frequently involved in epizootic outbreaks in cultured gilt-head sea bream and sea bass, causing fish mortality in Tunisian aquaculture farms (Abdallah et al., 2009a). In marine environment, *V. alginolyticus* and *V. parahaemolyticus* were considered as an important reservoir of *Vibrio cholerae* virulence genes (Abdallah et al., 2009b). According to Boyd et al. (2000) these genes may be horizontally transferred to *V. alginolyticus* in an aquatic environment. Indeed, the mobility of virulence genes may cause the transformation of non pathogenic strain to pathogenic strain. Xie et al. (2005) reported that *V. alginolyticus* often possess homologues of the *V. parahaemolyticus* and *V. cholerae* virulence genes such

*Corresponding author. E-mail: fetyben@yahoo.fr. Tel: + 21 6 73 466 244. Fax: + 216 73 461 830.

# These authors have equally contributed to this work

as *toxR*, *tlh* and VPI, suggesting that *V. alginolyticus* may be an important reservoir of many known virulence genes of other *Vibrio* species in the aquatic environment. It is probably that the aquatic environment harbours different virulence-associated genes scattered among environmental Vibrios.

In this work we searched, by polymerase chain reaction (PCR), for the presence of eight *V. cholerae* virulence genes, *toxR*, *toxS*, *toxRS*, *ctxA*, *zot*, *ace*, *toxT*, and virulence pathogenicity island (VPI), in the genome of *V. alginolyticus* and *V. parahaemolyticus* strains. The expression level of transferred virulence genes under UVC irradiation was achieved by reverse transcriptase polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Bacterial strains

Six *Vibrio* strains were used in this study including three reference strains: *V. alginolyticus* ATCC 33787 (S1), *V. alginolyticus* ATCC 17749 (S2), and *V. parahaemolyticus* ATCC 17802 (S5). In addition, *V. parahaemolyticus* strain (S6), isolated from the Calich estuary (Alghero, Italy), and two *V. alginolyticus* strains (S3 and S4) isolated, respectively from the internal organs of aquacultured diseased gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), in Tunisian aquaculture farm (Abdallah et al., 2009a), were included in this work.

UVC treatment

V. alginolyticus and *V. parahaemolyticus* were cultivated at 30°C in Tryptic soy broth 1% NaCl (TSB 1%) with shaking (150 rpm). The cultures of *Vibrio* strains grown to late log phase (OD_{600} = 0.6) were diluted and spread on Tryptic soy 1% NaCl (TSA 1%, Pronadisa, Spain) agar plates in glass Petri dishes. After 18 h of incubation at 30°C, bacterial colonies that appeared on the plates were exposed, in triplicate, to UV light according to the method described previously (Wang et al., 2004). The plates with even bacterial growth were covered with a piece of glass for non-UV treatment. The plates (covered and non-covered) were exposed to a 4-W UV lamp with a wavelength of 254 nm. The applied dose was 240 Joules m^{-2}. After exposure, 250-ml Erlenmeyer flasks containing 100 ml of TSB 1% were inoculated with a loopful of colonies from control and UV treated bacteria. All flasks were kept in at 30°C for 18 h with a shaking.

PCR detection of *Vibrio cholerae* virulence genes in *V. alginolyticus* and *V. parahaemolyticus* strains

Bacteria were cultured on TSA 1% for 24 h at 30°C. One colony was cultured in TSB 1% for 24 h at 30°C, and 1.5 mL was centrifuged. DNA was extracted using a Wizard genomic purification kit (Promega, Madison, WI) according to the manufacturer's instructions. The primers of *V. cholerae* virulence genes used in this study (Sechi et al., 2000) are listed in Table 1. Polymerase chain reaction (PCR) were performed in 25 µL containing 50 ng of extracted DNA, 5 µL Green *Go Taq* buffer (5×), 0.25 µL of each deoxynucleoside triphosphate (10 mM), 0.5 µL $MgCl_2$ (50 mM), 1 µL of each primer (25 pM), and 1U of *Go Taq* DNA polymerase (Promega). Reaction mixtures were incubated for 5 min at 94°C; followed by 35 cycles at 94°C for 45 s; annealing at 52°C for 45 s for *toxS*, *toxR*, and virulence pathogenicity island (VPI); 72°C for 1 min; and a final extension at 72°C for 10 min. The annealing temperature for the detection of the *toxRS* and *toxT* genes was 58°C, whereas for *ctxA*, *ace*, and *zot* the temperature was 60°C. PCR products (5 µL) were analyzed on 1% agarose gels stained with ethidium bromide (0.5 mg/mL) at 90 V for 1 h and viewed under ultraviolet transillumination. All PCR-positive strains, indicating the presence of the virulence genes, were confirmed by repeating the PCR three times.

