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**GENOME-WIDE EXAMINATION OF RADIATION STRESS RESPONSE IN SHEWANELLA ONEIDENSIS MR-1**

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# GENOME-WIDE EXAMINATION OF RADIATION STRESS RESPONSE IN SHEWANELLA ONEIDENSIS MR-1

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ABSTRACT

GENOME-WIDE EXAMINATION OF RADIATION STRESS RESPONSE IN SHEWANELLA ONEIDENSIS MR-1

By

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*Shewanella oneidensis* MR-1, a Gamma proteobacterium, is notable in the terminal electron acceptors it uses including some toxic metals and radionuclides. Thus it has a great potential for bioremediation. However, MR-1 is uniformly sensitive to UVC, UVB, UVA, natural solar radiation as well as ionizing radiation. I delineated the genomic response of *Shewanella oneidensis* MR-1 to five radiation stresses. A total of 4.2-, 3.9-, 8.1-, 28.0-, and 5.9% of the MR-1 genome showed differential expression following UVC, UVB, UVA, natural solar radiation, and ionizing radiation exposure at a dose that yields about 20% survival rate, respectively. The gene expression profile of MR-1 in response to ionizing radiation is more similar to that of UVC, which is characterized by a strong induction of the SOS response and of many prophage related genes, plus some oxidative stress response. Genomic response to UVB is a combination of the UVC and UVA patterns, which represents a shift from shorter wavelength of UVR-induced direct DNA damage and activation of prophages to longer wavelength of UVR-induced global photo-oxidative damages. I observed the traditional UVA-induced stress responses in MR-1 such as induction of antioxidant enzymes and proteins, sequestration of the transition metals and activation of the degradative pathways, however, the induction of heavy metal and multidrug efflux pumps is a previously unknown phenotype for this

stress. Consistent with natural solar UV radiation composition, genomic response to solar radiation is more similar to that of UVA but with more genes induced for detoxification. In addition, the number of differentially expressed genes from most functional categories increased greatly compared to either UVB or UVA or their sum. This unique gene expression profile indicates that natural solar radiation impacts biological processes in a much more complex way than previously thought.

Quantitative real time reverse transcription PCR (Q RT-PCR) assays were carried out in parallel for controls and irradiated samples for 16 selected genes that are involved in DNA recombination repair, nucleotide excision repair (NER), defending against oxidative stress, encoding heavy metal and multidrug efflux pumps, putative regulatory genes, transport genes, and metabolic genes. A good correlation was obtained between array-based transcriptional analysis and Q RT-PCR assays. I further demonstrated that mutagenic repair, photoreactivation and NER are functional in MR-1 although the expression of NER component genes is not damage inducible. Activation of prophages and DNA damage appear the major lethal factors in MR-1 following short wavelength UVR (UVC and UVB) and ionizing radiation exposure whereas global photo-oxidative damage contributes greatly to its UVA and solar radiation sensitivity. In addition, alteration in gene regulation, e.g. loss the damage inducibility of some DNA repair genes, perhaps as the consequence of lack of natural selection, may contribute to its high radiation sensitivity in general.

*To my son Evan Thomas Q Carter*

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# PREFACE

The research work on UV radiation presented in this dissertation was sampled at Dr. George W. Sundin's lab at Department of Plant Pathology, Michigan State University. The research work on ionizing radiation was sampled at Dr. Michael Daly's lab at Uniformed Services University of the Health Sciences at Bethesda, Maryland. The fluorescent images presented in this dissertation were taken by Shirley Owens, from Center for Advanced Microscopy at Michigan State University. The transmission electron microscopy images presented in this dissertation were taken by Alicia Pastor, from Center for Advanced Microscopy at Michigan State University. The whole genome microarray of *Shewanella oneidensis* MR-1 was generated at Dr. Jizhong Zhou's lab at Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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# CHAPTER 1

# INTRODUCTION AND RATIONALE

**Radiation and radiation induced biological effects in bacteria.**

Ultraviolation radiation (UVR) is probably the most common physical agent that damages DNA. Living organisms have had to cope with the genotoxic effects of solar UV radiation since the beginning of biological evolution on the earth. The UV radiation spectrum is commonly divided into three wavelength bands designated UVC (100-290 nm), UVB (290-320 nm) and UVA (320-400 nm). Solar UV radiation reaching earth's surface consists mainly of UVA (about 95%) and a small portion of UVB (about 5%) due to ozone filtration. However, unattenuated UV radiation prior to the accumulation of oxygen in the earth's atmosphere may have served as an important constraint during the evolution of terrestrial life.

Since the maximum absorption of DNA is at 260 nm, exposure to both UVB and UVC will induce the formation of a variety of photoproducts, resulting in various adverse biological effects. Cyclobutane pyrimidine dimer (CPD) and pyrimidine-pyrimidone (6-4) photoproduct ((6-4) PD) are two major DNA photoproducts in bacteria. CPD is produced from the formation of a four-membered ring structure resulting from saturation of the 5,6 double bonds of the two adjacent pyrimidines (Setlow 1966). The yield of CPD is influenced by nucleotide composition as well as the sequence context (Gordon et al. 1982; Michell et al. 1992). The (6-4) PD is produced by a covalent linkage between the C-6 position of one pyrimidine and the C4 position of the adjacent pyrimidine. Irradiation of (6-4) PD with 313 nm light leads to the formation of the Dewar isomer (Taylor and Cohrs 1987), which is a significant DNA photoproduct after solar light exposure (Perdiz et al. 2000). Other UVR induced DNA photoproducts include purine lesions, pyrimidine hydrates, and thymine glycol. Thymine glycol is also one of the major forms of DNA

base damage induced by ionizing radiation (Demple and Linn 1982; Fisher and Johns 1976; Gasparro and Fresco 1986; Porschke 1973; Setlow 1992; Varghese 1970). UV radiation also causes cross links and strand breaks. The frequency of stand breaks and DNA-protein cross-links is dramatically increased by longer wavelength UVR irradiation (Tyrrell 1991).

UV radiation can damage DNA indirectly through photosensitization, in which sensitizer molecules in the cell absorb the photons of UV radiation and transfer the energy to the base in the DNA. Thus long wavelength UV radiation (UVA) can also induce the formation of CPD (Perdiz et al. 2000). The sensitizer molecules can also transfer the energy to oxygen, resulting in highly reactive oxygen species (ROS), which can cause damage to a variety of molecules as well as physiological processes in the cell (Eisenstark 1987; Eisenstark 1989).

Similar to UV radiation, ionizing radiation has been a source of naturally occurring physical damage to the DNA of living organism since the beginning of life. Ionizing radiation has high energy and great penetrating ability, thus it can directly damage the cell by depositing energy randomly to any cellular components (Frankenberg-Schwager 1990; Goodhead 1989; Hutchinson 1985). Damage to DNA bases has been extensively studied in vitro. Many products and short-lived intermediates have been characterized (Teoule 1987). Ionizing radiation can also induce protein-DNA cross links, sugar damage and strand breaks, of which double strand breaks (DSBs) is the most lethal effect (Hutchinson 1985; Ilikis 1991; Ward 1988; Ward 1990).

Ionizing radiation can also induce the formation of ROS through the radiolysis of water, which has been suggested to be a major potential source of indirect damage to

DNA (Riley 1994; Ward 1990). It has been estimated that more than 80% of the energy of ionizing radiation deposited in the cell results in the abstraction of electrons from water. Superoxide radical will be produced when oxygen is present. Thus, similar to long wavelength UV radiation, ionizing radiation can induce oxidative stress in living organism.

Knowledge of DNA damage repair is primarily from *Escherichia coli* and *Deinococcus radiodurans*, an extremely radiation resistant bacterium. Several DNA repair pathways, e.g photoreactivation, base excision repair (BER), nucleotide excision repair (NER), mismatch repair, recombination repair, and the SOS response have been demonstrated to be important in repairing radiation-induced damage. Recently, Levin-Zaidman et al. proposed that an ATP-dependent ligase mediated a non-homologous end-joining pathway (NHEJ) as well as the presence of an unusual ring-like nucleoid conformation may facilitate the repair of DSBs in *D. radiodurans* (Levin-Zaidman et al. 2003). However, this explanation has been challenged by the research in Daly's lab, which indicates that high intracellular Mn/Fe ratio is the essential factor in *D. radiodurans* that contributes to the high radiation resistance (Daly et al. 2004).

*Shewanella oneidensis* MR-1, a Gamma proteobacterium, was originally isolated from the sediment of Oneida Lake, NewYork State (Myers et al. 1988). Extensive studies have been carried out on this bacterium due to its respiratory versatility: it can reduce a variety of compounds including some toxic metals and radionuclides (Liu et al. 2002; Middleton et al. 2003). Thus, it has great potential for bioremediation of inorganic pollutants. However, I found that MR-1 is uniformly sensitive to UVC, UVB, UVA, solar light and ionizing radiation. This extreme radiation sensitivity could not be simply

explained from the MR-1 genome content. A total of 2.8% of MR-1 genome is implicated in DNA replication, repair and recombination, which is comparable to that of *E. coli* K12 (2.7%) and *D. radiodurans* R1 (3.1%) (Blattner et al. 1997; Heidelberg et al. 2002; White et al. 1999). Compared to *E. coli*, MR-1 has most of the DNA repair pathways including photoreactivation; NER; BER; methyl directed mismatch repair; recombination repair and the SOS response, although a few *E. coli* DNA repair genes are not present on MR-1 genome (Table 1.1). Regarding defense against oxidative stress, MR-1 has a putative OxyR (SO1382) and the sigma factor RpoS, but it does not have the SoxR and SoxS regulators, which play a very important role in defending against superoxide radical induced oxidative damage in *E. coli*. As a respiratory generalist, MR-1 has 39 *c*-type cytochromes, which is much higher than *E. coli* (7) (Heidelberg et al. 2002). Cytochromes, along with other components of respiratory chain such as flavins, quinones, are potential photoreceptors for long wavelength UVR (UVA and UVB). In addition, Daly et al. showed that in contrast to *D. radiodurans*, MR-1 has a high intracellular ratio of Fe/Mn (Daly et al. 2004).

Why is MR-1 so sensitive to radiation? What are the crucial factors that contribute to this extreme radiation sensitivity in MR-1? Is it because of a lack of certain important DNA repair genes or is it because the cell is rich in photoreceptors and rich in Fe containing proteins? Furthermore, what are the important traits in determining bacterial radiation resistance or sensitivity? Do evolution and natural selection have any impact on bacterial radiation resistance or sensitivity and if so, in what manner?

**My research objectives.**

**Objective 1. Functional analysis of putative DNA damage repair pathways and DNA damage tolerance in *S. oneidensis* MR-1.**

Genome annotation indicates that MR-1 has a suite of DNA damage repair genes and damage tolerance systems (Table 1.1). However, since MR-1 has likely been screened from solar light for millions of years due to its sediment habitat, it is important to examine whether those putative genes are functional. The data obtained here will provide us a clue whether natural selection and evolution has an impact on the high UVR sensitivity in MR-1.

My research focus centers on three repair pathways: photoreactivation, NER and mutagenic DNA repair (MDR). Photoreactivation in bacteria involves a single enzyme called photolyase (*phrB*), which binds to CPDs, and in the presence of light (300-500 nm), reverses the dimer to its component monomers. NER is present from bacteria to humans and plays a critical role in protecting cells from a variety of DNA-damaging agents since it can recognize a broad range of DNA lesions including ionizing radiation induced purine damage, active oxygen species induced base loss and UV induced pyrimidine dimers. UmuDC-mediated MDR functions in translesion synthesis enabling bypass of DNA lesions that would normally block replication by DNA polymerase III. Translesion DNA synthesis provides the cell with an additional mechanism of survival, however, this process is accompanied by an elevation of the cellular mutation rate. The increase in cellular mutation frequency can be assayed by examining the increase in the occurrence of spontaneous mutants following irradiation.

Table 1.1 Comparison of major DNA repair genes between *E. coli* K-12 and *S. oneidensis* MR-1

| [a]Gene | ID in *E. coli* | Product | ID (gene) in MR-1 | [b]Pathways |
|---|---|---|---|---|
| *alkA* | b2068 | 3-methyl-adenine DNA glycosylase II, inducible | - | Adaptive response |
| *alkB* | b2212 | DNA repair system specific for alkylated DNA | SO1098* | Adaptive response |
| *dam* | b3387 | DNA adenine methylase | SO0289 (*dam*) | mMM |
| *dcm* | b1961 | DNA cytosine methylase | - | VSP |
| *dinB* | b0231 | DNA polymerase IV, damage inducible protein P | SO1114 (*dinP*) | SOS |
| *umuC* | b1184 | SOS mutagenesis and repair | SOA0012 (*umuC*) | SOS |
| *umuD* | b1183 | SOS mutagenesis; error-prone repair; processed to UmuD'; forms complex with UmuC | SOA0013 (*umuD*) | SOS |
| *dinF* | b4044 | DNA-damage-inducible protein F | SO4617 (*dinF*) | SOS |
| *dinG* | b0799 | ATP-dependent helicase | SO1819, SO2081 (*dinG*) | SOS |
| *dinD* | b3645 | DNA-damage-inducible protein | - | SOS |
| *dinI* | b1061 | damage-inducible protein I | - | SOS |
| *dinJ* | b0226 | damage-inducible protein J | - | SOS |
| *fis* | b3261 | site-specific DNA inversion stimulation factor; DNA-binding protein; a trans activator for transcription | SO0393 (*fis*) | Others |
| *lexA* | b4043 | regulator for SOS(lexA) regulon | SO4603 (*lexA*) | SOS |
| *ada* | b2213 | O6-methylguanine-DNA methyltransferase; transcription activator/repressor | SO3127 | Adaptive response |
| *oraA* | b2698 | regulator, OraA protein | SO3429 (*recX*) | Others |
| *mfd* | b1114 | transcription-repair coupling factor; mutation frequency decline | SO2255 (*mfd*) | NER |
| *mutH* | b2831 | methyl-directed mismatch repair | SO1330 (*mutH*) | mMM |
| *mutL* | b4170 | enzyme in methyl-directed mismatch repair | SO0601 (*mutL*) | mMM |
| *mutM* | b3635 | formamidopyrimidine DNA glycosylase | SO4726 (*mutM*) | BER |
| *mutS* | b2733 | methyl-directed mismatch repair | SO3431 (*mutS*) | mMM |
| *mutT* | b0099 | 7,8-dihydro-8-oxoguanine-triphosphatase, prefers dGTP, causes AT-GC transversions | SO0410 (*mutT*) | Others |
| *mutY* | b2961 | adenine glycosylase; G.C --> T.A transversions | SO3368 (*mutY*) | BER |
| *mug* | | thymine mismatch DNA glycosylase | - | BER |
| *tag* | b3549 | 3-methyl-adenine DNA glycosylase I, constitutive | SO0016 (*tag*) | BER |
| *ung* | b2580 | uracil-DNA-glycosylase | SO3654 (*ung*) | BER |
| *vsr* | b1960 | DNA mismatch endonuclease, patch repair protein | - | VSP |
| *nth* | b1633 | endonuclease III; specific for apurinic and/or apyrimidinic sites | SO2514 (*nth*) | BER |
| *nfo* | b2159 | endonuclease IV | - | BER |

| | | | | |
|---|---|---|---|---|
| *nfi* | b3998 | endonuclease V (deoxyinosine 3'endoduclease) | - | BER |
| *nei* | b0714 | endonuclease VIII and DNA N-glycosylase with an AP lyase activity | - | BER |
| *xthA* | b1749 | exonuclease III | SO3037 (*xth*) | BER |
| *xseA* | b2509 | exonuclease VII, large subunit | SO3294 (*xseA*) | Others |
| *xseB* | b0422 | exonuclease VII, small subunit | - | Others |
| *exo* | b2798 | 5'-3' exonuclease | SO1549 (*xni*) | Others |
| *ogt* | b1335 | O-6-alkylguanine-DNA/cysteine-protein methyltransferase | SO3126, SO2532 (*ogt*) | Others |
| *phrB* | b0708 | deoxyribodipyrimidine photolyase (photoreactivation) | SO3384 (*phrB*) | PR |
| *sms/radA* | b4389 | probable ATP-dependent protease | SO1226 (*radA*) | Others |
| *radC* | b3638 | DNA repair protein | SO4248, SO1455 (*radC*) | Others |
| *recA* | b2699 | DNA strand exchange and renaturation, DNA-dependent ATPase, DNA- and ATP-dependent coprotease | SO3430 (*recA*) | REM |
| *recB* | b2820 | DNA helicase, ATP-dependent dsDNA/ssDNA exonuclease V subunit, ssDNA endonuclease | SO2148 (*recB*) | REM |
| *recC* | b2822 | DNA helicase, ATP-dependent dsDNA/ssDNA exonuclease V subunit, ssDNA endonuclease | SO2149 (*recC*) | REM |
| *recD* | b2819 | DNA helicase, ATP-dependent dsDNA/ssDNA exonuclease V subunit, ssDNA endonuclease | SO2147 (*recD*) | REM |
| *recE* | b1350 | exonuclease VIII, ds DNA exonuclease, 5' --> 3' specific | - | REM |
| *recF* | b3700 | ssDNA and dsDNA binding, ATP binding | SO0010 (*recF*) | REM |
| *recG* | b3652 | DNA helicase, resolution of Holliday junctions, branch migration | SO4364 (*recG*) | REM |
| *recJ* | b2892 | ssDNA exonuclease, 5' --> 3' specific | SO0952 (*recJ*) | REM |
| *recN* | b2616 | protein used in recombination and DNA repair | SO3462 (*recN*) | REM |
| *recO* | b2565 | protein interacts with RecR and possibly RecF proteins | SO1350 (*recO*) | REM |
| *recQ* | b3822 | ATP-dependent DNA helicase | SO4241 (*recQ*) | REM |
| *recR* | b0472 | recombination and repair | SO2015 (*recR*) | REM |
| *recT* | b1349 | recombinase, DNA renaturation | - | REM |
| *rus* | b0550 | endodeoxyribonuclease RUS (Holliday junction resolvase) | - | REM |
| *ruvA* | b1861 | Holliday junction helicase subunit B; branch migration; repair | SO2430 (*ruvA*) | REM |
| *ruvB* | b1860 | Holliday junction helicase subunit A; branch migration; repair | SO2429 (*ruvB*) | REM |
| *ruvC* | b1863 | Holliday junction nuclease; resolution of structures; repair | SO2431 (*ruvC*) | REM |
| *sbcB* | b2011 | exonuclease I, 3' --> 5' specific; deoxyribophosphodiesterase | SO2790 (*sbcB*) | REM |
| *sbcC* | b0397 | ATP-dependent dsDNA exonuclease | SO2843 (*sbcC*) | REM |
| *sbcD* | b0398 | ATP-dependent dsDNA exonuclease | SO2844 (*sbcD*) | REM |
| *ssb* | b4059 | ssDNA-binding protein | SO4028 (*ssb*) | SOS |
| *uvrA* | b4058 | excision nuclease subunit A | SO4030 (*uvrA*) | NER |

