Identification of outer membrane proteins of Vibrio parahaemolyticus and Vibrio alginolyticus altered in response to γ-irradiation or long-term starvation.

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Identification of outer membrane proteins of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* altered in response to γ-irradiation or long-term starvation

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Abstract

Vibrio parahaemolyticus and *Vibrio alginolyticus* were subjected to γ-irradiation (0.5 kGy) or starvation by incubation for 8 months in seawater to study modifications in their outer membrane protein patterns. After treatment, outer membrane protein profiles of starved or γ-irradiated bacteria were found to be altered when analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Altered proteins were identified by mass spectrometry (MS and MS/MS) and analyses revealed that OmpU can be considered a starvation stress-induced protein. In addition, expression of OtnA, OmpW, OmpA and peptidoglycan-associated lipoprotein decreased to non-detectable levels in starved cells. Furthermore, MltA-interacting protein MipA appeared under γ-irradiation or starvation conditions. Thus, it can be considered to be a γ-irradiation, long-term starvation stress protein in some vibrios.

Keywords: Vibrio; Starvation; γ-Irradiation; Alteration; Outer membrane proteins; Mass spectrometry

1. Introduction

Vibrio, a food-borne pathogen, can sense and respond to changes in its external environment (Ben Kahla et al., 2008). The ability of *Vibrio* spp. to sense and respond effectively to changes in the environment is crucial for their survival (Morita, 1997). One such environmental parameter is the nutrient quantity in the extracellular medium. In general, microorganisms do not respond to nutrient deprivation or starvation by simply arresting all metabolic activities and stopping growth. Instead, they carry out starvation-induced activities that may include production of degradative enzymes and stress proteins (Siegele and Kolter, 1992). During nutrient deficiency, *Vibrio* spp. can survive for a long time by sequential changes in cell physiology and gradual changes in morphology (Morita, 1997). Srinivasan and Kjellberg (1998) found that, under starvation in natural environments, the response of vibrios indicated that the life cycle of bacteria broadly consisted of two major phases. The transition between these two major phases involves dramatic changes in gene expression, physiology and morphology.

Recently, gamma irradiation technology has shown positive effects in preventing decay by sterilizing microorganisms and improving the safety and shelf-stability of food products without compromising the nutritional or sensory quality (Lee et al., 2006). Andrews et al. (2003) reported that naturally incurred *Vibrio vulnificus* in oysters was reduced to non-detectable levels with ^{60}cobalt gamma radiation treatment at

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0.75 kGy. Treatment of oysters inoculated with *Vibrio parahaemolyticus* O3:K6 with 1.0–1.5 kGy also reduced contamination to non-detectable levels. Gamma irradiation causes damage to the DNA of cells (Villavicencio et al., 2004; Lee and Levin, 2008). The types of DNA damage experienced may be: i) direct physical effects of ionizing radiation with primary free radicals, and indirect biochemical effects from reactive oxygen species resulting in double-strand breaks; ii) single-strand breaks; and iii) base pair substituting mutations due to the conversion of pyrimidine bases to 5-(hydroxymethyl) uracil, 5-formyluracil, 5-hydroxycytosine, and 5-hydroxyuracil (Jung, 1997; Lydersen and Pettijohn, 1997; Min et al., 2000)

In Gram-negative bacteria, the outer membrane plays an important role in infection and pathogenicity toward the host (Tsolis, 2002). In the outer membrane, proteins play a crucial role during many cellular and physiological processes (Qian et al., 2008). Outer membrane proteins (OMPs) play a key role in adaptation to changes of external environments due to their location at the outermost area of the cell (Xu et al., 2005). It has been shown that when bacteria are transferred to a new environment, the synthesis of their OMPs changes (Kustos et al., 2007). In this way, Wu et al. (2006) demonstrated that OmpW and OmpV are required for environmental salt regulation in *Photobacterium damsela*. Furthermore, the altered osmolarity of the culture medium caused changes in the OMP patterns of *Vibrio alginolyticus* (Xu et al., 2005) and *V. parahaemolyticus* (Xu et al., 2004).