RT-PCR for virulence gene expression

To study the level of expression of *V. alginolyticus* and *V. parahaemolyticus* virulence genes before and after UV irradiation, semi-quantitative RT-PCR method was used. RNA from control and irradiated cells was extracted by SV total RNA isolation system (Promega) according to the manufacturer's instructions. RNA was quantified by Ultraspec spectrophotometer (Ultraspec 2100 pro; Amersham Biosciences Europe GmbH, France). RT-PCR was performed in triplicate using SuperScript™ One-Step RT-PCR with Platinum® Taq kit according to the manufacturer's recommendations (*Invitrogen*, Carlsbad, CA). For cDNA synthesis, 100 ng of RNA served as template. RT-PCR (25 µL reaction volume) was performed as follows: 50°C for 30 min; 94°C for 2 min; 35 cycles at 94°C for 45 s; annealing at 52°C for 45 s for *toxS*, *toxR*, and VPI; 72°C for 1 min; and a final extension at 72°C for 10 min. The annealing temperature for *ace* gene was 60°C. RT-PCR products (5 µL) were analyzed on 1% agarose gel stained with ethidium bromide (0.5 mg/mL) at 90 V for 1 h and viewed under ultraviolet transillumination. The amplification products were photographed and their sizes determined with 100 bp molecular size marker (Promega). Quantitative analysis of DNA bands was performed using imaging software (Gene Tools, Sygene, UK).

RESULTS

Virulence genes expression

PCR amplification of the eight *V. cholerae* virulence genes in investigated *Vibrio* strains showed that only *V. alginolyticus* (S1 and S3) and *V. parahaemolyticus* (S5 and S6) were positive for *toxR* and *toxS* genes. In addition, *V. alginolyticus* S3 and S4 were positive for VPI and *ace* genes, respectively.

Expression levels of detected genes before and after treatments with UVC irradiation were analyzed by semi-quantitative RT-PCR (Figure 1). After treatment, we observed a decrease in the expression level of *toxS* gene in *V. parahaemolyticus* (S6) isolated from the Calich estuary. In addition, the expression level of *toxR* gene was decreased in *V. parahaemolyticus* S6 but it was increased in *V. alginolyticus* S3. Further, the mRNA quantities of VPI and *ace* genes remained stable in *V. alginolyticus* strains S3 and S4, respectively.

DISCUSSION

The results developed in this work indicate wide dissemination among *V. alginolyticus* and *V. parahaemolyticus* of different *V. cholerae* virulence genes

Table 1. PCR primers selected for this study (Sechi et al., 2000).

Oligonucleotide sequence	Amplification region (bp)
*tox*RS	
toxR0, ATGAGTCATATTGGTACTTAAATT	1397
toxS2, AACAGTACCGTAGAACCGTGA	
*tox*S	
toxS1, CCACTGGCGGACAAAATAACC	640
toxS2, AACAGTACCGTAGAACCGTGA	
*tox*R	
toxR1, TTTGTTTGGCGTGAGCAAGGTTTT	595
toxR2, GGTTATTTTGTCCGCCAGTGG	
VPI	
VPI1, GCAATTTAGGGGCGCGACGT	680
VPI2, CCGCTCTTTCTTGATCTGGTAG	
toxT	
toxT1, TTGCTTGGTTAGTTATGAGAT	581
toxT2, TTGCAAACCCAGACTGATAT	
ace	
ace1, GCTTATGATGGACACCCTTTA	284
ace2, TTTGCCCTGCGAGCGTTAAAC	
zot	
zot1, ACGTCTCAGACATCAGTATCGAGTT	198
zot2, ATTTGGTCGCAGAGGATAGGCCT	
ctxA	
ctx2, CGGGCAGATTCTAGACCTCCTG	563
ctx3, CGATGATCTTGGAGCATTCCCAC	