| | | | | |
|---|---|---|---|---|
| *uvrB* | b0779 | DNA repair; excision nuclease subunit B | SO2506 (*uvrB*) | NER |
| *uvrC* | b1913 | excinuclease ABC, subunit C; repair of UV damage to DNA | SO1861 (*uvrC*) | NER |
| *uvrD* | b3813 | DNA-dependent ATPase I and helicase II | SO0467 (*uvrD*) | NER |
| *lig* | b2411 | DNA ligase (NAD) | SO2896 (*ligA*) | MP |
| *ybfD* | b0706 | putative DNA ligase | - | Not known |
| *yicF* | b3647 | putative DNA ligase | - | Not known |
| | | ATP-dependent DNA ligase | SO2204 | Others |
| *polA* | b3863 | DNA polymerase I, 3' --> 5' polymerase, 5' --> 3' and 3' --> 5' exonuclease | SO4669 (*polA*) | BER |
| *polB* | b0060 | DNA polymerase II | SO1820 (*polB*) | SOS |
| *dnaE* | b0184 | DNA polymerase III, alpha subunit | SO1644 (*dnaE*) | Others |
| *dnaN* | b3701 | DNA polymerase III, beta-subunit | SO0009 (*dnaN*) | Others |
| *holC* | b4259 | DNA polymerase III, chi subunit | SO3423 (*holC*) | Others |
| *holB* | b1099 | DNA polymerase III, delta prime subunit | SO2612 (*holB*) | Others |
| *holA* | b0640 | DNA polymerase III, delta subunit | SO1172 (*holA*) | Others |
| *dnaQ* | b0215 | DNA polymerase III, epsilon subunit | SO2245, SO2559 (*dnaQ*-1) | Others |
| *holD* | b4372 | DNA polymerase III, psi subunit | - | Others |
| *dnaX* | b0470 | DNA polymerase III, tau and gamma subunits; DNA elongation factor III | SO2013 (*dnaX*) | Others |
| *holE* | b1842 | DNA polymerase III, theta subunit | - | Others |
| *dnaG* | b3066 | DNA primase | SO1286 (*dnaG*) | Others |
| *topA* | b1274 | DNA topoisomerase type I, omega protein | SO2705 (*topA*) | Others |
| *topB* | b1763 | DNA topoisomerase III | SO3061 (*topB*) | Others |
| *gyrA* | b2231 | DNA gyrase, subunit A, type II topoisomerase | SO2411 (*gyrA*) | Others |
| *gyrB* | b3699 | DNA gyrase subunit B, type II topoisomerase, ATPase activity | SO0011 (*gyrB*) | Others |
| *parC* | b3019 | DNA topoisomerase IV subunit A | SO3897 (*parC*) | Others |
| *parE* | b3030 | DNA topoisomerase IV subunit B | SO3899 (*parE*) | Others |

[a] gene name is from *E. coli*. [b] abbreviation of DNA repair pathways: BER, base excision repair; NER, nucleotide excision repair; mMM, methylation dependent mismatch repair; MMY, *mut*Y-dependent mismatch repair; VSP, very short patch repair; RER: recombinational repair; SOS: SOS response; MP, multiple pathways. Others: related to DNA repair and replication. *distant homolog.

**Objective 2. Comparison of transcriptional profiles of MR-1 following UVC, UVB and UVA exposure.**

Historically, the investigation of UV radiation damage to DNA marks the beginning of the study of the repair and tolerance of DNA damage in bacteria. Since UV radiation at 254 nm is readily available from an ordinary germicidal lamp and it can efficiently induce DNA lesions, it has been used in most studies even though it has minor biological relevance. Previous studies had demonstrated that there was a significant difference between far UV (UVC) and near UV (UVB and UVA) induced lethal, mutagenic and physiological effects on bacteria (Eisenstark 1987; Eisenstark 1989; Jagger 1983; Webb 1977). However, there are still many unanswered questions and controversies left behind. With the concern of ozone depletion in the stratosphere, more studies have been focused on the biological effects of solar UVB on plants or phototrophic bacteria or the impact of solar UVB on ecosystems such as carbon and energy flow. There is no study so far emphasizing the understanding of the molecular basis of the difference in UVC, UVB and UVA induced damage in bacteria.

Upon DNA damage, many prokaryotes elicit the SOS response, which embodies the pleiotropic response when the cell is in stress. Many proteins induced as part of this process are involved in DNA replication, repair and control of cell division. Recently, Courcelle et al. compared the gene expression profile of *E. coli* MG1655 (a derivative of *E. coli* K12) and the *lexA* deficient mutant following UVC exposure and revealed that more than 30 genes are subject to SOS regulation (Courcelle et al. 2001). This study demonstrated that microarray-based gene expression profiling is a powerful tool in understanding the global stress response in bacteria. Comparison of transcriptional

profiles of MR-1 following UVC, UVB and UVA exposure will allow us to compare the global response to three different wavelengths of UVR in MR-1, and thus improve our understanding on the molecular basis of what are the common and the different damages induced by UVC, UVB and UVA. In addition, since the gene expression profile of *E. coli* following UVC exposure is available (Courcelle et al. 2001), comparison between *E. coli* and MR-1 will give us an opportunity to identifying the important factors that contribute to the difference in the UVC resistance and sensitivity between *E. coli* and *S. oneidensis* MR-1.

**Objective 3. Examination of transcriptional profiles of MR-1 following natural solar radiation exposure.**

Natural solar radiation contains about 3% UV radiation (wavelengths less than 400 nm), 37% visible light (wavelengths between 400-780 nm), and 60% infrared light (wavelengths longer than 780 nm). The deleterious effect of solar radiation is thought to be primarily caused by solar UV radiation (Diffey 1991), thus knowledge of the solar radiation induced biological effects comes primarily from the studies using either UVB or UVA as radiation sources. Is solar radiation induced biological effects a simple sum of UVB and UVA effects? *S. oneidensis* MR-1 is extremely sensitive to solar radiation. More than 80% of the cells die after exposure to the Michigan summer sun light for about 10 to 15 min. What are the primary lethal factors in MR-1 following solar radiation exposure, the DNA damage or the global photo-oxidative damage? By comparing the gene expression profiles of MR-1 among samples irradiated by solar radiation, UVA and UVB, I hope to gain an understanding of the molecular basis of what are the deleterious effects in MR-1 following natural solar radiation exposure. The knowledge obtained also

contributes to our understanding of what are the natural solar radiation induced biological effects in bacteria in general, a topic in its infacy.

**Objective 4. Examination of transcriptional profiles of MR-1 following ionizing radiation exposure.**

The high sensitivity of *S. oneidensis* MR-1 to ionizing radiation could be problematic in bioremediation of radionuclides wastes, e.g. U and Tc. Understanding the causes of this high sensitivity will provide us the knowledge for management of bioremediation. Ionizing radiation induced biological effects have been studied extensively in *D. radiodurans*. Until now, however, there is no conclusive explanation as to what are the major factors that contribute to the extreme radiation resistance in *D. radiodurans*. MR-1 could be an excellent model from the other end of the radiation resistance system for comparison. Recently, Liu et al. described the gene expression profile of *D. radiodurans* R1 following exposure to 15 kGy of gamma ray (Liu et al. 2003). Comparison of gene expression profiles between *D. radiodurans* and *S. oneidensis* will provide us insights into the molecular basis of what are the important factors that contribute to radiation resistance and sensitivity in bacteria. In addition, since ionizing radiation can damage DNA directly as well as induce oxidative stress, comparison of gene expression profiles of MR-1 among gamma ray, UVC and UVA will delineate the commonality and differences in biological effects induced by ionizing radiation and UV radiation, and thus provide clues on what are the major lethal factors in MR-1 following gamma ray exposure.

**My experimental approaches.**

Microarray based transcriptional analysis is a high throughput method, which allows analyzing thousands of genes in parallel, thus it is a powerful tool in investigating the global stress response in bacteria. Quantitative real time reverse transcription PCR (Q RT-PCR) based transcriptional analysis is a sensitive and quantitative method, which is powerful in detection and quantification of low abundant genes or transcripts. Ever since the introduction of microarray technology, there has been a tremendous discussion and rapid changes in array fabrication, array hybridization, data normalization and data analysis. For Q RT-PCR, issues regarding data normalization and quantification have been raised and discussed extensively. I will briefly review what I have learned about the technologies and the rationale of my choices for microarray hybridization, data normalization and analysis as well as the choice for internal controls in Q RT-PCR analysis.

**DNA microarray technology.**

DNA microarrays are basically a high-throughput format of a dot blot. Currently there are two major types: one is the oligonucleotide-based array and the other is the PCR product-based array. Oligonucleotides either can be synthesized insitu using photolithography as developed primarily by Affymetrix Inc (Santa Clara, CA) or first synthesized in a conventional way and then deposited on a glass surface (e.g. Operon Technologies). The PCR product-based microarray generally involves designing primers for each gene; PCR amplification and purification of the PCR products followed by spotting of the purified PCR products onto a glass surface. I used the PCR-products based

microarrays because they were the only *Shewanella* arrays available at the time and their performance and general acceptance were well established.

A DNA microarray experiment consists of array fabrication, probe preparation, hybridization and data analysis. A variety of methods have been developed for probe labeling (DeRisi et al. 1997; Gill et al. 2002; Hegde et al. 2000; Richmond et al. 1999). In the gene expression experiment, cDNA can be labeled by either directly incorporating fluorescent dye (Cy5 or Cy3) labeled nucleotides (DeRisi et al. 1997) into cDNA during the reverse transcription step or by a two-step labeling using aminoally-dUTP (Hegde et al. 2002), in which primary aliphatic amino groups are first incorporated during cDNA synthesis, and in the second step, the monofunctional N-hydroxylsuccinimide-activated fluorescent dye is coupled to cDNA by chemical reaction with the amino functional groups. Due to the increased labeling efficiency in this method, less starting material is needed (e.g. 2 μg of total RNA) than direct incorporation (10-20 μg of total RNA). In addition, since the substrate for the reverse transcription is identical for all samples, the two-step labeling can reduce the dye bias during incorporation. The hybridization and wash conditions have to be optimized to minimize the cross-hybridization. In general, array format, the GC content of the genome and nature of dye used for probe labeling are the important factors to consider for optimization of hybridization and washing conditions. I used aminoally-dUTP for labeling simply because it requires less amount of total RNA. This is particulary valuable for me because, due to the experimental limitation, e.g. volume and cell density for the UVR treatments, I had difficulty in obtaining the large quantity of total RNA. I was able to optimize the hybridization and washing conditions for my system based on the protocol developed by Hegde et al. (2000). I had

demonstrated that my experimental condition was stringent enough for detection of differentially expressed genes.

There are several systematic variables in a DNA microarray experiment that can affect the measurement of mRNA levels, which include the inherent errors from sample handling, slide to slide variation, difference in labeling or hybridization efficiency and variation during the image analysis. Normalization is a process to minimize these variations and establish a common base for comparison. A variety of methods have been described for normalization (Dozmorov et al. 2004; Faller et al. 2003; Hoffmann et al. 2002; Kepler et al. 2002; Park et al. 2003; Smyth and Speed 2003; Yang et al. 2002; Yang et al. 2001; Yoon et al. 2004). Intensity dependent normalization (often called non-linear or LOWESS normalization) is a technique that is used to eliminate dye-related artifacts in two-color experiments that cause the Cy5/Cy3 ratio to be affected by the total intensity of the spot. This normalization process attempts to correct for artifacts caused by non-linear rates of dye incorporation as well as inconsistence in the relative fluorescence intensity between some red and green dyes (Yang et al. 2002). Since I used the two dyes (Cy5 and Cy3) for probe labeling and there are more than 4000 genes on the array, based on recommendation from GeneSpring user manual, I used LOWESS method for normalization.

Cluster analysis is often employed to group genes with a similar expression pattern in a microarray based experiment. In an unsupervised mode, cluster analysis uses algorithms to arrange genes according to similarity in their expression pattern without applying predefined classes. In the supervised mode, the task is to construct a set of classification rules which assigns predefined classes to given expression profiles (Brazma

and Vilo 2000). Current major clustering methods include hierarchical clustering (Eisen et al. 1998), self-organizing maps (SOM) (Tamayo et al. 1999), K-means clustering (Tavazoie et al. 1999) and principle component analysis (Alter et al. 2000). Hierarchical clustering algorithms can be divided into two types: agglomerative and divisive. The agglomerative method is a bottom-up approach, where the algorithm starts with n separate clusters and successively combines clusters until only one is left. The divisive method, in contrast, is a top-down approach starting with one cluster and successively splitting clusters to produce others. The algorithm used to form the clusters must be defined. The two widely used algorithms are single linkage, which is also called nearest neighbor, and average linkage. A distance matrix must be calculated before the clustering is performed. The two most commonly used distance measurements are the Euclidean distance and the Pearson correlation coefficient. In non-hierarchical cluster analysis the data are divided into a given cluster number. The most common one is K-means, which identifies K points that function as cluster centers. Each data point is then assigned to one of these centers in a way that minimizes the sum of the distance between all points and their centers. Thus, the goal of K-means is to produce groups of genes with a high degree of similarity within each group and a low degree of similarity between groups. K-means is particular useful to identify unique classes of genes that are up- or down-regulated in a time dependent manner. SOM is similar to the K-means approach, but it has a geometrical configuration and the number of nodes predefines the configuration. Thus SOM illustrates the relationship between the groups by arranging them in a two-dimensional map in addition to dividing genes into groups based on their expression pattern. Since I was examining the gene expression in a time dependent manner, e.g. at 5,

20 and 60 min after irradiation, I used K-means for data analysis. The cluster number for K-means analysis was determined by pre-analyzing the data using an un-supervised hierarchical cluster method.

Since the development of microarray technology, it has been applied rapidly and widely in many other research areas including single nucleotide polymorphism and mutation detection (Gerry et al. 1999; Hacia et al. 1999), sequencing (Behr et al. 1999; Cheung et al. 1998), genetic linkage analysis and population genetics (Chakravarti 1999; Cheung et al. 1998; Gentalen and Chee 1999), comparative genomics (Behr et al. 1999; Murray et al. 2001), phylogenetic analysis (Kakinuma et al. 2003; Polz et al. 2003; Reyes-Lopez et al. 2003) as well as in environmental microbiology (Cho and Tiedje 2001; Cho and Tiedje 2002; Loy et al. 2002; Peplies et al. 2004; Small et al. 2001). Microarray technology marks a revolution in biology, and promotes biological research from gene level to genome level.

**Real time PCR technology**.

Real time PCR captures the sensitivity of PCR methodology and allows the quantification of target genes in a real-time manner by detecting the fluorescence that is either directly or indirectly associated with the accumulation of the newly amplified DNA. Currently fluorescence detection can be achieved by using either a double-stranded DNA binding dye such as SYBR green or with FRET-based probes such as Taqman 5' nuclease-sensitive probes or DNA binding probes (Ponchel et al. 2003; Walker 2002). When irradiated by UV light, SYBR green emits a fluorescent signal if it is intercalated into double-stranded DNA. The fluorescence emitted by the dye increases proportionally with the amount of amplified DNA. However, SYBR green is unable to discriminate

between target DNA and non-specific amplification. Thus, highly specific PCR primers are required. The specificity of PCR amplification can be checked by generating a melting temperature (Tm) curve after a PCR run (Ririe et al. 1997). The Tm is the temperature at which 50% of the double stranded DNA separates. The Tm value is dependent on the length and the nucleotide composition of the amplicon. A single peak at the corresponding Tm of the amplicon indicates a specific amplification, whereas additional peaks or broad peaks indicate the presence and the significance of non-specific amplification.