The aim of this work was to study modifications in the OMPs profiles of starved or gamma-irradiated *V. parahaemolyticus* and *V. alginolyticus* strains. OMPs profiles were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In order to identify altered OMPs, mass spectrometry (MS and MS/MS) was used.

2. Materials and methods

2.1. 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 a Tunisian aquaculture farm (Ben Abdallah et al., 2009) were included in this work.

2.2. Starvation stress

V. alginolyticus and *V. parahaemolyticus* were grown at 30 °C in tryptic soy broth (Pronadisa, Spain) supplement with 1% NaCl (TSB 1%) for 24 h. The natural seawater (100 ml) was sterilized by membrane filtration (pore size, 0.22 μm; Millipore Corp., Bedford, MA, USA) and autoclaving (121 °C for 20 min). *Vibrio* cells were washed three times (13,000 rpm for 10 min at 20 °C) and then suspended in 10 ml of sterilized seawater. The microcosms (100 ml) were inoculated with these suspensions (10^9 Colony Forming Unit (CFU)/ml) and then incubated at room temperature (22–25 °C) for 8 months.

2.3. Gamma irradiation treatment

V. alginolyticus and *V. parahaemolyticus* tested strains were cultivated at 30 °C in TSB 1% with shaking (150 rpm). The cultures of *Vibrio* strains grown to late log phase (O.D.600 = 0.6) were divided into 1 ml aliquots without change of broth and were exposed, in triplicate, on ice to a ^{60}cobalt γ-irradiator (point source, AECL, IR-79, Technopole, Sidi Thabet, Tunisia). The applied dose was 0.5 kGy. The control cells were introduced in ice during the time of irradiation. The irradiated and non-irradiated *Vibrio* were diluted 50-fold by using 50 ml of fresh medium and then harvested after 24 h of incubation at 30 °C with shaking (150 rpm).

2.4. Outer membrane protein extraction

OMPs of *V. alginolyticus* and *V. parahaemolyticus,* before and after irradiation, were prepared according to the method described previously (Sabri et al., 2000). Briefly, the bacterial cells were harvested by centrifugation at 4000 × *g* for 15 min at 4 °C. The cells were then washed three times in 40 ml of sterile saline water (0.9% NaCl) and then resuspended in 5 ml sterile saline water. Cells were disrupted by intermittent sonic oscillation in ice bath (350 W, 10 min × 3). Unbroken cells and cellular debris were removed by centrifugation at 5000 × *g* for 20 min. Supernatant was collected and was further centrifuged at 100 000 × *g* for 40 min at 4 °C. The pellet was resuspended in 10 ml of 2% (w/v) sodium lauryl sarcosinate (Sigma, St Louis, MO) and incubated at room temperature for 1 h, followed by centrifugation at 100 000 × *g* for 40 min at 4 °C. The resulting pellet was resuspended in 200 μl of sterile saline water. The concentration of the OMPs in the final preparation was determined using the Bradford Kit (Sigma).

2.5. SDS-PAGE

OMPs of starved (2 μg) and γ-irradiated (1 μg) *V. alginolyticus* and *V. parahaemolyticus* were analyzed in triplicate by SDS-PAGE (Laemmli, 1970) with 15% acrylamide in the separating gel and 5% acrylamide in the stacking gel. After separation, the proteins were visualized according to standard procedures by staining with Coomassie brilliant blue G250 (Sigma).

2.6. Protein identification by MS

After staining with colloidal Coomassie blue, 1D gel bands were manually excised from gels and collected in a 96-well plate. Destaining, reduction, alkylation and trypsin digestion of the proteins followed by peptide extraction were carried out

with the Progest Investigator (Genomic Solutions, Ann Arbor, MI, USA). After the desalting step (C18-μZipTip, Millipore), peptides were eluted directly using the ProMS Investigator, (Genomic Solutions, Ann Arbor, MI, USA) onto a 96-well stainless steel MALDI target plate (Applied Biosystems/MDS SCIEX, Framingham, MA, USA) with 0.5 μl of α-ciano-4-hydroxycinnamic acid (CHCA) matrix (2.5 mg/ml in 70% acetonitril (ACN)/30% H_2O/0.1% trifluoracetic (TFA)).