such as *toxR*, *toxS,* VPI and *ace*, which suggests that *V. alginolyticus* may be an important reservoir of many known virulence genes of other *Vibrio* species in the aquatic environment. It is probable that the aquatic environment harbours different virulence-associated genes scattered among environmental Vibrios. Similar results have been reported in some *Vibrio* species (Nishibuchi et al., 1996; Sechi et al., 2000). According to Boyd et al. (2000), these genes may be horizontally transferred, leading to new pathogenic strains. Indeed, the mobility of the virulence genes and a successful transfer may cause the transformation of a nonpathogenic strain to pathogenic strain (Faruque et al., 1998; Boyd et al., 2000). Previous studies have shown the distribution of different virulence genes among *V. cholerae* O1 (Colombo et al., 1994; Sechi et al., 2000). Most *V. cholerae* strains isolated from cholera patients simultaneously carry *ctxA*, *tcp* and *toxR* genes (Faruque et al., 1998). Three *V. cholerae* non-O1/non-139 isolates have been found to contain the three associated virulence genes (*ctxA*, *tcpA* and *toxR*) (Ghosh et al., 1997). It is now clear that *V. cholerae* might be continually undergoing genetic change by the acquisition of DNA, facilitated, at least in part, by temperate phages such as those associated with some of its critical virulence factors (Sechi et al., 2000).

Many vibrios are pathogenic for humans and or marine vertebrates and invertebrates, with the virulence mechanisms reflecting the presence of enterotoxin, haemolysin, cytotoxin, and various enzymes such as protease and lipase (Zhang and Austin, 2005). Among them in this work, the relative expressions of *toxR, toxS*, VPI and *ace* virulence genes in UVC-irradiated *V. alginolyticus* and *V. parahaemolyticus* cells were investigated, and variations were observed in the expression levels of *toxR* and *toxS* genes after irradiation. However,