Quantification by real time PCR can be absolute or relative. Absolute quantification determines the PCR template copy number by relating the detection signal to a standard curve. During the PCR amplification process, fluorescence values are recorded during every cycle and represent the amount of amplified DNA at that point. The more template present at the beginning of the reaction, the fewer number of cycles it takes to reach a point in which the fluorescence signal is first recorded as statistically significant above background (Gibson et al. 1996). This point is defined as the Ct, and will always occur during the exponential phase of amplification, thus the quantification is not affected by any reaction components becoming limited as occurs in the plateau phase. The quantity of template DNA can be obtained by interpolation of its Ct value versus a linear standard curve of Ct value obtained from a serially diluted standard solution. Since it is crucial to have the same amplification efficiency of target DNA as with standard DNA, a DNA fragment that contains the target DNA is usually used for constructing the standard curve. In general, the standard curves are linear over more than five orders of magnitude. Relative quantification describe the changes in nucleic acid level between

target DNA and a reference sample by comparing their Ct values directly without referring to a standard curve. Thus the fold change is more important in this case.

Quantification of mRNA by real time PCR involves two steps. The mRNA is first converted to cDNA by reverse transcription reaction, and the cDNA is used as template for PCR amplification. An internal control gene is usually required for normalizing the difference in reverse transcription efficiency. A gene that does not exhibit a change in expression at the condition examined may serve as an internal control. Common internal control genes are house keeping genes, e.g. glyceraldehyde-3-phosphate dehydrogenase (GAPDH), albumin, β-actin, γ-actin, and ribosomal *rrn* genes (Thellin et al. 1999). However, the use of ribosomal genes as the internal control is a concern due to their high abundance in the cell. For choice of a housing keeping gene, it is important to evaluate its suitability first under the experimental condition examined (Schmittgen and Zakrajsek 2000; Savli et al. 2003). Recently, Vandesompele et al. (2002) recommended a normalization strategy to obtain an accurate RT-PCR expression profiling by geometric averaging of at least three internal control genes. However, it is not very practical since a tremendous number of reactions are added for each PCR run.

To obtain an absolute quantification of mRNA, the efficiency of reverse transcription for the gene that is used to construct the standard curve has to be considered. The RNA standard curve can be obtained by sub-cloning the amplicon behind a T7 or SP6 RNA polymerase promoter and the sense RNA transcript is in-vitro transcribed. Alternatively, the sense-strand oligodeoxynucleotides of up to 100 nt can be used for construction of the RNA standard curve (Bustin 2000). The absolute quantification is time-consuming, however, and not practical for a high throughput format since it requires

the construction of an absolute standard curve for each individual gene. Various relative quantification methods have been developed. The standard curve method is similar to absolute quantification; however, the standard can be any nucleic acid as long as its concentration and length of amplicon are known. This standard curve is only used for calibration of Ct of each target RNA (cDNA). The comparative Ct method detects the relative gene expression with the formula $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001). This formula is based on the assumption that the amplification efficiencies of the target genes and the internal control gene are the same, which is not true in most cases. A new comparative Ct method has been proposed by Liu and Saint (2002) by simulating the kinetics of real time PCR using experimentally determined parameters. The new method has demonstrated improved the accuracy of quantification.

The candidate internal control genes for my experiments may come from those house keeping genes that express constantly under my experimental conditions. The relative abundance of the internal control is another factor to consider. I chose *ldhA* (lactate dehydrogenase) because its expression level remains unchanged in MR-1 after irradiation. In addition, the basal level of *ldhA* is close to most of genes I planned to quantify, which allowed me to use the same concentration of cDNA for PCR amplification and yielded a better quantification (less errors introduced by dilutions). To compare, I also used the 16S ribosomal gene as an internal control in all analysis. Since I was interested in the absolute abundance of NER component genes *uvrA*, *uvrB* and *uvrD* and key DNA damage repair gene *rec*A in MR-1 before and after UVC irradiation, I constructed the standard curves for each of them. For other genes that were used for

validation of microarray analysis, their cDNA copies were interperated by using the standard curve of *recA*.

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# CHAPTER 2

# SURVIVAL OF SHEWANELLA ONEIDENSIS MR-1 AFTER UV RADIATION EXPOSURE

**By**

**Xiaoyun Qiu, George W. Sundin, Benli Chai and James M. Tiedje (2004)**

**(Applied and Environmental Microbiology 70: 6435-6443)**

**Abstract**

We systematically investigated the physiological response as well as DNA damage repair and damage tolerance in *Shewanella oneidensis* MR-1 following UVC, UVB, UVA and solar light exposure. MR-1 showed the highest UVC sensitivity among *Shewanella* strains examined, with $D_{37}$ and $D_{10}$ values of 5.6- and 16.5% of *Escherichia coli* K12. Stationary cells did not show an increased UVA resistance compared to exponential phase cells, instead, they were more sensitive at high UVA dose. UVA irradiated MR-1 survived better on TSA than LB plates regardless the growth stage. A 20% survival rate of MR-1 was observed following doses of 3.3 J $m^{-2}$ of UVC, 568 J $m^{-2}$ of UVB, 25 kJ $m^{-2}$ of UVA and 558 J $m^{-2}$ of solar UVB respectively. Photoreactivation conferred an increased survival rate to MR-1 as much as 177-365 fold, 11- 23 fold and 3-10 fold following UVC, UVB and solar light irradiation, respectively. A significant UV mutability to rifampin resistance was detected in both UVC and UVB treated samples with the mutation frequency in the range of $10^{-5}$ to $10^{-6}$. Unlike in *E. coli*, the expression of the nucleotide excision repair (NER) component genes *uvrA*, *uvrB* and *uvrD* was not damage inducible in MR-1. Complementation of *Pseudomonas aeruginosa* UA11079 (*uvrA*$^-$) with *uvrA* of MR-1 increased the UVC survival of this strain more than three orders of magnitude. Loss of damage inducibility of the NER system appears to contribute to the high sensitivity of this bacterium to UVR as well as other DNA-damaging agents.

## Introduction

Solar ultraviolet radiation (UVR) is lethal and potentially mutagenic to all organisms at species-specific levels. The stratospheric ozone layer absorbs UVC (<290 nm) effectively, however, both UVA (320 to 400 nm) and UVB (290 to 320nm) wavelengths penetrate to the earth's surface. UVR-induced damage is greatly dependent on the sources of radiation and the time of exposure. Photons of UVB and UVC wavelengths cause direct DNA damage by inducing the formation of DNA photoproducts such as cyclobutyl pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidinone ((6-4) PD) (37). The accumulation of DNA photoproducts can be lethal through the blockage of DNA replication and transcription. UVA can cause photodamage to a variety of molecules as well as physiological processes directly or indirectly by inducing the production of reactive oxygen species (ROS) (5, 6, 18, 53). Distinct differences between Far- (UVC) and Near-UV (UVA and UVB) damage have been observed in both bacteria and bacteriophages (6).

Bacteria are particularly vulnerable to UVR damage due to their small size and unicellular structure. Thus, the possession of mechanisms to repair UVR-induced damage as well as other sheltering strategies, e.g. pigments and sunscreen molecules, are essential contributors to the ecological fitness of organisms that are regularly exposed to solar UVR. Several mechanisms have evolved in bacteria to repair or tolerate UVR-induced DNA damage. Photoreactivation and nucleotide excision repair (NER) are the two primary mechanisms that accurately repair UVR-damaged DNA whereas mutagenic DNA repair (MDR) is a determinant that increases damage tolerance (11). In addition, many of the genes involved in DNA damage repair are inducible through the SOS

response (29). Approximately 30 genes were reported to belong to the SOS regulon of *E. coli*. (4, 8).

Photoreactivation in bacteria involves a single enzyme called photolyase, which binds to CPDs and, in the presence of light (300-500 nm), reverses the dimer to its component monomers (26). CPD photolyases are widely but also sporadically distributed among Bacteria, Archaea and eukaryotes (56). NER is present from bacteria to humans and plays a critical role in protecting cells from a variety of DNA-damaging agents since it can recognize a broad range of DNA lesions including ionizing radiation induced purine damage, active oxygen species induced base loss and UV induced pyrimidine dimers (41). During the repair process, NER component enzymes hydrolyze two phosphodiester bonds, one on either side of the lesion, to generate an oligonucleotide carrying the damage. The excised oligonucleotide is released from the duplex, and the resulting gap is filled and ligated (28, 42, 43). In *E. coli*, the NER component genes *uvrA*, *uvrB*, and *uvrD* are subject to SOS regulation (4, 10, 22, 23, 45). UmuDC-mediated MDR functions in translesion synthesis enabling bypass of DNA lesions that would normally block replication by DNA polymerase III (46, 50, 51). Translesion DNA synthesis provides the cell with an additional mechanism of survival, although the process is accompanied by an elevation of the cellular mutation rate (46, 55). Expression of the *umuDC* operon is regulated by the SOS response in many bacteria (46).

*Shewanella oneidensis* MR-1, a Gamma proteobacterium, was originally isolated from the sediment of Oneida Lake, NewYork State (35). Extensive studies have been carried out on this bacterium due to its respiratory versatility: it can reduce a variety of compounds including toxic metals and radionuclides (30, 31). This unique feature offers

potential for bioremediation by immobilization of soluble metal species at contaminated sites. To succeed, MR-1 has to tolerate toxic levels of pollutants, and exposure to ionizing or solar radiation. Recently, the genome sequence of MR-1 has been completed (15). It consists of a 4,969,803-bp chromosome with 4,758 predicted ORFs and a 161,613-bp plasmid with 173 ORFs. Three prophages, lambdaSo (51,857 bp), MuSo1 (34, 551bp) and MuSo2 (35,666 bp) are present on MR-1 chromosome (15). Compared to *Escherichia coli* K12, MR-1 has most of genes in repairing DNA damages and defending oxidative stress. Knowledge on bacterial UV resistance and repairing mechanisms is predominantly from *Escherichia coli*. Limited knowledge on molecular and physiological responses to UVR is available for environmentally relevant bacteria. Here, we report the responses of MR-1 following UVC, UVB, UVA and natural sunlight exposure. We found that MR-1 was uniformly sensitive to all wavelengths of UVR. We also evaluated the contribution of photoreactivation, nucleotide excision repair and mutagenic repair to the survival of MR-1 following UVR exposures. An inefficiently expressed NER system in MR-1 appears to contribute to its high sensitivity to both UVB and UVC.

## Materials and methods

**Bacterial strains, plasmids, and culture conditions.** The bacterial strains, plasmids, and PCR primers used in this study are listed in Table 2.1. *E. coli* and *Pseudomonas aeruginosa* strains were grown in Luria-Bertani medium (pH 7.2) at 37 °C. All *Shewanella* strains were grown at 30 °C in tryptic soy broth except *S. algae,* which was grown in a modified marine broth (5 g peptone, 2 g yeast and 17 g sea salts in 1 liter, pH 7.2). For gene expression experiments, *S. oneidensis* MR-1 was grown in Davis

medium (Difco) supplemented with 15 mM of lactic acid. Ampicillin (100 μg $ml^{-1}$) was used to grow *E. coli* carrying plasmids pJJK20, pJB321, pXQ01 and pXQ03 whereas carbenicillin (200 μg $ml^{-1}$) was used to grow *P. aeruginosa* carrying the plasmids described above.

**Molecular techniques.** Genomic and plasmid DNA isolation, restriction digestion, gel purification, ligation and transformation were performed using standard techniques (40). PCR primers (Table 2.1) were designed using the Primer 3 program (http://www.broad.mit.edu/cgi-bin/primer/primer3.cgi/) and synthesized at the Genomic Technology Center of Michigan State University.

**UV irradiation, photoreactivation and MDR assays.** UVA, UVB, and UVC assays were performed using previously described methods (47, 49). The UVA, UVB, and UVC sources used were XX-15L, XX-15M, and XX-15 lamps (UVP Products; San Gabriel, Calif.), respectively. The energy output of each lamp was monitored with a UV-X radiometer (UVP Products) fitted with the appropriate sensor. The UVB lamp was filtered through cellulose diacetate (Kodacel; Eastman Kodak; Rochester, NY) to eliminate stray UVC wavelengths. During irradiation, cell suspensions were mixed continuously to avoid shading effects. In experiments comparing the UVA sensitivity at different physiological stages, cells grown in Davis medium to exponential phase were used directly for UVA treatment whereas stationary phase cells were diluted with Davis medium to an OD600 of about 0.2 (the density at mid-exponential phase in Davis medium). Photoreactivation assays and MDR assays were conducted as described previously (24, 25).

**Solar radiation sensitivity assays.** Solar radiation sensitivity assays were conducted by exposing cell suspensions to ambient solar radiation. The suspensions were maintained in sterile boxes constructed of 64 mm thick Acrolyte OP-4 plastic (Professional Plastics, Austin, TX). The Acrolyte OP-4 plastic transmits greater than 90% of the total radiation throughout the UVA and UVB wavelengths (Acrolyte OP-4 technical data sheet; Cyro Industries, Arlington, NJ). Replicate boxes were maintained on ice on a rocking platform during the exposures. Solar UVB radiation was measured with a UVB detector (SED240/UVB-1/W) attached to an IL-1700 research radiometer (International Light, Newburyport, Mass.). UVB radiation was measured every second, and the readings were integrated over the exposure period yielding a quantitative output in J $m^{-2}$. At appropriate time points, the boxes were temporarily shaded from sunlight exposure, and two samples (5 ml) were taken. One sample was plated in the dark and the other was plated following a photoreactivation treatment as described previously (25).

**Transcriptional analysis NER using a cDNA microarray.** *S. oneidensis* MR-1 whole genome DNA arrays were produced by Liyou Wu and Jizhong Zhou at Oak Ridge National Laboratory (Oak Ridge, TN). Mid-exponential phase cells (80 ml) grown in Davis medium were split to two parts, one was used for UVR treatments and the other was used as controls. The exposure doses were 3.3 J $m^{-2}$ for UVC, 568 J $m^{-2}$ for UVB and 25 KJ $m^{-2}$ for UVA, which yielded about 20% survival rate. After irradiation, cells were transferred to a 100 ml flask, and incubated at 30 °C on a shaker (200 rpm). An aliquot of cells (12 ml) was transferred to a centrifuge tube after 5, 20 and 60 min of incubation, concentrated by centrifugation at 4 °C. The cell pellet was resuspended in 2 ml of supernatant and mixed with 4 ml of bacterial RNA protection reagent (Qiagen, Valencia,

CA). The cell suspension was kept at room temperature until all the samples were collected (within 2 h). Cells were then pelleted and stored at -80°C until RNA extraction. Controls were treated in the same way except the UVR irradiation. Total RNA was isolated using a Qiagen RNeasy mini Kit (Qiagen), digested with RNase-free DNaseI (Invitrogen, Carlsbad, CA ) at 25°C for 30 min, extracted with phenol, phenol: chloroform (1:1), and chloroform, and stored in ethanol at -80°C until use. Both PCR and gel electrophoresis were used to confirm the complete digestion of any contaminating DNA. We confirmed both RNA purity and quality by the 260 nm to 280 nm absorbance ratio and gel electrophoresis before the reverse transcription reaction. Prehybridization and RNA labeling were performed as described by Schroeder *et al* (44) with a 2:3 ratio of 5-(3-aminoallyl)-dUTP and dTTP. Hybridization and washing were carried out as described by Hegde *et al* (14). The array was scanned using Axon 4000B scanner (Axon Instruments, Inc. Union City, CA). The data were imported into GeneSpring (Silicon Genetics, Redwood City, CA) for analysis. Data were normalized both per chip and per gene (Lowess method). Spots with less than 55% of pixels great than background plus 2SD were not include in data analysis (34).

**Functional analysis of *uvrA* in *P. aeruginosa* strain UA11079.** The *uvrA* gene (SO4030, Gene Bank accession No: NP_719560) from *S. oneidensis* MR-1 was amplified from 50 ng of genomic DNA using primers *uvrA* NdeI 5' and *uvrA* BamHI 3' (Table 2.1), and cloned into pJJK20 (25), creating plasmid pXQ01 (Table1). A 3.6-kb SphI and BamHI fragment from pXQ01 containing the 0.75-kb *umuDC* promoter from *E. coli* and the 2.85-kb *uvrA* gene from MR-1 was cloned into pJB321, creating pXQ03 (Table 2.1). pXQ03 was transferred from *E. coli* DH10B to *P. aeruginosa* UA11079 by tri-parental

mating as described by Kim and Sundin (25). The survival after UVC exposure was assayed as described above.