2.7. MS and MS/MS analysis

Raw data for protein identification were obtained on the 4800 MALDI TOF/TOF Analyzer (Applied Biosystems/MDS SCIEX, Framingham, MA, USA) and were analyzed by GPS Explorer 3.6 software (Applied Biosystems/MDS SCIEX, Framingham, MA, USA). For positive-ion reflector mode spectra 3000 laser shots were averaged. Autolysis peaks of trypsin ([M + H]+ = 842.5100 and 2211.1046) were used as internal calibrators for MS calibration. Monoisotopic peak masses were automatically determined within the mass range 800–4000 *m/z* with a signal to noise ratio minimum set to 30. Up to twelve of the most intense ion signals were selected as precursors for MS/MS acquisition, excluding common trypsin autolysis peaks and matrix ion signals. In MS/MS positive ion mode, 4000 spectra were averaged, collision energy was 2 kV, collision gas was air and default calibration was set using the Glu1-Fibrino-peptide B ([M + H]+ = 1570.6696) spotted onto fourteen positions of the MALDI target. Combined peptide mass fingerprinting (PMF) and MS/MS queries were performed using the MASCOT search engine 2.1 (Matrix Science Ltd., UK) embedded into GPS Explorer Software 3.6 (Applied Biosystems/MDS SCIEX, Framingham, MA, USA) on the UNIPROT database (downloaded 2009 06 25; 9064751 sequences; 2941541906 residues) with the following parameter settings: 50 ppm peptide mass accuracy, trypsin cleavage, one missed cleavage allowed, carbamidomethylation set as fixed modification, oxidation of methionine was allowed as variable modification, MS/MS fragment tolerance was set to 0.3 Da. Protein hits with MASCOT Protein score ≥82 and a GPS Explorer Protein confidence index = 95% were used for further manual validation.

3. Results

3.1. Effect of starvation on OMPs

OMPs of the four starved *V. alginolyticus* and two *V. parahaemolyticus* cells were analyzed by SDS-PAGE (Fig. 1) and MS (Table 1). Before their incubation in seawater, each *Vibrio* strain presents its specific OMPs profile. In addition, almost 15 bands were detected in each profile containing from three to six major OMPs. After 8 months of incubation in seawater, we noted that only *V. alginolyticus* ATCC 33787 (S1) and *V. parahaemolyticus* (S6), isolated from the Calich estuary (Alghero), preserved their initial OMPs profiles. Whereas, for *V. alginolyticus* ATCC 17749 (S2), we observed that the band corresponding to porin, putative and long-chain fatty acid transport protein was reduced, whereas OmpU became less abundant after starvation. Similar results have been observed in starved *V. parahaemolyticus* ATCC 17802 (S5), which respond to nutrient limitation by increasing the expression of OmpU. The major alterations in the OMP profile of the studied strains were observed in the two *V. alginolyticus* strains S3 and S4 isolated from the internal organs of aquacultured-diseased gilthead sea bream and sea bass, respectively. For strain S3, five bands were distinctly altered, showing that expression of OtnA, OmpW and peptidoglycan-associated lipoprotein decreased to non-detectable levels. In addition, the expression of OmpU was induced and the abundance of OmpK and outer membrane protein was decreased. In the OMPs profile of the