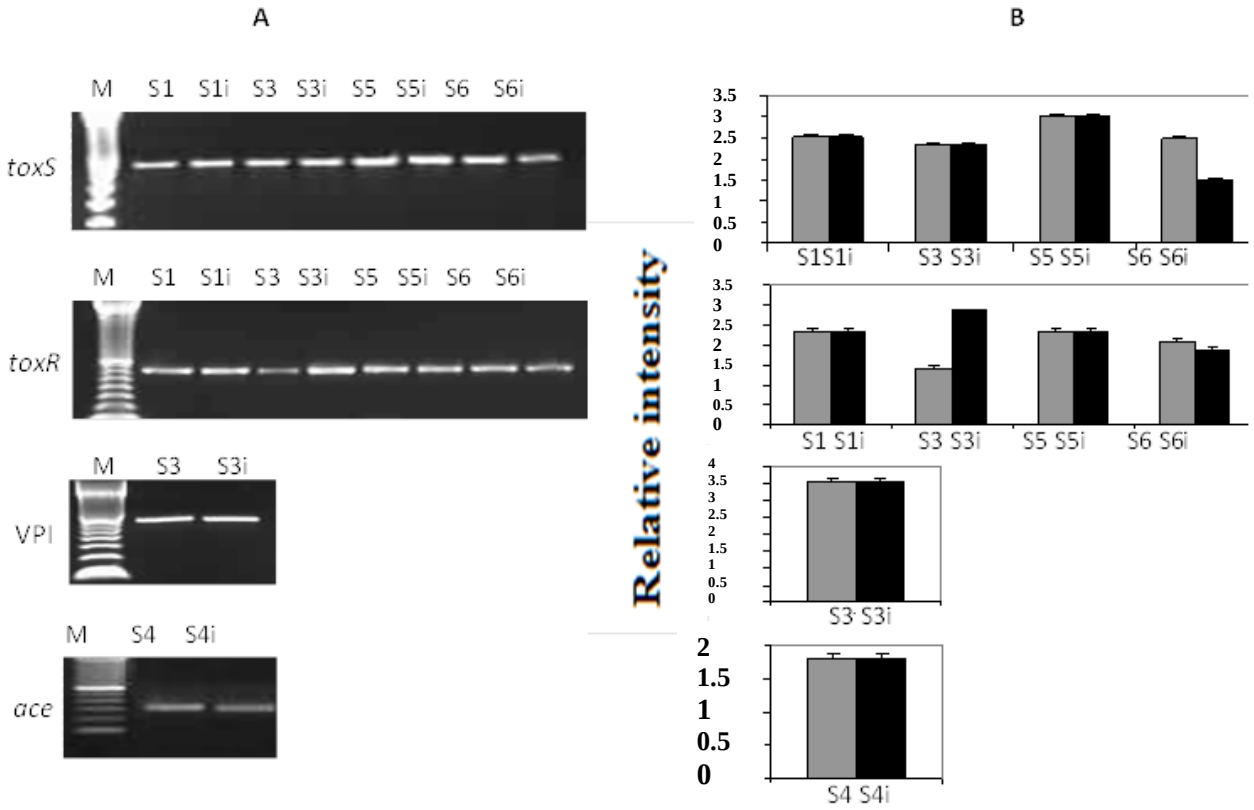

Figure 1. Virulence genes expression of *V. alginolyticus* and *V. parahaemolyticus* cells exposed to UVC radiation. M: 100 bp DNA ladder (Promega); S1 and S3: *V. alginolyticus* strains; S5 and S6: *V. parahaemolyticus* strains. i: strain irradiated; A: 1% agarose gels analysis; B: relative expression of virulence genes.

the mRNA quantities of VPI and *ace* genes remained. These results are in accordance with those developed by Qiu et al. (2005), who demonstrated that UVC-induce genes grouped into 11 functional categories, of which both “hypothetical proteins” and “conserved hypothetical protein” in *Shewanella oneidensis*. According to Jeffrey et al. (1990), UVC creates damage in the DNA by altering the nucleotide base pairing, thereby creating new linkages between adjacent nucleotides on the same DNA strand. In addition, based on the work of Miller et al. (1999), UV radiation can cause indirect damage to DNA by generating reactive oxygen species that then damage bases, break strands, and cross-link DNA and proteins. In response to stress conditions *Vibrio* modulates its virulence genes expression (Peterson and Mekalanos, 1988). This modulation is essential for *in vivo* survival since strains lacking this ability due to a mutation in the *toxR* gene, the product of which is involved in signal-dependent virulence gene expression, are deficient in intestinal colonization of human volunteers (Herrington et al., 1988). During radiation treatment, DNA molecules are heavily damaged, preventing them from functioning normally. Ionizing radiation is a physical agent that targets DNA molecules either via direct interaction or via production of free radicals and reactive oxygen species. Bacterial cells exposed to radiation will normally react in different ways: arrest cell cycle progression and repair of DNA lesion (Caillet et al., 2008). The genes expression instability of *Vibrios* under environmental stress conditions has also been demonstrated (Asakura et al., 2006). Gonzalez-Escalona et al. (2006) presented the interesting finding that the incubation temperature significantly affects the gene expression profile of *V. cholerae*. These authors compared the transcription of some target genes in the viable but nonculturable (induced at 4°C) and starvation (15°C) states and showed that key enzymes for cellular metabolism, such as *tuf* and *relA* or *rpoS* (stress response genes), were present at higher levels in bacteria that had entered the viable but nonculturable (VBNC) state.

In summary, the successful transfer of *V. cholerae* virulence genes and their expression may cause the transformation of a nonpathogenic *V. alginolyticus* and *V. parahaemolyticus* strains to pathogenic strain. This may explain the pathogenicity of *V. alginolyticus* to cultured gilt-head sea bream and sea bass in Tunisian aqua-culture farms. In addition, instability in virulence gene expression under UVC irradiation, widely used in these farms was also observed. These reflect on the stability of these virulence factors under stress conditions.

REFERENCES

Asakura H, Ishiwa A, Arakawa E, Makino S, Okada Y, Yamamoto S, Igimi S (2006). Gene expression profile of *Vibrio cholerae* in the cold stress-induced viable but non-culturable state. Environ. Microbiol., 9: 869–879.

Abdallah Ben F, Chaieb K, Kallel H, Bakhrouf A (2009a). RT-PCR assays for *in vivo* expression of *Vibrio alginolyticus* virulence genes in cultured gilthead *Dicentrarchus labrax* and *Sparus aurata*. Ann. Microbiol., 59: 1–5.

Abdallah Ben F, Kallel H, Bakhrouf A (2009b). Enzymatic, outer membrane proteins and plasmid alterations of starved *Vibrio parahaemolyticus* and *Vibrio alginolyticus* cells in seawater. Arch. Microbiol., 191: 493–500.