Table 2.1. Bacterial strains, plasmids and primers used in this study

| Strain, plasmid or primer | Relevant characteristics or nucleotide sequence | Source or reference |
|---|---|---|
| Bacterial strains | | |
| *E. coli* DH10B | Plasmid free strain used for cloning | 13 |
| *E. coli* K12 | Reference strain | DSM498 |
| *S. oneidensis* MR-1 | Lake Oneida, NY, sediment | 34 |
| *S. oneidensis* DLM7 | Lower Green Bay sediment, Lake Michigan, Wisconsin | 51 |
| *S. oneidensis* MR-4 | Black sea water column, 5m | 51 |
| *S. putrefaciens* 200 | Crude oil pipeline, Canada | ATCC |
| *S. algae* | Red algae, Japan | ATCC |
| *P. aeruginosa* PAO1 | Prototrophic, no UV-resistance plasmid present | A. M. Chakrabarty |
| *P. aeruginosa* UA11079 | [a] Same as PAO1, but Rif$^r$, *uvrA*::ΩHg (Hg$^r$), plasmid free strain | 38 |
| Plasmids | | |
| pJJK20 | [a] Ap$^r$, *E. coli umuDC* promoter source | 23 |
| pJB321 | [a] Cb$^r$, broad-host-range cloning vector | 3 |
| pRK2013 | helper plasmid for triparental matings | 9 |
| pXQ01 | [a] Ap$^r$, cloned MR-1 *uvrA* coding sequence as NdeI-BamHI in pJJK20 | This study |
| pXQ03 | [a] Ap$^r$, *umuDC* promoter and MR-1 *uvrA* coding sequence from pXQ01 as SphI-BamHI in pJB321 | This study |
| Primers | Sequence | Relative position |
| *uvrA* NdeI 5' | [b] GATCCATATGGATAAGATTGAAATACGCGGTGC | ATG: start codon |
| *uvrA* BamHI 3' | [b] GATCGGATCCCTACTGCTGTTTGGTTAGC | CTA: stop codon |
| *uvrA*1F | TAACGGTCGTAAGGGTGAGC | 465 |
| *uvrA*1R | GAGAGTTCGAGTGCGGTTTC | 683 |
| *uvrA*2F | GCTTATTCACCTGCGTGACA | 1898 |
| *uvrA*2R | GTATAAGTGGCGGGGTTTGA | 2114 |
| *uvrB*1F | CACCATCGCCAATGTGATAG | 144 |
| *uvrB*1R | CAATCAGCACCACATCCTTG | 421 |

| | | |
|---|---|---|
| *uvrB*2F | TAGTGCCTAAAGGGGTGGTG | 1754 |
| *uvrB*2R | CCCTTAAGCGTTTCACCTCA | 2002 |
| *uvrD*1F | AAGACCAAGGATTACGGCCT | 449 |
| *uvrD*1R | ATCCAAGCGTATTGAATGGC | 698 |
| *uvrD*2F | CAAGAGCTCACGTTATGGCA | 1297 |
| *uvrD*2R | CTCTTCAGGCATTTCGAAGG | 1572 |

[a]Phenotype resistance (r) abbreviations are: Ap, ampicillin; Cb, carbenicillin; Hg, mercury; Rif: rifampin. [b]For primer oligonucleotide sequences, the restriction sites incorporated in primers are underlined. CATATG, NdeI; GGATCC, BamHI.

## Results

**UVC sensitivity in *Shewanella* strains.** The sensitivity to UVC of five *Shewanella* strains from different natural habitats was examined. *Escherichia coli* strain K12 was used as the control (Figure 2.1). Both *S. algae* and *S. oneidensis* strain MR-4 were more tolerant to UVC radiation whereas *S. putrefaciens* 200 and *S. oneidensis* strains DLM-7 and MR-1 were more sensitive to UVC radiation. To compare the degrees of resistance of the five strains with UVC treatments, we calculated the $D_{37}$ and $D_{10}$ values from the regression line of the exponential slope of each survival curve (Table 2.2). *S. algae* showed the highest UVC resistance with a $D_{37}$ of 4.0 J $m^{-2}$ and a $D_{10}$ of 8.2 J $m^{-2}$, respectively, which was about 74.1 and 79.6 % of that for *E.coli* K12. *S. oneidensis* strain MR-1 showed the highest UVC sensitivity with a $D_{37}$ of 0.3 J $m^{-2}$ and a $D_{10}$ of 1.7 J $m^{-2}$, respectively, which was about 5.6 and 16.5 % of *E. coli* K12 values (Table 2.2). The UVC resistance and sensitivity within the *Shewanella* genus correlated well with the radiation exposure in the habitat from which they were isolated. Both MR-1 and DLM-7, isolated from lake sediment, and *S. putrefaciens* 200, isolated from crude oil pipeline, were from habitats with limited solar radiation exposure, whereas *S. algae*, isolated from the surface of a red alga, and *S. oneidensis* MR-4, isolated from the top 5 m of the Black Sea, were from habitats with more solar radiation exposure (Table 2.1).

Table 2.2 [a]D-values (J $m^{-2}$) and slopes of survival curves from various bacterial strains.

| Bacterial strains | Slope | $R^2$ | $D_{37}$ (%) | $D_{10}$ (%) |
|---|---|---|---|---|
| *E. coli* K12 | -0.272 ± 0.037 | 0.964 ± 0.013 | 5.4 ± 0.5 (100) | 10.3 ± 1.1 (100) |
| *S. algae* | -0.311 ± 0.031 | 0.989 ± 0.005 | 4.0 ± 0.5 (74.1) | 8.2 ± 0.9 (79.6) |
| *S. oneidensis* MR-4 | -0.330 ± 0.020 | 0.990± 0.007 | 2.9 ± 0.8 (53.7) | 6.8 ± 0.9 (66.0) |
| *S. putrefaciens* 200 | -0.690 ± 0.014 | 0.991 ± 0.004 | 1.9 ± 0.1 (35.2) | 3.8 ± 0.1 (36.9) |
| *S. oneidensis* DLM7 | -0.879 ± 0.044 | 0.994 ± 0.005 | 1.0 ± 0.2 (18.5) | 2.5 ± 0.2 (24.3) |
| *S. oneidensis* MR-1 | -0.937 ± 0.028 | 0.962 ± 0.022 | 0.3 ± 0.2 (5.6) | 1.7 ± 0.2 (16.5) |

[a]D-values are the radiation dose that reduced a cell population to a specified percentage of the original number of the cells (32). $D_{37}$ is the radiation does required to inactivate 63% of a bacterial population. The $D_{10}$ is the radiation dose which inactivates 90% of the bacterial population. The D-values were calculated from the regression line of the exponential slope of the survival curve.

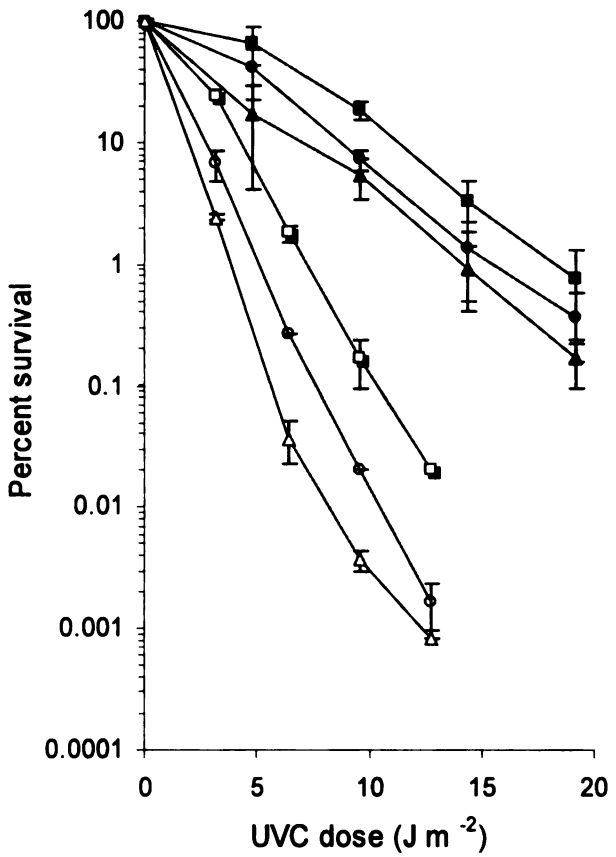


Figure. 2.1. Survival of *E. coli* K12 and *Shewanella* strains after UVC irradiation. (■): *E. coli* K12; (●): *S. algae*; (□): *S. putrefaciens* 200; (▲): *S. oneidensis* MR-4; (o): *S. oneidensis* DLM-7; (Δ): *S. oneidensis* MR-1. Plates used for measuring CFU are LB for *E. coli* K12; marine agar for *S. algae* and TSA for others. Each datum represents the mean (± the standard error of the mean) from three replicates.

**UVA sensitivity in *S. oneidensis* MR-1.** Sensitivity to UVA has been reported to depend greatly on the physiological conditions of the cell. Exponential cells were more sensitive to near-UV damage than stationary cells due to their active DNA replication (6), while the stationary phase triggers numerous protective pathways as well as enzymatic activities expected to confer some degree of UVA resistance to cells (6, 7, 32). Since UVA induces oxidative damage to cells, the survival rate is greatly dependent on the medium used to recover after irradiation. MR-1 survived much better on TSA plates than

LB plates for both exponential and stationary cells (Figure 2.2). No significant difference in UVA sensitivity was observed between exponential cells and stationary cells at lower UVA doses. At high UVA dose, exponential cells were slightly more resistant to UVA. The difference in survival rate on LB plates was more than 10-fold at the dose of 30 kJ $m^{-2}$ (Figure 2.2).

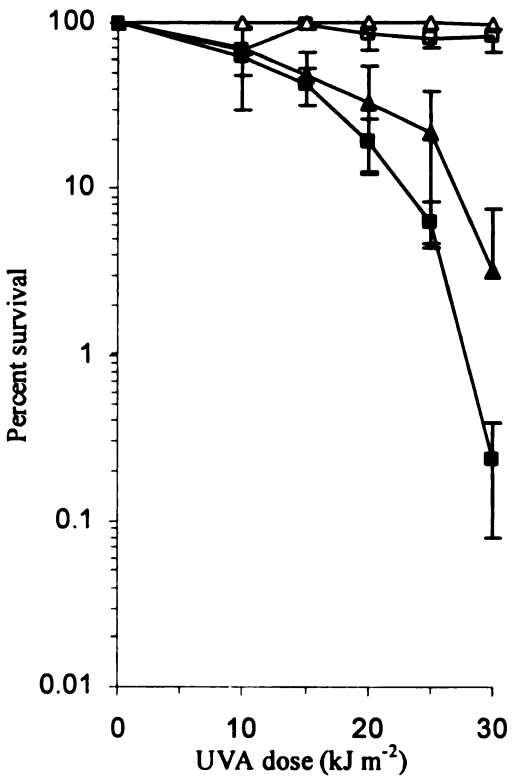


Figure 2.2. Survival of *S. oneidensis* MR-1 after UVA irradiation. Both log- and stationary phase MR-1 grown in Davis medium were irradiated with various UVA doses and plated on both LB and TSA plates to measure CFU. (▲): exponential, LB plates; (Δ): exponential, TSA plates; (■): stationary phase, LB plates; (□): stationary phase, TSA plates. Each datum represents the mean (± the standard error of the mean) from at least three replicates.

**Contribution of photoreactivation to survival of *S. oneidensis* strain MR-1 after UVR exposure.** Annotated photolyase (512 aa) in *S. oneidensis* MR-1 shares 44% identity to that of *E. coli* K12 (472 aa). The amino-terminus contains the conserved domain for binding light harvesting cofactor and the carboxyl terminus contains the

conserved FAD binding domain of DNA photolyase. The tryptophans at enzyme active sites (W306 in *E. coli* K12) and the one involved in substrate Pyr<>Pyr specific binding (W277 in *E. coli* K12) are conserved in the photolyase of MR-1 (W342 and W312 in MR-1, respectively), which may indicate a similar catalyzing mechanism with that of *E. coli* K12 (26). Photoreactivation conferred a significantly increased survival rate to *S. oneidensis* MR-1 in both UVB and UVC irradiated cells: as much as 177- and 365-fold after irradiation at UVC doses of 12 and 15 J $m^{-2}$ (Figure 2.3A) and 11- to 23-fold after irradiation at UVB doses of 774 to 1032 J $m^{-2}$ (Figure 2.3B). For solar light irradiated cells, further incubation under visible light for 1h increased the survival rate 3- and 10-fold at solar UVB doses of 640 and 800 J $m^{-2}$ (Figure 2.3C) compared to those plated in the dark immediately after treatments.

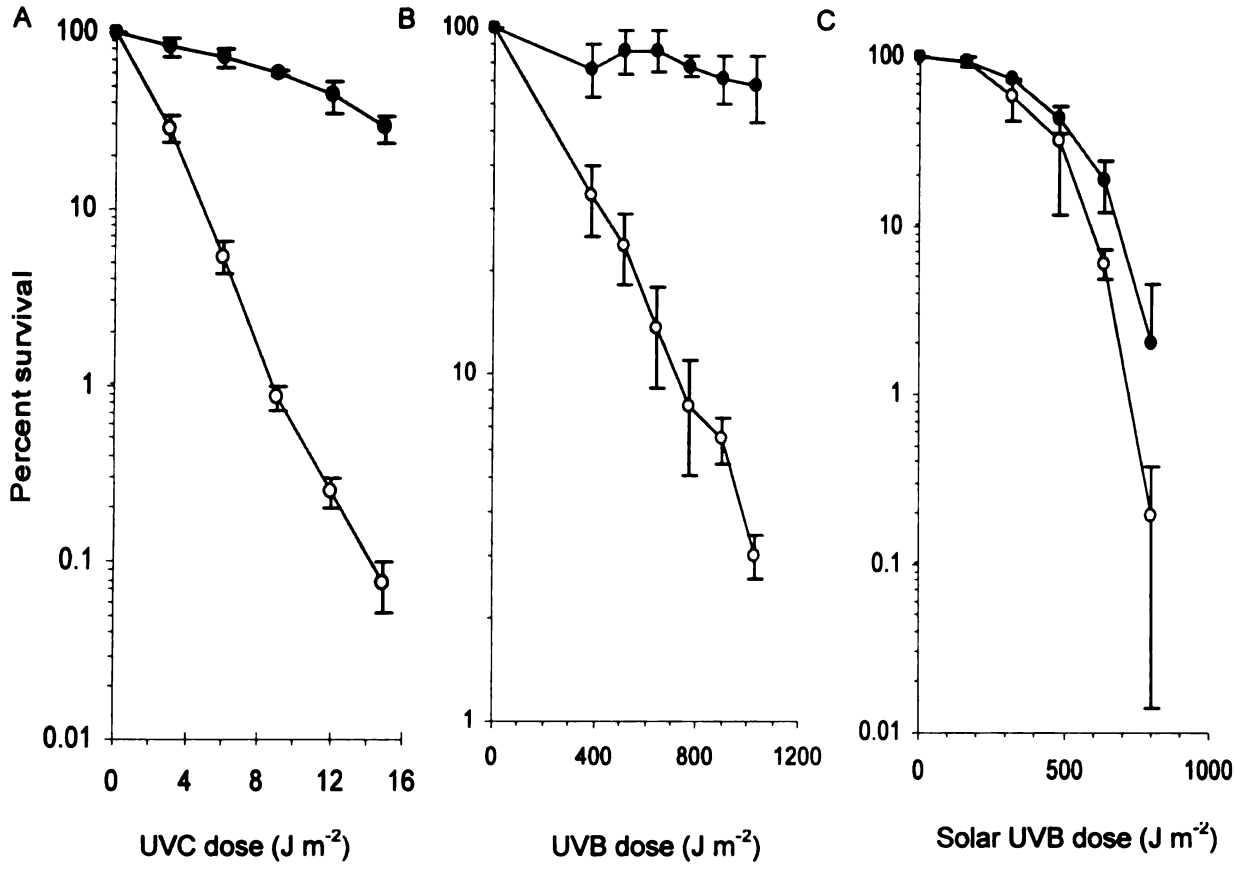


Figure 2.3. Survial of *S. oneidensis* MR-1 following photoreactivation (●) or in the dark (o) after UVC (A), UVB (B) and solar light (C) irradiation. LB plates were used for measuring CFU. Each datum represents the mean (± the standard error of the mean) from three replicates.

**MDR activity in *S. oneidensis* MR-1.** The *umuDC* operon in *S. oneidensis* MR-1 is located on the mega plasmid. The by-product of MDR, an increase in cellular mutation frequency, can be assayed by examining the increase in the occurrence of spontaneous mutants following irradiation. We examined the occurrence of $Rif^r$ mutants in both UVC and UVB treated samples (Figure 2.4). The overall frequency was slightly higher in UVC treated samples (Figure 2.4A) than those in UVB treated samples (Figure 2.4B) over the UV dose-range used in this study. A mutation frequency as high as $6.6 \times 10^{-6}$ was observed at 16.5 J $m^{-2}$ of UVC (Figure 2.4A). This result indicates that MDR-mediated translesion synthesis is functional in *S. oneidensis* MR-1.