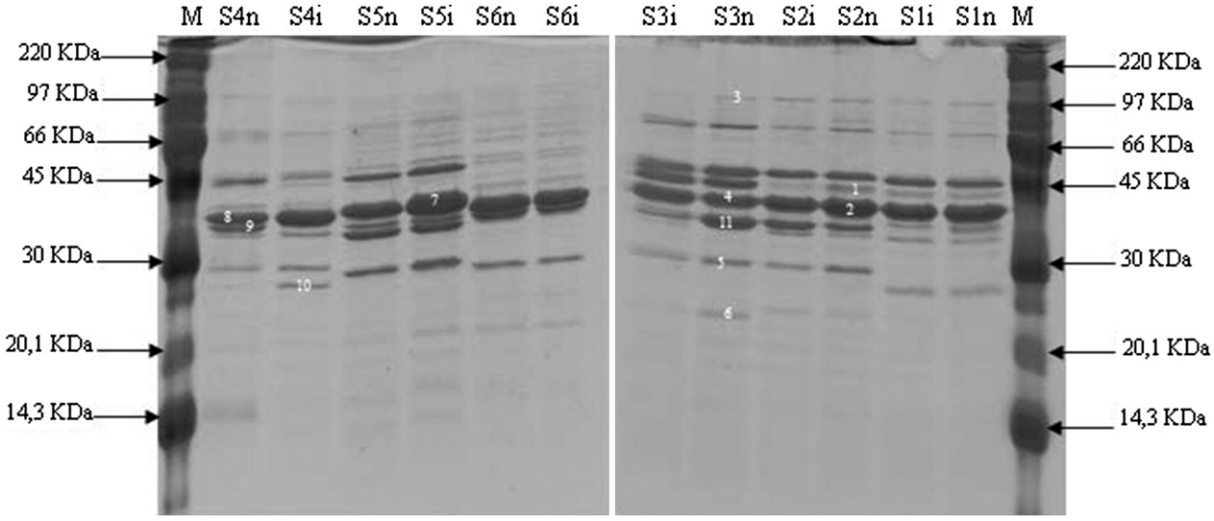

Fig. 1. OMPs of *V. alginolyticus* and *V. parahaemolyticus* cells exposed to starvation for eight months in seawater. M: high-range rainbow (Amersham, Little Chalfont, Buckinghamshire, UK); S1, S2, S3 and S4: *V. alginolyticus* strains; S5 and S6: *V. parahaemolyticus* strains. n :strain before incubation in seawater; i :strain incubated for eight months in seawater microcosms.

Table 1
List of altered OMPs of starved *V. alginolyticus* and *V. parahaemolyticus* identified using MS–MS/MS.

Strain	Band number	Identified protein	Accession number (Uniprot)	Mascot score	Molecular mass (Da)	Expression level
S2	01	Long-chain fatty acid transport protein	Q1VA77|Q1VA77_VIBAL	175	46030.4	−
		Porin, putative	A7K0N2|A7K0N2_9VIBR	487	39676	−
	02	Outer membrane protein OmpU	Q1V395|Q1V395_VIBAL	728	37067.4	−
S3	03	OtnA protein	Q1V7L9|Q1V7L9_VIBAL	605	99671.3	−
	04	Outer membrane protein OmpU	Q1V395|Q1V395_VIBAL	641	37067.4	+
	05	Outer membrane protein = Ompk	Q4PNS4|Q4PNS4_VIBAL	477	31311.9	−
	06	Peptidoglycan-associated lipoprotein	Q1V5X7|Q1V5X7_ VIBAL	237	18740.4	−
		Outer membrane protein OmpW	Q58IE1|Q58IE1_ VIBAL	122	23315.6	−
	11	Outer membrane protein	A7JZ07|A7JZ07_9 VIBR	375	36074.2	−
S4	08	Outer membrane protein = OmpU	Q4ZIM7|Q4ZIM7_ VIBPA	866	36189.9	−
	09	Outer membrane protein OmpA	Q1VAZ6|Q1VAZ6_VIBAL	246	36182.2	−
	10	MltA-interacting protein MipA	A7JZF9|A7JZF9_9 VIBR	336	31026.2	+
	12	Outer membrane channel protein = tolC	Q1V4T2|Q1V4T2_VIBAL	395	47846.3	−
S5	07	Outer membrane protein OmpU	Q1V395|Q1V395_ VIBAL	368	37067.4	+

S2: *V. alginolyticus* ATCC 17749, S3: *V. alginolyticus* isolated from diseased gilthead *Sparus aurata*, S4: *V. alginolyticus* isolated from diseased gilthead *Dicentrarchus labrax*, S5 : *V. parahaemolyticus* ATCC 17802. −: downregulated; +: upregulated.

starved *V. alginolyticus* (S4), we observed the appearance of MltA-interacting protein MipA, whereas OmpA was decreased to non-detectable levels. Furthermore, OmpU was induced and the expression level of outer membrane channel protein TolC was reduced.