Boyd EF, Moyer KE, Shi L, Waldor MK (2000). Infectious CTXphi and the *Vibrio* pathogenicity island prophage in *Vibrio mimicus*: evidence for recent horizontal transfer between *V. mimicus* and *V. cholerae*. Infect. Immun., 68: 1507-1513.

Caillet S, Millette M, Dussault D, Shareck F, Lacroix M (2008). Effect of gamma radiation on heat shock protein expression of four foodborne pathogens. J. Appl. Microbiol., 105:1384–1391.

Colombo MM, Mastrandea S, Santona A, De Amdrade AP, Uzzau S, Rubino S, Cappuccinelli P (1994). Distribution of the *ace, zot* and *ctxA* toxin genes in clinical and environmental *Vibrio cholerae*. J. Fish. Dis., 170: 750-751.

Faruque SM, Albert MJ, Mekalanos JJ (1998). Epidemiology, genetics and ecology of toxigenic *Vibrio cholerae*. Microbiol. Mol. Biol. Rev., 62: 1301-1314.

Ghosh C, Nandy RK, Dasgupta SK, Nair GB, Hall RH, Ghose AC (1997). A search for cholera toxin (CT), toxin coregulated pilus (TCP), the regulatory element ToxR and other virulence factors in non-O1/non-O139 *Vibrio cholerae*. Microb. Pathog., 22: 199-208

Gonzalez-Escalona NA, Fey MG, Hofle RT, Guzman C (2006). Quantitative reverse transcription polymerase chain reaction analysis of *Vibrio cholerae* cells entering the viable but nonculturable state and starvation in response to cold shock. Environ. Microbiol., 8: 658–666.

Herrington DA, Hall RH, Losonsky GA, Mekalanos JJ, Taylor RK, Levine MM (1988). Toxin, toxincoregulated pilus and the toxR regulon are essential for *V. cholerae* pathogenesis in human volunteers. J. Exp. Med., 168: 1487–1492.

Jeffrey JB, Huai-Shu X, Colwell R (1990). Viable but nonculturable bacteria in drinking water. Appl. Environ. Microbiol., 57: 875–878.

Miller RV, Jeffrey W, Mitchell D, Elasri M (1999). Bacterial responses to ultraviolet light. A.S.M. News., 65: 535–541.

Nishibuchi M, Janda JM, Ezaki T (1996). The thermostable direct hemolysin gene (tdh) of *Vibrio hollisae* is dissimilar in prevalence to and phylogenetically distant from the tdh genes of other vibrios: implications in the horizontal transfer of the tdh gene. Microbiol. Immunol., 40: 59-65.

Peterson KM, Mekalanos JJ (1988). Characterization of the *Vibrio cholerae* ToxR regulon: identification of novel genes involved in intestinal colonization. Infect. Immun., 56: 2822- 2829.

Qiu X, Sundin GW, Wu L, Zhou J, Tiedje JM (2005). Comparative analysis of differentially expressed genes in *Shewanella oneidensis* MR- following exposure to UVC, UVB, and UVA radiation. J. Bacteriol., 187: 3556–3564.

Said Ben M, Masahiro O, Hassen A (2010). Detection of viable but non cultivable *Escherichia coli* after UV irradiation using a lytic Qβ phage. Ann. Microbiol., 60:121–127.

Sechi LA, Dupre I, Deriu A, Fadda G, Zanetti S (2000). Distribution of *Vibrio cholerae* virulence genes among different *Vibrio* species isolated in Sardinia, Italy. J. Appl. Microbiol., 88: 475- 481.

Wang Y, Leung PC, Qian P, Gu JID (2004). Environment effects of UV, H_2O_2 and Fe^{3+} on the growth of four environmental isolates of *Aeromonas* and *Vibrio* species from a Mangrove. Microbes. Environ., 19: 163–171.

Xie ZY, Hu CQ, Chen C, Zhang LP, Ren CH (2005). Investigation of seven *Vibrio* virulence genes among *Vibrio alginolyticus* and *Vibrio parahaemolyticus* strains from the coastal mariculture systems in Guangdong, China. Lett. Appl. Microbiol., 41: 202-207.

Zhang XH, Austin B (2005). Haemolysins in *Vibrio* species. J. Appl. Microbiol., 98: 1011–1019.

Zimmer JL, Slawson RM (2002). Potential repair of *Escherichia coli* DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. Appl. Environ. Microbiol., 68: 3293–3299.