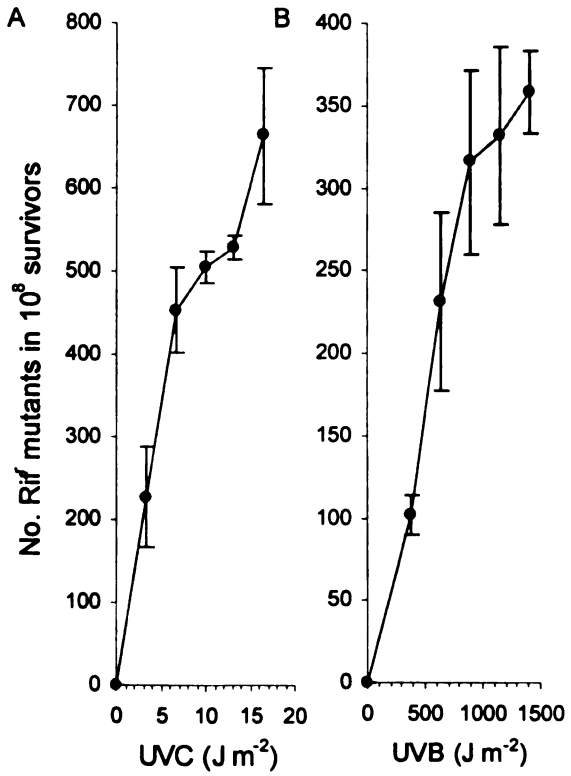


Figure 2.4. Analysis MDR in *S. oneidensis* MR-1 following UVC (A) and UVB (B) exposure. The number of spontaneous mutations conferring $Rif^r$ (rifampin resistance) in the absence of UVR irradiation has been subtracted. LB plates were used for measuring CFU. Each datum represents the mean (± the standard error of the mean) from three replicates.

**Expression of NER component genes after UVR exposure.** Expression of NER component genes (*uvrA*, *uvrB* and *uvrD*) after UVC, UVB and UVA irradiation were examined using a microarray that contains about 95% of MR-1 open reading frames. In contrast to NER system of *E. coli*, we did not observe any induction in any of the three UVR treatments at any of the incubation times. The ratio of irradiated sample to control (unirradiated sample) was in the range of 0.9-1.2 (Figure 2.5). To confirm that the *uvr* genes are truly transcribed, we designed the gene specific primers that targeted both amino terminal and carboxyl terminal fragments of *uvrA*, *uvrB* and *uvrD* (Table 2.1). Positive amplicons were detected in all UVR irradiated cells as well as in the controls (unirradiated samples) by RT-PCR (data not shown). This result indicates that, unlike in *E. coli* K12, the *uvrA*, *uvrB* and *uvrD* of MR-1 were not damage inducible. In agreement with above observation, we were unable to identify any *E. coli*-like SOS box near the translation region (-200 to +40) (8, 27) for all three genes examined whereas putative LexA binding sites were found for *recA*, *lexA*, *umuDC* and *dinP*, a homolog of *umuC*, with a relative low HI value (Table 2.3).

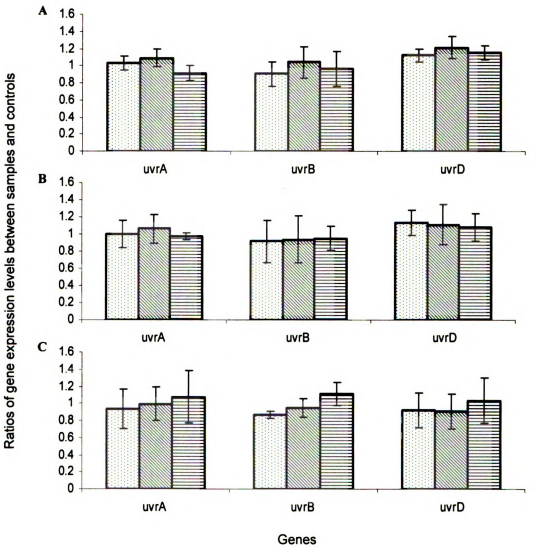


Figure 2.5. Relative gene expression of NER component genes *uvrA*, *uvrB* and *uvrD* at 5 min (filled with dots), 20 min (filled with stripes) and 60 min (filled with lines) after UVC (A), UVB (B) and UVA (C) irradiation. Ratios were UVR irradiated samples to unirradiated samples at the same time points. Each datum is the mean (± the standard error of the mean) of eight to twelve of data points from three biological replicates and two technical replicates.

Table 2.3 Examples of putative *E. coli*-like SOS box in *S. oneidensis* MR-1

| Protein (Gene) | Putative SOS Box | [a] HI value | [b] Relative Position |
|---|---|---|---|
| LexA repressor (*lexA*) | TACTGTATATACTAACAGTA | 1.09 | -46 |
| LexA repressor (*lexA*) | AACTGTATAGAAAAACAGGA | 6.37 | -27 |
| RecA protein (*recA*) | TACTGTATGATTGTACAGTA | 4.05 | -127 |
| UmuD protein (*umuD*) | AACTGTATATTTATACAGTT | 5.20 | -32 |
| DNA-damage-inducible protein P (*dinP*) | AACTGTTTTTATATACAGTA | 3.59 | -45 |

[a] HI (heterology index) is the value to indicate the affinity of LexA to the SOS box (26). Low HI indicates a strong binding of the LexA to the SOS box. [b] distance to a putative translation start condon.

**Functional analysis UvrA of *S. oneidensis* MR-1.** To confirm that the NER system of MR-1 is truly functional, we attempted to complement *Pseudomonas aeruginosa* UA11079 (*uvrA*$^-$) (Table 2.1) with *uvrA* of MR-1. Since we were not sure if the promoter of *uvrA* from MR-1 was functional in *P. aeruginosa*, we used the *umuDC* promoter from *E. coli,* which has been demonstrated to be functional in *P. aeruginosa* (24), for the expression of *uvrA* from MR-1. Complementation increased the UVC survival of the mutant more than three orders of magnitude, but not to the level of the wild-type PAO1 strain (Figure 2.6). The $D_{37}$ (0.43 J $m^{-2}$) and $D_{10}$ (2.90 J $m^{-2}$) values of the complemented strain were about 11.5- and 42.8% of that for PAO1 ($D_{37}$: 3.73 J $m^{-2}$ and $D_{10}$: 6.78 J $m^{-2}$). Nonetheless, this result demonstrates that UvrA from MR-1 is functional in repairing UVC induced damage although the efficiency is not as high as with UvrA from PAO1.

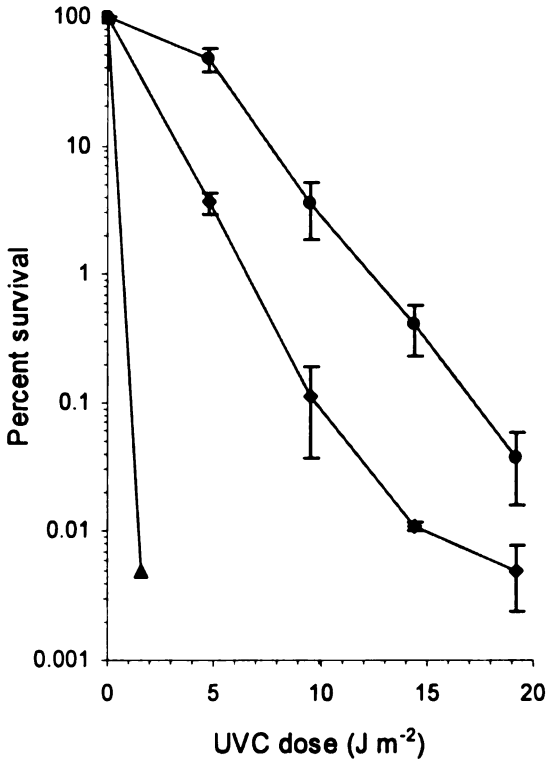


Figure 2.6 Complementary analysis of *uvrA* of *S. oneidensis* MR-1 in *P. aeruginosa* UV11079 (*uvr*A deficient) alter UVC irradiation. Figure shows the survival of *P. aeruginosa* UA11079 (▲), *P. aeruginosa* UA11079 complemented with *uvrA* of MR-1(♦) and *P. aeruginosa* PAO1 (●) after UVC irradiation. LB plates were used for measuring CFU. Each datum represents the mean (± the standard error of the mean) from three replicates.

## Discussion

We evaluated the phenotypic responses important to all relevant wavelength groups of UVR and solar UVR in the environmentally relevant bacterium *S. oneidensis* MR-1. An analysis of the MR-1 genome (NC_004347 and NC_004349) indicated that this organism possesses genes that could encode major DNA repair systems including nucleotide excision repair and recombinational repair, and that MR-1 also encodes a photolyase enzyme and a plasmid-borne mutagenic DNA repair determinant. Regarding UVA survival, MR-1 contains several genes encoding proteins relevant to the removal of

reactive oxygen species such as catalase (SO0725, SO4405, SO1771.2), superoxide dismutase (SO2881), and proteins of the organic hydroperoxide resistance (Ohr) family (SO0976, SO3409) et al. The potential use in bioremediation, the availability of the genome sequence, and the phylogenetic relationship of *S. oneidensis* to other well-characterized organisms, suggest that this strain is an effective model for physiological and genetic studies of UV and ionizing radiation effects on an environmental bacterium.

While the UVC resistance and sensitivity within the *Shewanella* genus correlated well with the radiation exposure in the habitat from which they were isolated, the uniform sensitivity of *S. oneidensis* MR-1 to UVA, UVB, UVC and solar UVR may or may not be a result of lack of UVR exposure. For example, bacteria regarded as tolerant or resistant to UVR have been recovered from solar-radiation exposed habitats including aquatic habitats and the plant phyllosphere (17, 21, 47), but little or no correlation was observed between UVR resistance and the natural levels of solar radiation exposure (12). Great variability in sensitivity to UVR was observed from marine bacterial isolates (2, 21). UVR-tolerant organisms with active photoreactivation mechanisms were prevalent from deep subsurface bacteria which have been screened from solar radiation for more than one million years (1). Thus, the habitat of isolation is not always an indicator of the UVR sensitivity of an organism. The uniform UVR sensitivity of MR-1, however, could not be explained by gene content either. MR-1 possesses most of important repair pathways and determinants compared to phylogenetically related *E. coli*, and has even more DNA repair genes than *D. radiodurans*, a radiation extremely resistant bacterium (http://www.usuhs.mil/pat/deinococcus/FrontPage_DR_Web_work/Pages/DNA_repair/d

na_repair_pathways.htm). However, the resistance to UVC of *D. radiodurans* is more than three magnitudes higher than that of MR-1 (12, 33).

The sensitivity to DNA-damaging UVC and UVB wavelengths in MR-1 could be offset by photoreactivation. The contribution of photoreactivation to MR-1 survival is very similar to that observed in other bacteria (25, 56). Photoreactivation makes a larger contribution to survival following irradiation with UVB or UVC wavelengths in vitro compared to the increase in survival following exposure to solar UVR. This result is probably due to the additional lethal effects of UVA wavelengths present in solar UVR but also has implications for physiological studies aimed at determining the ecological importance of photoreactivation in microbial communities (20). Dramatic difference in survival rate between LB and TSA plates following UVA exposure indicates potential membrane damage caused by UVA (18, 53). We also observed additional decrease in survival rate when the irradiated MR-1 was plated on old LB plates (relative dry). Sensitivity to UVA radiation in MR-1 was also dose dependent. At lower doses, the survival of exponential phase and stationary phase cells was similar whereas exponential phase cells were more resistant to higher radiation doses. This result agrees with findings in studies using 4-thiouridine mutants that showed mutants possessing more DNA replication forks (similar to exponential growth cells) are more resistant to high UVA doses than are wild-type bacteria (19). This could explain the dramatic change in UVA induced photodamage at lower and higher UVA doses.

The plasmid-encoded MDR determinant *umuDC*$_{So}$ contributed to UVR-induced mutability in MR-1, but the contribution of this determinant to UVR survival is unclear. Although most MDR determinants transiently increase the mutation rate of cells

following UVC irradiation, the contribution of these determinants to increased cell survival is only apparent in some cases. For example, the MDR determinant *rulAB* confers tolerance to UVC wavelengths in *P. syringae* (48), but deletion of MDR determinants such as *umuDC* and *samAB* from *E. coli* and *Salmonella typhimurium*, respectively, does not affect their cellular UVC sensitivity (36, 55).

Our investigation on the sensitivity to DNA damaging UVC and UVB wavelengths centered on the NER system of MR-1. This system is probably functional as organisms harboring mutations in NER component genes (e.g. *uvrA, uvrB*) are typically exquisitely sensitive to UVC (41). Indeed, we confirmed the functionality of UvrA through the ability of this protein to complement the UvrA defect in *P. aeruginosa* UA11079 (Figure 2.6). Loss of the damage inducibility of the NER system in MR-1 may contribute to the UVR sensitivity of this organism. For example, in *E. coli*, the expression of the *uvrA, uvrB*, and *uvrD* genes is significantly induced following DNA damage. However, in *P. aeruginosa*, an organism that is more sensitive to UVC than *E. coli*, both *uvrA* and *uvrB* are not DNA damage inducible although this bacterium possesses an SOS-like system (38, 39). In MR-1, we observed strong SOS induction following UVB or UVC exposure which included increases in transcript levels of *lexA*, *recA* as well as the *umuDC* operon (unpublished data). The gene expression level of *uvrA*, *uvrB* and *uvrD*, however, remained constant following DNA damage.

We next examined the regulation of NER component genes among five organisms that are phylogenetically related to *S. oneidensis*, including *E. coli*, *Haemophilus influenzae, Pasteurella multocida, Pseudomonas aeruginosa*, and *Vibrio cholerae* (15). Since LexA and RecA are highly conserved among these bacteria, it is reasonable to

hypothesize that a similar mechanism is present in the regulation of the SOS response. Using the *E. coli* SOS box consensus sequence and three SOS box searching patterns (8), we searched for putative SOS box near to a putative translation start codon (-200 to +40) of either *uvrA*, *uvrB* or *uvrD* gene in five organisms. As expected, an SOS box was identified for all three genes in *E. coli* (Table 2.4). In *Vibrio cholerae*, a strong putative SOS box was identified upstream of the *uvrA* gene, but no putative SOS box was identified upstream of both *uvrB* and *uvrD* (Table 2.4). Relatively strong putative SOS boxes were identified upstream of both *uvrA* and *uvrD* but not *uvrB* in both *Haemophilus influenzae* and *Pasteurella multocida* (Table 2.4). Similar to MR-1, no putative SOS box was identified upstream of *uvrA*, *uvrB* or *uvrD* in *P. aeruginosa* PAO1 (Table 2.4). In agreement with their UVC sensitivity, both *S. oneidensis* MR-1 and *P. aeruginosa* PAO1 lost the damage inducibility of the NER system. Alternatively, the functional efficiency of the UvrABCD complex in NER may be diminished in both *P. aeruginosa* PAO1 and *S. oneidensis* MR-1. Further work is needed to understand the evolution and maintenance of NER in these organisms.

Table 2.4. *E. coli*-like SOS box in strains that are phylogenetically close to *S. oneidensis* MR-1.

| Strain | [f] *E. coli*-like SOS box (HI value) | | |
|---|---|---|---|
| | *uvrA* | *uvrB* | *uvrD* |
| [a] *V. cholerae* | AACTGTTTTTTTATCCAGTA(2.7) | - | - |
| [b] *E. coli* | TACTGTATATTCATTCAGGT (9.6) | AACTGTTTTTTTATCCAGTA (2.7) | ATCTGTATATATACCCAGCT (9.4) |
| [c] *P. aeruginosa* | - | - | - |
| [d] *P. multocida* | AACTGGATATTTGCACAGTT(7.8) | - | TACTGTATAAAAAAACAGTT(4.1) |
| [e] *H. influenzae* | AACTGGATATTTGCACAGAT (10.7) | - | AACTGTAAATTTAAACAGAT (7.0) |

[a] *Vibrio cholerae* O1 biovar eltor str. N16961 (Genome accession number: NC_002505.1) The SOS box of *uvrA* is from 420543 to 420562 (complement); [b] *Escherichia coli* K12 (Genome accession number: NC_000913.1). The SOS box of *uvrA*, *uvrB* and *uvrD* is from 4271534 to 4271553 (complement), 812655 to 812674 and 3995520 to 3995539, respectively; [c] *Pseudomonas aeruginosa* PA01 (Genome accession number: NC_002516.1); [d] *Pasteurella multocida* (Genome accession number: NC_002663.1). The SOS box of *uvr*A and *uvr*D is from 2187327 to 2187346 and 480407 to 480426, respectively; [e] *Haemophilus influenzae* Rd (Genome accession number: NC_000907.1). The SOS box is from 282331 to 282350 for *uvr*A and from 1255918 to 1255937 for *uvr*D. [f] HI (heterology index) is the value to indicate the affinity of LexA to the SOS box (26). Low HI indicates a strong binding of the LexA to the SOS box.

It is very well known that UVR can induce prophage into lytic cycle. Kidambi reported that UVB can activate D3 prophage in *Pseudomonas aeruginosa* in a RecA dependent manner (16). The novel *Shewanella* phage lambdaSo shares syntenic regions with *Pseudomonas aeruginosa* D3 and enterobacteria HK022 (15). Whether or not activation of prophage(s) on MR-1 genome contributes to its high sensitivity to UVR needs be investigated.