3.2. Effect of γ-irradiation on OMPs

OMPs of γ-irradiated *V. alginolyticus* and *V. parahaemolyticus* were analyzed by SDS-PAGE (Fig. 2) and MS (Table 2). After γ-irradiation, several OMPs were altered. Our study showed that only γ-irradiated *V. parahaemolyticus* (S6) isolated from the Calich estuary (Alghero, Italy) preserved its initial profile of OMPs. For *V. alginolyticus* (S1, S2 and S4) and *V. parahaemolyticus* (S5), we observed that OmpU became less abundant. Similar results were obtained in *V. alginolyticus* (S1 and S4) and *V. parahaemolyticus* (S5), which respond to γ-irradiation by reducing the expression of OmpA. We also observed a reduction in the expression level of outer membrane channel protein TolC for strains S1, S2, S3 and S4, OMP for strains S2 and S3 and maltoporin LamB for *V. parahaemolyticus* (S5). In *V. alginolyticus* ATCC 17749 (S2), the expression level of porin, a putative and/or long-chain fatty acid transport protein, was increased. The major alterations in the OMP profile of the studied strains were observed in the two *V. alginolyticus* S3 and S4strains isolated from the internal organs of aqua-cultured-diseased gilthead sea bream and sea bass, respectively. Indeed, in addition to the modifications already cited, we observed that the vitamin B12 receptor and OmpK were decreased to non-detectable levels in *V. alginolyticus* S4, whereas their expression levels were decreased in *V. alginolyticus* S3. Moreover, peptidoglycan-associated lipoprotein and OmpW were decreased to non-detectable levels in strain S3, whereas MipA was expressed in strain S4.

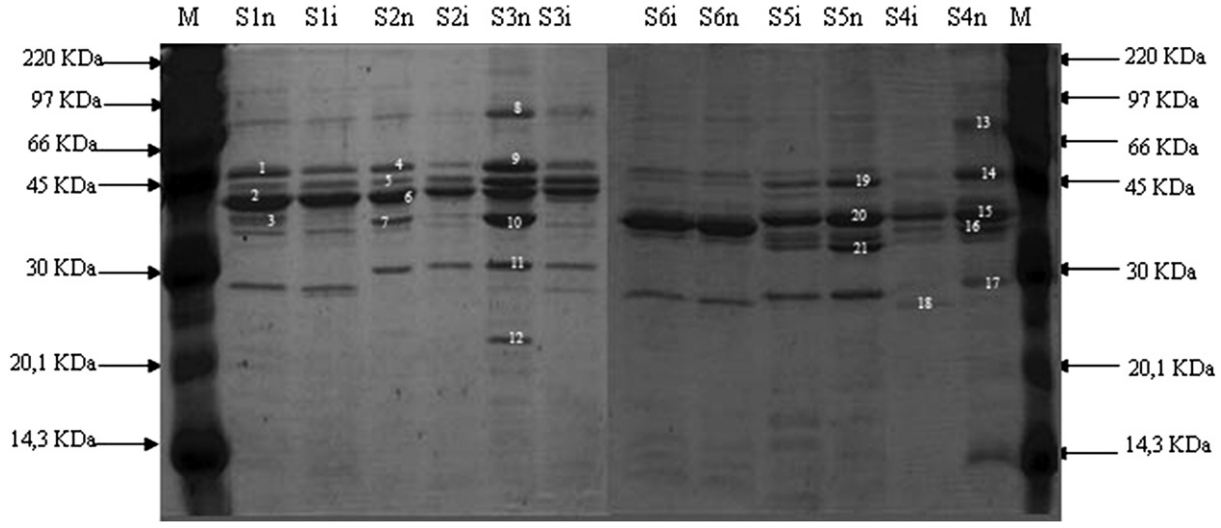

Fig. 2. OMPs of *V. alginolyticus* and *V. parahaemolyticus* cells exposed to γ-irradiation. M, high-range rainbow (Amersham); S1, S2, S3, and S4, *V. alginolyticus* strains; S5 and S6, *V. parahaemolyticus* strains; n, strain non-irradiated; i, strain irradiated.

Table 2
List of altered OMPs of γ-irradiated *V. alginolyticus* and *V. parahaemolyticus* identified using MS–MS/MS.