Despite possessing the relevant repertoire of oxidative damage repair genes, the results of our study indicate that *S. oneidensis* MR-1 is one of the most UVA-sensitive organisms known. Genome analysis showed that MR-1 has more c-type cytochromes than many organisms including *E. coli*, *V. cholerae*, and *P. aeruginosa* (15). Cytochromes, along with flavins, quinones, are potential chromophores for UVA (5, 18, 53). Whether or not the high cytochrome content of MR-1 contributes to its high UVA sensitivity needs detailed investigation. As expected, MR-1 is also highly sensitive to ionizing radiation (Michael Daly, personal communication). The radiation sensitivity of MR-1 may pose potential problems for environmental uses of this strain or its indigenous relatives in bioremediation of toxic metals or radionuclides since a variety of DNA-damaging agents as well as ionizing radiation may be present at contaminated sites. Relatively little is known of the interrelationship of genetic systems and mechanisms involved in repairing cellular damage caused by UVR and ionizing radiation in organisms other than *D. radiodurans*. MR-1 is an excellent model to compare and understand the cellular function and regulation in response to various radiation stresses. This knowledge will contribute greatly to our fundamental understanding of the important traits in determining bacterial radiation resistance.

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# CHAPTER 3

# COMPARATIVE ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN SHEWANELLA ONEIDENSIS MR-1 FOLLOWING EXPOSURE TO UVC, UVB AND UVA RADIATION

**Abstract**

I delineated the cellular response of *Shewanella oneidensis* MR-1 to ultraviolet radiation damage by analyzing the transcriptional profile following UVC (254 nm), UVB (290-320 nm) and UVA (320-400 nm) irradiation at a dose that yields 20% survival rate, respectively. About 8% of the MR-1 genome was differentially expressed in response to UVA whereas only about 4% of the genome showed differential expression after UVC or UVB exposure. The response to UVA was immediate with most genes showing induction at 5 min. In contrast, the response to UVC was relatively slow with most genes showing induction at 60 min. Two induction peaks were observed after UVB exposure, at 5 min and at 60 min. Almost 70% of UVB-induced genes were up-regulated in the UVC treatment whereas only about 40% of UVB-induced genes were up-regulated in the UVA treatment. Although the SOS response was observed in all three treatments, the induction was more robust in response to short-wavelength UVR (UVB and UVC). Similarly, more prophage-related genes were induced by short-wavelength UVR. MR-1 showed an active detoxification mechanism in response to UVA, which included the induction of antioxidant enzymes and iron sequestering proteins to scavenge reactive oxygen species. The activation of prophages by UVC and UVB and the induction of multidrug and heavy metal efflux pumps and production of toxins following UVB and UVA irradiation highlight the differences in response to stress induced by different wavelengths of UVR.

**Introduction**

The deleterious effect of ultraviolet radiation (UVR) is highly dependent on the wavelength of radiation. DNA is the major chromophore following exposure to short-wavelength UVR. Both UVC (< 290 nm) and UVB (290-320 nm) can induce the formation of cyclobutyl pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidinone ((6-4) PD) photoproducts, which are mutagenic and lethal to bacteria if unrepaired (36). Damage induced by long wavelength UVR is more complex since a variety of non-DNA photoreceptors with λmax in the range of 290-400 nm are present in the cell (11, 21). In addition, both UVB and UVA can produce reactive oxygen species (ROS), causing oxidative damage to a variety of molecules in the cell (10, 11).

Bacteria have evolved various mechanisms to cope with UVR-induced damage. In *Escherichia coli*, both photoreactivation and nucleotide excision repair (NER) are highly efficient in removing CPDs (23, 40) whereas *recA*-mediated recombination repair can bypass CPDs during DNA replication, thus improving DNA damage tolerance (14, 27). The LexA-RecA mediated SOS response is a global response to DNA damage involving the induction of more than 30 unlinked genes, many of which are involved in DNA replication and repair and in the control of cell division (8, 24). *E. coli* also possesses a variety of glycosylases to repair oxidative DNA damage through the base excision repair (BER) pathway (14). In addition, several regulatory genes are involved in protecting cells from oxidative stress. For example, OxyR, a LysR family protein, can be converted into a transcriptional activator by the formation of a disulfide bond between two reactive cysteine residues (52), activating the transcription of genes involved in peroxide metabolism and protection (*katG*, *ahpC*, *ahpF*, *dps*), in redox balance (*gor*, *grxA*,

*trxC*) and genes encoding regulators such as *fur* and *oxyS* (42). The *E. coli* SoxRS regulon provides defense against oxidative damage caused by superoxide anions. Regulation of the *soxRS* regulon occurs by two-step transcriptional activation. First, SoxR is oxidized and becomes an active form, which can stimulate the transcription of *soxS*. SoxS in turn activates transcription of target genes by binding to their promoter region (34, 49). More than 10 genes including *nfo* (endonuclease IV) and *sodA* (Mn-superoxide dismutase) belong to the SoxRS regulon (2). Sigma factor 38 (*rpoS*) is another important regulator in *E. coli* in response to oxidative stress (20). Some genes that are under control of OxyR are also regulated by RpoS (12). Similar oxidative stress regulators have been identified in many other bacteria as well as pathogenic bacteria (7, 9, 30, 35, 39, 45).

Although extensive studies have focused on distinguishing different genes and regulons in response to far UV (UVC) and near UV (UVB and UVA), global genetic information remains limited due to the complexity of UVR-induced damage and limitations in the technologies used in the past studies. Microarray technology, however, allowed me to systematically investigate the global transcriptional response to different UVR wavelengths and hence enhance our understanding of the effects of global damage induced by different wavelengths of UVR.

*Shewanella oneidensis* MR-1, an environmental Gamma Proteobacterium, can reduce a variety of compounds including toxic metals and radionuclides (26, 29). Previous data indicated that MR-1 is highly sensitive to all wavelengths of UVR, solar UV and ionizing radiation (38). However, this sensitivity could not be explained by its genome content. Similar to *E. coli*, which is more radiation resistant, MR-1 encodes the

major DNA damage repair and damage tolerance systems including SOS response, recombination repair, mutagenic repair, nucleotide excision repair, mismatch repair and a DNA photolyase (17). MR-1 also encodes a suite of genes potentially involved in protection from UVA-induced oxidative stress including *rpoS* and a homolog of OxyR (SO1328). For scavenging ROS, MR-1 has genes encoding for catalase (*katB*), catalase/peroxidase (*katG*-1 and *katG*-2), organic hydroperoxide resistance protein (*ohr*), alkyl hydroperoxide reductase (*ahpC* and *ahpF*), and a Dps protein (*dps*). For repair of oxidative DNA damage, the MR-1 genome contains putative genes of *tag*, *ung*, *mutM*, *mutY*, *mutT*, *nth* and *xthA* that are important in removing the damaged bases (17).

*S. oneidensis* MR-1, with its response to UVR previously characterized (38), and full genome sequence known, represents an excellent candidate bacterium for a comprehensive analysis of genomic response to varied UVR wavelengths. Here, I examined the global gene expression profiles in response to UVC (254 nm), UVB (290-320 nm) and UVA (320-400 nm) in MR-1 using a whole genome microarray containing approximately 95% of total ORFs. My results indicate there are similarities in genomic response between MR-1 and *E. coli*; however, there are distinct differences which may contribute to the increased UVR sensitivity of MR-1. In addition, induction of multidrug and heavy metal efflux pumps and production of toxins following UVA irradiation highlights previously unknown phenotypes for this stress.

**Materials and methods**

***S. oneidensis* MR-1 whole genome cDNA array.** The *S. oneidensis* MR-1 whole genome cDNA arrays containing about 95% of total *S. oneidensis* MR-1 ORFs

were produced at Oak Ridge National laboratory (15). In brief, a total of 4197 PCR amplicons and 451 oligonucleotides were deposited onto Corning Ultra GAPS slides (Corning, Corning, NY) using a Microgrid II arrayer (Matrix, Hudson, NH) with 16 (4 x 4) SMP2.5 pins (Telechem, Inc., Sunnyvale, CA). The arrays were printed with two replicates, each containing a 4 x 4 subgrid with the spot distance of 210 microns and the spot size of 140-180 microns. A total of 276 control spots including black (no DNA deposited) and 10 different *Arabadopis* genes (Strategene, La Jolla, CA) and 4 genomic DNA at each subgrid were also included on the array. After cross-linking the DNA to the surface of array by UV (250 mJ) using a Stratagene Stratalinker (Strategene), arrays were stored in a desiccator.

**Microarray hybridization and data analysis**. Gene expression profiling experiments were performed as described previously (38). Briefly, MR-1 was grown in Davis medium with 15 mM lactate as carbon source until $OD_{600}$ reached 0.2-0.3. The culture was split into two portions. One was used for UVR irradiation (3.3 J $m^{-2}$ for UVC, 568 J $m^{-2}$ for UVB, and 25 kJ $m^{-2}$ for UVA) and the other one was used as control. After irradiation, both samples and controls were incubated at 30°C on a shaker. Cells were collected at 5 min, 20 min and 60 min of incubation for RNA extraction. Both UVC and UVB samples were collected in a dark room to avoid photoreactivation. Prehybridization and RNA labeling were performed as described by Schroeder et al. (41) with a 2:3 ratio of 5-(3-aminoallyl)-dUTP and dTTP. Hybridization and washing were carried out as described by Hegde et al. (16). At each time point of each treatment, six hybridizations from three biological replicates and two technical replicates (dye-swap) were performed. GENESPRING 6.0 software (Silicon Genetics, Redwood City, CA) was used to analyze

all microarray hybridization data. Only those spots with more than 55% of pixels greater than background plus 2SD (standard diveration) in either the cy5 or cy3 channel were used for analysis (28). Data were normalized both per chip and per gene (Lowess method). Those genes that showed a statistically significant change in gene expression ($P<0.05$) and a > 2-fold change in magnitude were regarded as significant. The number of clusters for K-means analysis was determined by pre-analyzing data using hierarchical cluster analysis in GeneSpring.

**Quantitative real time reverse transcription PCR (Q RT-PCR).** Q RT-PCR analysis were performed for 12 selected genes (Table 3.1) using the same RNA samples as used for microarray analysis. Two micrograms of total RNA from each sample was converted to cDNA in the same condition as used for the microarray experiment except that dTTP instead of a mixture of aa-dUTP and dTTP was used. After hydrolyzing total RNA, total cDNA was purified using Qiagen PCR purification kit (Qiagen, Valencia, CA) and quantified using a spectrophotometer. Gene specific primers (Table 3.1) were designed using Primer Express® 1.0 software (Applied Biosystems, Foster City, CA). All amplicons were in the range of 90-100 bp. The specificity was first checked by blasting the primer sequences against the MR-1 genome. Both primer and template concentration for each gene were optimized in 1X SYBR Master Mixture (Applied Biosystems) using an ABI 7900HT (Applied Biosystems) Sequence Detection System (Table 3.1). The reaction specificity was further confirmed by examining the dissociation curves after each PCR run. Standard curves for *recA*, *uvrA*, *uvrB* and *uvrD* were constructed using purified PCR products. Since I am interested in absolute quantification of NER component genes, copies of *uvrA*, *uvrB* and *uvrD* in each sample were interpolated from

their corresponding standard curve. For other genes, copies were calculated from the standard curve for *recA* gene. Both 16S rrn gene and *ldhA* were used as internal controls to normalize the difference in reverse transcription efficiency (43). Duplicate runs were performed for each sample.

**Staining phages with SYBR Green I.** MR-1 was grown in TSB medium until $OD_{600}$ reached 1.0. One ml of culture was collected by centrifuging at 8,000 rpm for 3 min, washed once in 1 ml of saline buffer (0.85% NaCl) and resuspended in 10 ml of saline buffer. The cell suspension was exposed to 3.3 J $m^{-2}$ of UVC, after which 1 ml of cell suspension was transferred into 1 ml of 2X TSB medium. The culture was incubated at 30 °C on a shaker for 5 h in the dark. The control was performed in the same way except for the UVC irradiation. After incubation, 100 µl of each culture (UVC-irradiated sample and the control) were treated with 250 U of DNaseI (sigma D7291) and 250 U of RNaseA (Sigma R4642) in a final volume of 1 ml at 25 °C for 30 min to remove free nucleic acids (3). Sample fixation and staining with SYBR Green I were performed as described by Noble et al. (33). Bacteriophage Lambda strain W60 (ATCC 97537) was used as a positive control. The samples were observed using a Zeiss LSM-Pascal (Carl Zeiss, Germany) microscope with a plan-aprochromat 63X oil objective (N. A. = 1.4). The phage were viewed at an excitation wavelength of 488 nm. The images were viewed using Zeiss software Laser Scanning Microscope LSM 5 Pascal Version 3.2 SP2 (Carl Zeiss).

**Examining phages by transmission electron microscopy (TEM).** MR-1 was grown in TSB and irradiated by UVC or UVB as described above for SYBR Green I staining. After 5 h incubation in the dark, 0.8 ml of chloroform was added to 20 ml of

UVR-irradiated MR-1 culture and continued incubation for 15 min to lyse the cells. Both RNaseA and DNaseI was added to a final concentration of 2 μg/ml, respectively, and incubated at room temperature for 30 min to remove MR-1 genomic DNA and RNA. The proteins in the solution were precipitated by adding NaCl to a final concentration of 1.0 M and incubated on ice for 30 min. The cell debris was removed by centrifugation at 5000 rpm for 10 min at 4°C. The supernatant was collected for TEM examination. Phage were fixed with glutaraldehyde at a final concentration of 1%. Five microliter of fixed phage suspension was added directly onto a Formvar and carbon-coated electron microscopy grid. The grid was stained for 30 s with uranyl acetate (2%) and phage were examined with a JEOL 100CX (Japan) transmission electron microscope at an accelerating voltage of 100 kV. Images in this dissertation are presented in color.

Table 3.1. Genes and corresponding primers in Q RT-PCR analysis

| Gene | Forward primer (5'-3') | Reverse primer (5'-3') | Primer conc. (nM) | Template conc. (pg) |
|---|---|---|---|---|
| 16S | CGACGATCCCTAGCTGTTCTG | AATATTCCCCACTGCTGCCTC | 250 | 1 |
| *ldhA* | TGCCGAAGGTTTCGAGGTC | TGATTTTAGTGCCGCCCTTG | 250 | 500 |
| *recA* | CCTCAAGCAATCGAACACTCTG | CACCCGTTGTGGTTTCTGG | 250 | 500 |
| *radC* | ATGGCGATTAAGGATTGGCC | CCAGCAATTCTGCATCCGA | 500 | 1000 |
| *uvrA* | TGAAGCGCAGCGTATTCG | GATGCAAACCGATGGATGG | 1000 | 500 |
| *uvrB* | ACGGGCTCTGGTAAGACGTTC | CGGCGAGTGTTTTGTTTGG | 250 | 500 |
| *uvrD* | AGCGTGTTTACGGCTGCTG | CTGCAGGGTGACGATAAGATCC | 250 | 500 |
| *ohr* | GGTCGTCAAGGCCAAGTCAG | TGGTCGCAAGCCCACTTC | 250 | 500 |
| *katB* | AATCGCAGCAAGGCGTAGTG | ACCACCATATCGCGCTGAG | 250 | 500 |
| SO1923 | AGCCGCCTGTTGCCACTAG | GACCTCCGCGGGTGTAGAG | 250 | 500 |
| SO1924 | CGGTACCTAATGCCTCTGAAGC | ACGCCTGAGAGTGCCTCTAAAC | 250 | 500 |
| SO4328 | GCGAACAACTATCAGTCAGCTGTG | TCTTCGATGGTGGCAACTTG | 250 | 500 |
| SOA0154 | CCCTAAGGCCGTACTGATCT | CCGTGTGCTCTTGTGTGTTC | 1000 | 500 |

## Results

**Global gene expression trends**. I observed distinct gene expression trends in MR-1 following UVC and UVA exposure. The response to UVA was fast: the maximal differentiation appeared at 5 min with a total of 239 induced genes and 92 repressed genes (Figure 3.1A). In contrast, the response to UVC was much slower: the greatest differentiation occurred at 60 min with 128 induced genes and 51 repressed genes (Figure 3.1A). The UVB-induced gene expression trend appeared to be a combination of the "UVA-pattern" and the "UVC-pattern": two response peaks were observed at 5 min ("UVA-pattern") and 60 min ("UVC-pattern"), respectively (Figure 3.1A). The genetic response to UVA was more global with approximately 8% of the genome showing differential expression whereas only about 4% of the genome was differentially expressed after UVC or UVB exposure (Figure 3.2). In all three treatments, a greater number of genes were induced than repressed (Figure 3.2). Almost 70% of UVB-induced genes were up-regulated in the UVC treatment whereas only about 40% of UVB-induced genes were up-regulated in the UVA treatment (Figure 3.1B), which indicates that the UVB-induced stress response in MR-1 is more similar to that of UVC. A total of 31 genes were induced by all three UVR wavelengths, which have been clustered into three groups (Table 3.2). Cluster I (16 genes) contained SOS responding genes, a site specific recombinase gene and seven hypothetical and conserved hypothetical genes. Cluster II (11 genes) contained genes involved in replication of prophages, transposition, and eight hypothetical and conserved hypothetical genes. Cluster III (4 genes) contained an ISSo12 transposase and three conserved hypothetical genes (Table 3.2).