Strain	Band number	Identified protein	Accession number (Uniprot)	Mascot score	Molecular mass (Da)	Expression level
S1	01	Outer membrane channel protein = tolC	Q1V4T2|Q1V4T2_VIBAL	395	47846.3	−
	02	Outer membrane protein OmpU	Q1V395|Q1V395_VIBAL	672	37067.4	−
	03	Outer membrane protein OmpA	Q1VAZ6|Q1VAZ6_VIBAL	164	36182.2	−
		Putative outer membrane protein	Q1V592|Q1V592_ VIBAL	150	37991.1	−
S2	04	Outer membrane channel protein = tolC	Q1V4T2|Q1V4T2_VIBAL	475	47846.3	−
	05	Porin, putative	Q1VA77|Q1VA77_VIBAL	283	39676	−
		Long-chain fatty acid transport protein	A7K0N2|A7K0N2_ 9VIBR	188	46030.4	−
	06	Outer membrane protein OmpU	Q1V395|Q1V395_VIBAL	680	37067.4	−
	07	Outer membrane protein	A7JZ07|A7JZ07_9VIBR	188	36074.2	−
S3	08	Vitamin B12 receptor	Q1V4E1|Q1V4E1_VIBAL	1080	68061.3	−
	09	Outer membrane channel protein = tolC	Q1V4T2|Q1V4T2_VIBAL	383	47846.3	−
	10	Outer membrane protein	A7JZ07|A7JZ07_9VIBR	347	36074.2	−
	11	Outer membrane protein = Ompk	Q4PNS4|Q4PNS4_VIBAL	520	31311.9	−
	12	Peptidoglycan-associated lipoprotein	Q1V5X7|Q1V5X7_VIBAL	210	18740.4	−
		Outer membrane protein OmpW	Q58IE1|Q58IE1_ VIBAL	145	2095.1	−
S4	13	Vitamin B12 receptor	Q1V4E1|Q1V4E1_VIBAL	703	68061.3	−
	14	Outer membrane channel protein = tolC	Q1V4T2|Q1V4T2_VIBAL	480	47846.3	−
	15	Outer membrane protein OmpU	Q1V395|Q1V395_VIBAL	386	37067.4	−
	16	Outer membrane protein OmpA	Q1VAZ6|Q1VAZ6_VIBAL	260	36182.2	−
	17	Outer membrane protein OmpK	Q1VF49|Q1VF49_VIBAL	107	29310	−
	18	MltA-interacting protein MipA	A7JZF9|A7JZF9_9 VIBR	336	31026.2	+
S5	19	Maltoporin = lamB	C3W4N2|C3W4N2_VIBPA	662	46349.8	−
	20	Outer membrane protein = ompU	Q4ZIM7|Q4ZIM7_VIBPA	953	36189.9	−
	21	Outer membrane protein OmpA	Q87RL4|Q87RL4_VIBPA	192	34165.9	−

S1: *V. alginolyticus* ATCC 33787, S2: *V. alginolyticus* ATCC 17749, S3: *V. alginolyticus* isolated from diseased gilthead *Sparus aurata*, S4: *V. alginolyticus* isolated from diseased gilthead *Dicentrarchus labrax*, S5: *V. parahaemolyticus* ATCC 17802. −: downregulated; +: upregulated.

4. Discussion

All organisms respond to environmental stress by modifying the rate of synthesis of certain proteins. In this study, we observed that long-term starvation or γ-irradiation induced several alterations in OMPs patterns of marine food-borne pathogens *V. alginolyticus* and *V. parahaemolyticus* cells. These alterations were manifested by the appearance and/or disappearance of proteins as well as in the level of expression of certain proteins.