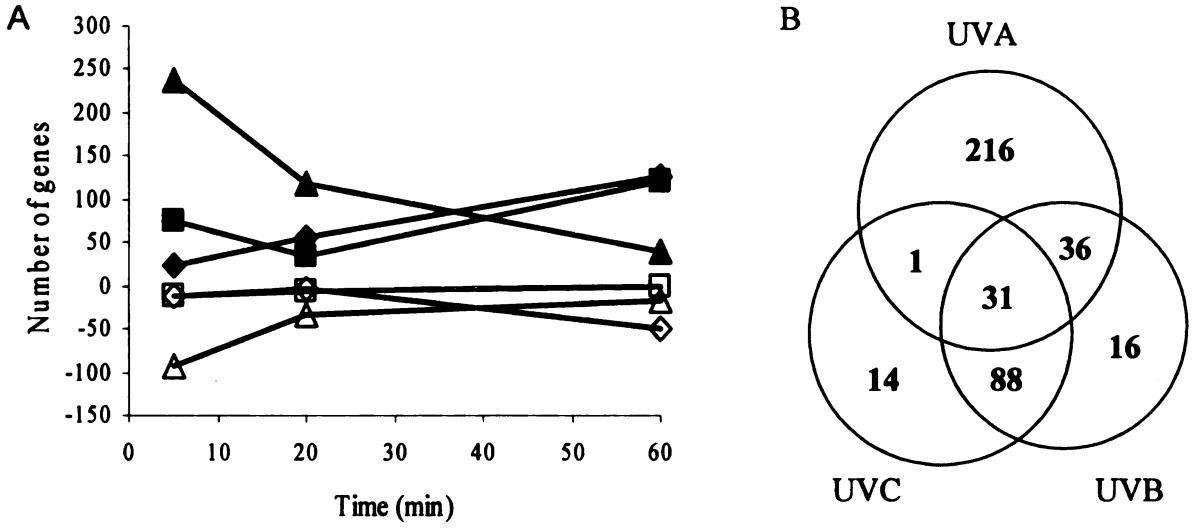


Figure 3.1. A: the global gene expression trends in response to UVC, UVB and UVA during a 1 h recovery period after exposure: up- (◆) and down-regulated (◇) genes in response to UVC; up- (■) and down-regulated (□) in response to UVB and up- (▲) and down-regulated (△) genes in response to UVA. Positive number indicates the number of up-regulated genes whereas the negative number indicates the number of down-regulated genes. B: Venn diagram of up-regulated genes in response to UVC, UVB and UVA irradiation.

**Gene expression profile following UVC irradiation.** Based on the TIGR annotation (17), one hundred and thirty four up-regulated genes in response to UVC were grouped into 11 functional categories, of which both "hypothetical proteins" (41.8%) and "conserved hypothetical proteins" (23.1%) were dominant (Figure 3.2, UVC). Other large groups included "other categories" (13.4 %), which mainly are prophage-related genes and transposases, "DNA metabolism" (8.2%), "protein fate" (3%) and "unknown function" (3%). Three major clusters were revealed using K-means analysis (Supplemental Figure 3.1, UVC). The first cluster (39 genes) represented immediate-responding genes: the induction was observed at 5 min as well as at 20 and 60 min (Supplemental Figure 3.1, UVC, I; Supplemental Table 3.1, cluster I). The second cluster (42 genes) represented intermediate-responding genes: the induction was observed at 20 and 60 min (Supplemental Figure 3.1, UVC, II; Supplemental Table 3.1, cluster II). The third cluster (52 genes) was late-responding genes: no induction was observed until at 60 min (Supplemental Figure 3.1, UVC, III; Supplemental Table 3.1, cluster III).

Seventy-three down-regulated genes in response to UVC were grouped into 16 functional categories (Figure 3.2, UVC). Besides "hypothetical proteins" (20.5%) and "conserved hypothetical proteins" (13.7%), a large number of repressed genes belonged to "energy metabolism" (15.1%), "transport and binding proteins" (13.7%), "regulatory function" (6.8%), "biosynthesis" (5.5%) and "cell envelope" (5.5%) categories. Since my focus is primarily on those genes that are induced, which will probably encode proteins that are most directly involved in DNA repair and detoxification to overcome the cellular damage, I did not analyze the down-regulated genes in detail.

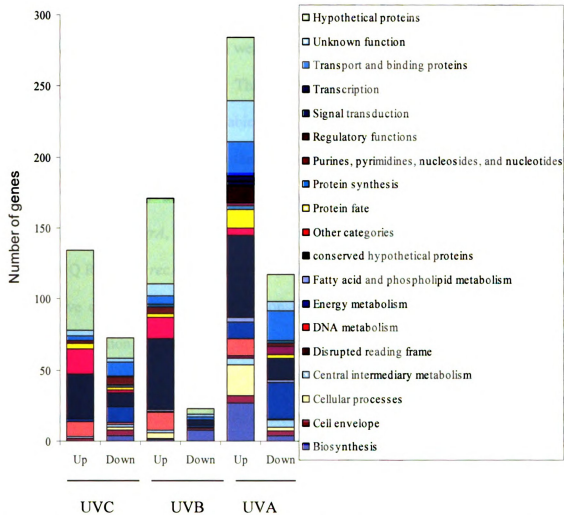


Figure 3.2. Distribution of the differentially expressed genes in various functional categories following UVC, UVB and UVA exposure. The total number of induced (Up) and repressed (Down) genes were 134 and 73 for UVC, 171 and 23 for UVB and 284 and 117 for UVA, respectively (This image is presented in color).

**Induction of DNA damage repair genes after UVC exposure**. DNA is the major target of UVC, thus DNA damage is the main mutagenic and lethal effect induced by UVC. Correspondingly, a strong induction of *recA* and *lexA* was observed following UVC exposure, which indicated the induction of the SOS response in MR-1 (Table 3.2). In addition, I observed a similar strong induction of *recN*, *recX*, *topB*, *dinP* and the *umuDC* operon. Induction of the *umuDC* operon correlated very well with my prior

observation of increased mutability in MR-1 following UVC exposure (38). Although no induction was observed for *ruvAB*, a weak induction of *recG* (2-2.5 fold), which encodes a specific helicase, was observed. This result suggests that recombination repair is functional in MR-1 (Supplemental Table 3.1).

My previous work suggests that the NER component genes of MR-1 may be expressed constitutively at a relative low level (38). I attempted to quantify the expression levels of *uvrA*, *uvrB* and *uvrD* in both UVC-irradiated and non-irradiated samples by Q RT-PCR. *recA* was used as a positive control (induced) and *radC* was used as a negative control (non-induced). A consistent result was observed by Q RT-PCR except the induction fold for *recA* measured by microarray hybridization was lower compared to Q RT-PCR assay (Table 3.2 and Table 3.3). This result is consistent with a previous report on validating cDNA microarray data by Q RT-PCR (50). A better correlation was obtained using *ldhA* as internal control ($R^2$: 0.9478) than 16S rrn gene ($R^2$: 0.7394). *uvrA* was present in about 500 copies and both *uvrB* and *uvrD* in about 200 copies in 500 pg of total cDNA. The basal expression level of all three genes was lower than that of *recA*, which averaged about 2500 copies in 500 pg of total cDNA in MR-1 prior to UVC irradiation (Table 3.3).

Table 3.2 Common up-regulated genes in response to UVC, UVB and UVA radiation

| SO ([a]Cluster) | Product | UVC ([b]SD) | | | UVB([b]SD) | | | UVA([b]SD) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 min | 20 min | 60 min | 5 min | 20 min | 60 min | 5 min | 20 min | 60 min |
| SOA0013 (I) | UmuD protein | 5.1 (1.1) | 15.2 (1.7) | 16.1 (2.9) | 4.0 (1.0) | 11.0 (3.0) | 19.8 (3.5) | 5.0 (2.3) | 4.3 (1.3) | 4.3 (2.0) |
| SO0089 (I) | Hypothetical protein | 3.1 (0.7) | 6.7 (1.7) | 6.9 (0.9) | 2.3 (0.4) | 5.1 (2.1) | 7.0 (2.5) | 2.3 (1.0) | 2.3 (1.1) | 1.6 (0.4) |
| SOA0012 (I) | UmuC protein | 3.6 (1.3) | 8.4 (1.7) | 8.1 (0.7) | 2.6 (0.5) | 6.3 (1.9) | 8.0 (2.0) | 2.6 (0.5) | 3.3 (0.9) | 2.1 (0.5) |
| SO3061 (I) | DNA topoisomerase III | 4.1 (0.8) | 8.0 (0.9) | 9.9 (3.3) | 2.9 (0.3) | 6.9 (0.5) | 9.8 (1.2) | 3.5 (0.2) | 3.4 (0.5) | 2.1 (0.4) |
| SO2603 (I) | Conserved hypothetical proteins | 3.2 (0.2) | 5.2 (0.3) | 5.7 (0.6) | 2.6 (0.3) | 4.8 (0.3) | 5.7 (0.5) | 2.7 (0.2) | 2.4 (0.4) | 1.6 (0.3) |
| SO2604 (I) | Conserved hypothetical proteins | 3.7 (0.5) | 6.1 (0.8) | 6.7 (0.9) | 3.0 (0.8) | 5.5 (1.6) | 7.3 (0.9) | 3.5 (0.6) | 3.1 (0.4) | 2.1 (0.4) |
| SO1114 (I) | DNA-damage-inducible protein P | 3.6 (0.3) | 6.8 (0.7) | 6.2 (0.8) | 3.6 (0.6) | 5.8 (1.6) | 12.3 (1.8) | 4.2 (0.9) | 4.7 (1.3) | 2.9 (0.5) |
| SO3462 (I) | DNA repair protein RecN | 7.9 (1.6) | 16.0 (3.7) | 15.0 (1.4) | 7.4 (1.2) | 11.5 (1.8) | 20.3 (1.0) | 6.8 (0.7) | 6.3 (1.0) | 3.7 (0.9) |
| SO3429 (I) | Regulatory protein RecX | 4.8 (1.3) | 7.8 (0.3) | 7.4 (1.4) | 4.1 (0.7) | 6.3 (1.2) | 9.6 (1.2) | 3.5 (0.3) | 3.3 (0.2) | 2.1 (0.2) |
| SO4603 (I) | LexA repressor | 6.7 (0.6) | 12.2 (1.2) | 11.8 (2.1) | 8.0 (1.1) | 9.9 (2.0) | 17.6 (1.8) | 5.6 (1.8) | 6.1 (2.0) | 3.4 (0.8) |
| SO4604 (I) | Conserved hypothetical protein | 6.9 (0.8) | 11.8 (1.9) | 12.5 (1.3) | 7.6 (1.9) | 9.5 (2.7) | 20.1 (1.7) | 7.0 (1.0) | 7.1 (2.1) | 3.8 (0.8) |
| SO3430 (I) | RecA protein | 5.0 (1.2) | 8.0 (1.2) | 9.4 (2.1) | 4.0 (0.6) | 6.8 (0.9) | 10.2 (2.6) | 3.5 (0.5) | 3.6 (0.6) | 2.3 (0.2) |
| SO4605 (I) | Hypothetical protein | 5.7 (1.6) | 8.3 (2.9) | 6.4 (2.0) | 5.1 (2.3) | 9.5 (4.2) | 8.7 (3.3) | 3.0 (1.7) | 3.2 (0.4) | 2.3 (0.4) |
| SO1757 (I) | Conserved hypothetical protein | 13.6 (2.8) | 22.6 (2.9) | 16.7 (3.0) | 20.7 (8.9) | 17.2 (7.8) | 32.7 (3.9) | 23.2 (7.7) | 33.0 (18.8) | 14.5 (5.7) |
| SO3327 (I) | Hypothetical protein | 2.0 (1.0) | 5.0 (1.1) | 4.5 (0.8) | 2.5 (0.6) | 2.0 (1.3) | 5.4 (3.1) | 3.8 (1.4) | 1.5 (0.5) | 1.8 (0.7) |
| SO2037 (I) | Site-specific recombinase, phage intergrase family | 1.7 (0.7) | 1.9 (0.6) | 2.4 (0.4) | 1.2 (0.4) | 1.6 (0.4) | 2.2 (0.6) | 1.8 (0.6) | 2.3 (1.0) | 1.5 (0.5) |
| SO2998 (II) | Hypothetical protein | 0.9 (0.1) | 2.2 (0.5) | 15.2 (4.2) | 0.9 (0.2) | 1.3 (0.3) | 14.7 (4.6) | 0.8 (0.2) | 1.4 (0.1) | 2.2 (0.4) |
| SO3000 (II) | Conserved hypothetical protein | 1.0 (0.4) | 3.1 (1.2) | 25.3 (5.9) | 0.9 (0.2) | 1.8 (0.5) | 37.5 (5.4) | 1.0 (0.1) | 1.5 (0.3) | 3.1 (0.8) |
| SO2993 (II) | Prophage LambdaSo, type II DNA modification methyltransferase | 0.9 (0.3) | 3.0 (1.1) | 26.2 (4.9) | 1.0 (0.1) | 1.7 (0.1) | 35.6 (5.4) | 1.6 (0.2) | 1.5 (0.3) | 2.9 (0.9) |
| SO2997 (II) | Hypothetical protein | 0.9 (0.2) | 2.6 (0.6) | 17.3 (3.7) | 0.9 (0.2) | 1.5 (0.5) | 19.5 (5.7) | 1.0 | 1.2 (0.2) | 2.5 (0.5) |
| SO2988 (II) | Conserved hypothetical protein | 1.1 (0.2) | 2.5 (0.5) | 21.0 (3.7) | 0.9 (0.1) | 1.3 (0.2) | 21.6 (3.9) | 1.2 (0.4) | 1.4 (0.4) | 2.1 (0.6) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SO3001 (II) | Hypothetical protein | 1.0 (0.1) | 2.3 (0.6) | 23.1 (10.7) | 0.8 (0.1) | 1.3 (0.2) | 19.1 (8.7) | 0.8 (0.3) | 1.3 (0.1) | 2.2 (0.7) |
| SO3002 (II) | Conserved hypothetical protein | 1.1 (0.3) | 2.2 (0.7) | 21.5 (3.8) | 0.9 (0.1) | 1.4 (0.3) | 24.8 (8.4) | 0.9 (0.2) | 1.4 (0.2) | 2.1 (0.3) |
| SO2984 (II) | Conserved hypothetical protein | 1.0 (0.2) | 1.8 (0.3) | 14.3 (1.9) | 1.0 (0.2) | 1.3 (0.2) | 14.8 (1.0) | 1.0 (0.2) | 1.0 | 2.2 (0.5) |
| SO2985 (II) | Prophage LambdaSo, replication protein O | 1.1 (0.4) | 2.0 (0.6) | 15.8 (2.8) | 1.0 (0.1) | 1.3 (0.2) | 15.9 (2.8) | 1.2 (0.2) | 1.4 (0.5) | 2.2 (0.4) |
| SO2983 (II) | Hypothetical protein | 1.1 (0.1) | 1.8 (0.4) | 16.0 (3.7) | 0.8 (0.2) | 1.3 (0.2) | 17.3 (1.0) | 1.3 (0.1) | 1.1 (0.2) | 2.1 (0.3) |
| SO0644 (II) | Prophage MuSo1, DNA transposition protein, putative | 1.0 (0.1) | 2.2 (0.6) | 13.3 (1.2) | 1.0 (0.4) | 1.4 (0.4) | 19.0 (2.0) | 2.3 (0.7) | 1.2 (0.5) | 1.3 (0.1) |
| SO1759 (III) | Conserved hypothetical proteins | 10.0 (4.6) | 10.7 (1.1) | 6.7 (1.0) | 13.3 (3.5) | 11.3 (4.2) | 11.3 (2.5) | 11.1 (3.7) | 8.3 (3.2) | 4.4 (2.4) |
| SO3849 (III) | Conserved hypothetical proteins | 9.7 (3.9) | 9.8 (1.2) | 5.2 (0.5) | 11.1 (3.8) | 11.9 (8.5) | 9.3 (1.4) | 7.2 (1.9) | 5.5 (3.1) | 1.7 (0.3) |
| [c]SO1761 (III) | Conserved hypothetical proteins | 2.8 (1.4) | 3.4 (2.4) | 2.2 (0.7) | 5.3 (3.4) | 4.0 (2.2) | 2.8 (1.1) | 4.3 (2.3) | 3.9 (2.1) | 3.9 (0.8) |
| SO3854 (III) | ISSo12, transposase | 8.0 (3.5) | 8.0 (3.6) | 3.8 (1.0) | 3.0 (1.0) | 5.7 (2.0) | 4.3 (2.0) | 2.5 (1.2) | 1.5 (0.6) | 1.1 (0.2) |

[a]Cluster analysis was performed using either standard correlation or distance to calculate the similarities. [b]SD (standard deviation) was calculated from six data points which included three independent biological samples and two technical replicates for each biological sample. [c]The only gene that showed different clusters (cluster I or cluster III) using two methods.