Modifications observed in cells incubated for 8 months in the seawater microcosm are probably due to nutrient deficiency in seawater. Indeed, it is now clear that changes in the environment induce several alterations in bacterial function and protein expression (Kustos et al., 2007). After the beginning of an adverse effect such as starvation, protein synthesis is inhibited and cell division is interrupted. In parallel, expression of several proteins increases; these are the so-called stress proteins (Kustos et al., 2007). Furthermore, in this study, OmpU can be considered as a starvation-inducible protein, since this protein becomes more abundant in some of the *Vibrio* strains tested. In addition, MipA was visualized and thus it can also be considered a stress protein in some vibrios. Generally, environmental changes influence the expression of OMPs, and a rise in temperature may induce significant changes in the OMP expression of *Escherichia coli* (Molloy et al., 2000), *Stenotrophomonas maltophilia* (Rahmati-Bahram et al., 1996), *Leptospira interrogans serovar Lai* (Cullen et al., 2002), *Borrelia burgdorferi* (Obonyo et al., 2002) and *Serratia marcescens* (Puig et al., 1993). Martinez et al. (2001) also found expression of new OMP in *E. coli* strains by increasing the temperature from 30 to 37 °C. Acidic pH induced expression of new proteins on the surface of *Yersinia pestis* (Feodorova and Devdariani, 2001) and OMP expression in *B. burgdorferi* was also altered at different pH values (Carroll et al., 1999). Our study showed that expression of OtnA, OmpW, OmpA and peptidoglycan-associated lipoprotein decreased to non-detectable levels when cells were submitted to starvation. It appears that these proteins can be regulated by the availability of nutrients, either directly or indirectly. Studies have shown that the expression of OmpW in *Vibrio cholerae* was regulated by nutrients and other environmental conditions, including temperature and osmolarity (Nandi et al., 2005). Furthermore, OmpW from *V.parahaemolyticus* (Xu et al., 2004), *V. alginolyticus* (Xu et al., 2005) and *P. damselae* (Wu et al., 2006) were all found to be upregulated in high NaCl concentrations (3.5% and 4%). In addition, in this work, several OMPs such as OmpK, outer membrane protein, putative porin and/or long-chain fatty acid transport protein were reduced due to nutrient deprivation. The composition of the culture medium may have significant effects on protein expression. Furthermore, Yoshida et al. (2002) revealed changes in concentrations of OmpR and P, while Contreras et al. (1995) demonstrated an increase in OmpC and a decrease in OmpF expression of *E. coli*. The effects of magnesium limitation on OMP expression in *Pseudomonas aeruginosa* (Shand et al., 1988) and *Salmonella* strains (Hwang et al., 2002) have been also observed.

Our study showed alterations in OMP patterns of *V. alginolyticus* and *V. parahaemolyticus* after the cells had been

irradiated. In addition, we did not observe γ- induced proteins, since the expression level of all altered OMPs identified was reduced, whereas MipA was detected; therefore, it may be involved in a γ-irradiation stress response of some vibrios. According to Filali-Mouhim et al. (1997), γ-irradiation is capable of causing irreversible alterations in protein conformations at the molecular level, such as fragmentation or aggregation. Such an alteration due to γ-irradiation in aqueous solutions is related to the formation of free radicals by water radiolysis (Assemand et al., 2004). Caillet et al. (2008) reported that γ-rays influence the synthesis of heat shock proteins in foodborne pathogens in a way that critically depends on the radiation dose.

Many vibrios are pathogenic for humans and/or marine vertebrates and invertebrates (Zhang and Austin, 2005). OMPs, whose production is often regulated by environmental cues, play important roles in bacterial pathogenesis by enhancing the adaptability of the pathogens to various environments. Thus, OMPs contribute to the virulence of bacteria and play essential roles in bacterial adaptation to host niches, which are usually hostile to invading pathogens (Lin et al., 2002). The various alterations observed in the OMP profiles of starved and γ-irradiated *Vibrio* reflect the stability of these virulence factors under stress conditions.

In the present study, we noted that alterations in OMPs profiles of vibrios confronted with starvation and γ-irradiation are variable. Furthermore, based on the number of altered proteins, the effect of γ-irradiation on expression of OMPs is more accentuated compared to starvation. In addition, compared to *V. parahaemolyticus*, most modifications are observed in *V. alginolyticus*, and especially strains isolated from the internal organs of aquacultured-diseased gilthead sea bream and sea bass. We also observed that these modifications are variable between the *V. alginolyticus* cells examined. This might be related to the genomic plasticity of the tested strains.

In summary, starvation or γ-irradiation causes damage to the expression of OMPs in the foodborne pathogen *V. alginolyticus* and *V. parahaemolyticus* cells. The response of *Vibrio* to starvation is different from that to γ-irradiation, but MipA was detected in two cases. This protein can be considered as a γ-irradiation and long-term starvation stress protein in some vibrios.

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