Table 3.3 The expression levels of selected DNA damage repair genes quantified by Q RT-PCR before and after UVC irradiation

| Gene | [a]C5 | [a]S5 | [b]T5 | [a]C20 | [a]S20 | [b]T20 | [a]C60 | [a]S60 | [b]T60 |
|---|---|---|---|---|---|---|---|---|---|
| *recA* | 1803 ± 1721 | 30584 ± 14966 | 7.8 ± 0.8 | 3034 ± 846 | 32774 ± 13179 | 16.8 ± 7.7 | 2285 ± 1916 | 32098 ± 6634 | 17.7 ± 3.5 |
| *radC* | 21 ± 15 | 35 ± 14 | 0.8 ± 0.5 | 28 ± 6 | 18 ± 7 | 1.1 ± 0.5 | 27 ± 24 | 21 ± 7 | 1.0 ± 0.3 |
| *uvrA* | 364 ± 291 | 779 ± 196 | 0.9 ± 0.1 | 618 ± 128 | 492 ± 151 | 1.3 ± 0.7 | 464 ± 365 | 476 ± 122 | 1.3 ± 0.4 |
| *uvrB* | 142 ± 137 | 332 ± 89 | 1.1 ± 0.0 | 269 ± 63 | 174 ± 70 | 1.0 ± 0.4 | 224 ± 215 | 180 ± 44 | 1.1 ± 0.3 |
| *uvrD* | 146 ± 92 | 387 ± 74 | 1.1 ± 0.2 | 221 ± 35 | 187 ± 67 | 1.3 ± 0.5 | 167 ± 145 | 167 ± 10 | 1.3 ± 0.3 |

[a]C5, C20 and C60 were gene expression levels of controls at 5 min, 20 min and 60 min whereas S5, S20 and S60 were gene expression levels of UVC-irradiated samples at 5 min, 20 min and 60 min. The data were the average copies of genes in 500 pg of total cDNA except for *radC*, which were the average copies in 1000 pg of cDNA estimated by using standard curve of *recA*. SD was calculated from six data points which included three independent biological samples and two technical replicates for each biological sample. [b]T5, T20 and T60 are the ratio of UVC-irradiated sample to the control at the corresponding time point. The data reported here was normalized using *ldhA* as internal control (43).

**Induction of prophage-related genes by UVR.** It is well known that short-wavelength UVR can induce the lytic cycle of lysogenic bacteriophage. I observed the induction of a great number of prophage-related genes in MR-1 after UVC exposure with the largest percentage (74.7%) of genes induced from the LambdaSo genome (Table 3.4). In addition, induction of "early genes" which are involved in LambdaSo replication and transcription was observed from 5-20 min whereas induction of "later genes" which encode phage structural proteins was observed only at 60 min. A similar expression pattern was observed for prophage MuSo1, but not for MuSo2. A total of 15 genes (SO0643-SO0652 and SO0674-SO0678) were induced from the MuSo1 genome. The activation of genes responsible for transposition and a positive regulator of later transcription (SO0643-SO0652) indicated a potential activation of phage MuSo1. Indeed, gene products of SO0674-SO0678 are structural proteins of Mu. A total of 16 genes (SO2653-SO2668) were induced from the MuSo2 genome, which include genes responsible for transposition and a positive regulator of later transcription. However, no genes encoding structural proteins of phage MuSo2 were induced (Supplemental Table 3.1).

The effect of UVB on phage gene induction was comparable to that of UVC for all three MR-1 prophages (Table 3.4). In contrast, UVA exposure induced the expression of few genes including only 11 of 75 genes of LambdaSo, and 1 of 42 and 2 of 53 genes in MuSo1 and MuSo2, respectively (Table 3.4).

Table 3.4. The number of induced prophage-related genes following UVC, UVB and UVA exposure

| Prophage | [a]Total ORF | Total induced ORF | | | Hypothetical proteins | | | | Conserved hypothetical proteins | | | | Others | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Total | Induced | | | Total | Induced | | | Total | Induced | | |
| | | UVC | UVB | UVA | | UVC | UVB | UVA | | UVC | UVB | UVA | | UVC | UVB | UVA |
| LambdaSo | 75 | 56 | 51 | 11 | 38 | 29 | 26 | 5 | 16 | 14 | 14 | 4 | 21 | 13 | 11 | 2 |
| MuSo1 | 42 | 15 | 15 | 1 | 21 | 6 | 6 | 0 | 8 | 4 | 4 | 0 | 14 | 5 | 5 | 1 |
| MuSo2 | 53 | 16 | 15 | 2 | 20 | 9 | 9 | 0 | 17 | 3 | 3 | 1 | 16 | 4 | 3 | 1 |

[a]according to Heidelberg et al. (17).

Gene expression data strongly suggested that UVC may induce the lytic cycle of LambdaSo in MR-1 (Table 3.4). Using SYBR Green I staining, I observed phage particles in the cultures exposed to UVC (Figure 3.3B), but not in control cultures (Figure 3.3A). In addition, cells exposed to UVC were greatly enlarged (Figure 3.3B) compared to the control samples (Figure 3.3A). This observation is consistent with the previous observation that inhibition of cell division is a consequence of the UVC-induced stress response in many bacteria (18). In a suspension from UVC irradiated MR-1 cells, I observed phage with a head and a tail structure by TEM (Figure 3.3C). Similar phage particles were seen in the UVB irradiated samples (data not shown).

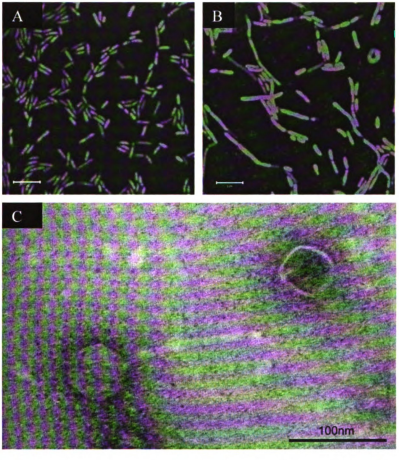


Figure 3.3. SYBR Green I staining MR-1 (A), MR-1 treated with UVC (B) and TEM images for phage isolated from UVC irradiated MR-1 (C). The scan zoom was 4.0 for images A and B.

**Gene expression profile following UVA irradiation.** Unlike the UVC gene expression profile, two hundred and eighty four up-regulated genes were distributed in 16 functional categories more evenly (Figure 3.2, UVA). The top six large groups were "conserved hypothetical proteins" (19.7%); "hypothetical proteins" (15.5%); "biosynthesis" (11.6%); "unknown functions" (10.2%); "transport and binding proteins" (8.8%) and "cellular process" (7.8%) (Figure 3.2, UVA). Compared to the UVC transcriptional profile, genes in "DNA metabolism" and "other categories" were reduced from 8.2- and 13.4% to 4.2- and 1.8%, respectively, whereas genes in "regulatory function", "signal transduction", "transcription" and "metabolism" categories showed a slight increase in percentage (Figure 3.2, UVA). One hundred and seventeen down-regulated genes in response to UVA were grouped into 15 functional categories. Similar to UVC expression profile, the largest four functional groups were "energy metabolism" (21.4%), "transport and binding proteins" (17.9%), "hypothetical proteins" (16.2%) and "conserved hypothetical proteins" (12.8%) (Figure 3.2, UVA).

As expected, genes involved in repairing DNA damage were induced in MR-1 following UVA irradiation (Table 3.2). Induction of key genes of the SOS regulon was less substantial compared to UVC. In addition, I observed a strong induction (20 fold) of *phrB*, which encodes a DNA photolyase mediating photoreactivation, and a weak induction of *mutL* (2.1 fold) which encodes a component of DNA mismatch repair.

**Scavenging of UVA-induced reactive oxygen species in MR-1.** The removal of reactive molecules that result from photo-oxidation is a challenge faced by organisms in coping with UVA-induced stress. The induction of antioxidant enzymes and proteins is a common strategy in bacteria to scavenge ROS. In *S. oneidensis* MR-1, I observed at 5

min the induction of genes encoding a catalase/peroxidase HPI (SO0725: 3.8 fold), alkyl hydroperoxide reductase subunit C (SO0958: 4.3 fold), a cytochrome c551 peroxidase (SO2178: 2.8 fold), an organic hydroperoxide resistance protein (SO0976: 8.7 fold) and a putative glutathione peroxidase (SO1563: 4.7 fold) (Supplemental Figure 3.1, UVA, II; Supplemental Table 3.2, cluster II). In addition, I observed the strong induction of SO1773 (8.0 fold), which encodes a catalase related protein and SO3349 (11.9 fold), which encodes a second putative glutathione peroxidase. Induction of these two ORFs occurred at 5 min and lasted until 20 min (Supplemental Figure 3.1, UVA, III; Supplemental Table 3.2, cluster III). Although MR-1 possesses a *katB* (SO1070) and another *katG* (SO4405), no induction of either gene was observed. Since the overall hybridization signals of *katB* were lower than most of the spots on the array, Q RT-PCR was performed. No induction of *katB* following UVA exposure was observed (Table 3.5).

The intracellular iron pool plays an important role in near UVR induced damage. First, iron-containing proteins may act as chromophores, becoming excited and thereby damaged directly (10, 21). Ferrous iron can catalyze the formation of hydroxyl radicals through the Fenton reaction, influencing the generation of ROS following UVA irradiation (19, 37). Hence, regulation of iron uptake and metabolism and iron sequestration are important protection mechanisms against UVA-induced oxidative damage. Indeed, I observed the induction of several iron sequestering proteins such as SO1158 (ferritin-like Dps protein: 3.6 fold), *bcp* (bacterioferritin comigratory protein: 7.0 fold), and *hemH* (ferrochelatase: 10.4 fold), which encodes the enzyme that inserts iron into protoporphrin IX to make heme. Correspondingly, genes involved in iron up-take were strongly repressed at 5 min after irradiation (SO3669-SO3675: 0.25-, 0.37-, 0.27-,

0.38-, 0.46- and 0.30 fold) (Supplemental Figure 3.1, UVA, V; Supplemental Table 3.2, cluster V). Also, the expression of SO4077, which encodes a putative TonB dependent receptor, was repressed more than 3-fold during the 1 h recovery period. The expression of SO3669 (*hugA*), SO3670 (*tonB*1) and SO3671 (*exbB*1) increased slightly at 20 min (2.3, 2.2 and 2.3 fold, respectively), which may indicate the requirement of iron for the synthesis of new proteins in MR-1 following UVA irradiation (Supplemental Table 3.2).

**Induction of toxin and toxin secretion related genes after UVA exposure.** The MR-1 genome contains a putative pore-forming RTX (repeats in toxin) toxin operon (SO4146-SO4149) and a gene cluster (SO4317-SO4319) that is related to RTX production and secretion. MR-1 also contains a gene encoding a putative hemolysin (SO1354). Hemolysin can bind to and lyse mammalian cell membranes and, at low concentration, perturb cell signal transduction causing the release of inflammatory mediators (44, 47, 48). I observed the induction of SO4149, which encodes a RTX (2.0 fold) and SO4148 (4.9 fold), which encodes a HlyD family secretion protein involved in secretion of toxin and SO1354 (2.6 fold) (Supplemental Table 3.2).

Secretion of RTX toxins requires three gene products in *E. coli*: HlyB, HlyD and TolC. Both HlyB and HlyD are inner membrane proteins, functioning as an ATPase (HylB) and an adaptor (HylD), whereas TolC is an outer membrane exit duct protein (4, 13, 46). This tripartite machinery transports toxins directly across the entire cell envelope. Interestingly, MR-1 is highly redundant in *hlyD*. There are a total of 17 ORFs encoding HylD family proteins, of which six (SO1881, SO1925, SO3483, SO4015, SO4327 and SO4693) are located closely with genes coding for RND (the resistance-nodulation-cell division) antiporter AcrB/AcrD/AcrF family protein. High induction was observed in

MR-1 following UVA irradiation for SO1925 (5.4 fold) and SO4327 (10.0 fold) (Supplemental Table 3.2).

**Induction of multidrug and heavy metal efflux pumps after UVA exposure.** Similar to HlyD, MR-1 is also highly redundant (nine copies) in genes encoding AcrB/AcrD/AcrF family proteins (17), and has a gene (SO4328) encoding a truncated AcrB/AcrD/AcrF family protein (629 aa) due to an authentic frameshift. In *E. coli*, AcrAB-TolC is a major, constitutively-expressed, multidrug efflux pump that provides resistance to structurally unrelated noxious molecules (1, 32). AcrB functions as an antiporter which uses proton flux as the source of energy whereas AcrA functions as an adaptor and TolC works in the same way as it does in type I secretion pathway (HlyBD-TolC) (1, 51). Strong induction of SO1923 (7.8 fold), SO1924 (10.2 fold) and SO4328 (10.2 fold) were observed after exposure to UVA (Supplemental Figure 3.1, UVA, II; Supplemental Table 3.2, cluster II). In addition, SO0525, which encodes an EmrB/QacA family protein, showed a 4.5-fold induction. EmrB of *E. coli* is an integral membrane translocase which mediates drug extrusion (25). MR-1 also carries a chromosome-borne (SO4597 and SO4598) and a plasmid-borne (SOA0153 and SOA0154) heavy metal efflux pump. Both SO4598 and SOA0153 encode a CzcA family protein, which is a cation/proton antiporter of the RND family protein, whereas both SO4597and SOA0154 encode a putative heavy metal efflux membrane fusion protein (M. Romine pers. comm.). CzcA along with CzcB, a membrane fusion protein and CzcC, an outer membrane protein, confers resistance to cobalt, zinc and cadmium ions (31). Strong induction (6.0-7.0 fold) was observed for all four ORFs after UVA exposure (Supplemental Figure 3.1, UVA, III;

Supplemental Table 3.2, cluster III). These data suggest that heavy metal and multidrug efflux pumps may function as a method of detoxification in UVA-irradiated MR-1 cells.

Due to the high similarities among ORFs encoding the same or similar products, I may have observed cross-hybridization in my microarray-based gene expression experiments. To validate my observations, I designed gene specific primers for four genes (SO1923, SO1924, SO4328 and SOA0154) that encode heavy metal and multidrug efflux pumps described above (Table 3.1). I also included *ohr* (highly induced), *recA* (moderately induced) and *radC* (no induction) in Q RT-PCR analysis for validation and comparison. Consistent results were observed by the Q RT-PCR assay (Table 3.5). Again, a better correlation was obtained using *ldhA* as internal control ($R^2$: 0.8953) than 16S rrn genes ($R^2$: 0.8). I also confirmed less induction of *recA* than with UVC and no induction of *radC* in UVA-irradiated samples (Table 3.5).

**Induction of other stress related genes following UVA irradiation.** Other stress related genes that were induced by UVA exposure included those involved in cell motility (SO1989: 5.5 fold; SO3247: 2.8 fold; SO3248: 6.1 fold; SO3282: 2.4 fold; SO3241: 2.1 fold), in cell signaling (SO4170: 13.3 fold) and in producing antibiotic resistance (SO4299: 2.8 fold; SO0837: 2.1 fold). I also observed a slight induction of some heat shock and chaperone proteins such as HslU (SO4160: 2.1 fold), HtpG (SO2016: 2.1 fold) and DnaK (SO1126: 2.2 fold) (Supplemental Table 3.2).

Table 3.5. The relative expression of selected genes following UVA exposure quantified by microarray hybridization and Q RT-PCR

| Gene | [a]T5 | | [a]T20 | | [a]T60 | |
|---|---|---|---|---|---|---|
| | Array | Q RT-PCR | Array | Q RT-PCR | Array | Q RT-PCR |
| *recA* | 3.5 ± 0.5 | 4.4 ± 1.8 | 3.6 ± 0.6 | 4.5 ± 0.7 | 2.3 ± 0.2 | 2.6 ± 0.3 |
| *radC* | 0.8 ± 0.3 | 1.4 ± 0.5 | 0.8 ± 0.2 | 1.5 ± 0.3 | 1.4 ± 0.5 | 1.2 ± 0.3 |
| *ohr* | 8.7 ± 4.3 | 49.4 ± 22.5 | 1.5 ± 0.6 | 2.9 ± 0.8 | 0.8 ± 0.6 | 0.8 ± 0.1 |
| SO1923 | 7.8 ± 4.7 | 21.0 ± 7.1 | 2.8 ± 0.5 | 5.8 ± 0.4 | 0.9 ± 0.3 | 1.0 ± 0.1 |
| *katB* | 0.9 ± 0.4 | 1.9 ± 0.4 | 1.1 ± 0.8 | 1.2 ± 0.2 | 0.9 ± 0.2 | 1.2 ± 0.3 |
| SO1924 | 10.2 ± 3.6 | 15.9 ± 3.4 | 3.1 ± 0.6 | 6.8 ± 2.3 | 1.1 ± 0.5 | 1.3 ± 0.2 |
| SO4328 | 10.2 ± 5.1 | 16.4 ± 8.0 | 3.0 ± 1.3 | 5.8 ± 1.5 | 0.9 ± 0.1 | 1.0 ± 0.1 |
| SOA0154 | 6.5 ± 3.2 | 11.5 ± 4.9 | 3.2 ± 1.3 | 7.3 ± 5.5 | 1.6 ± 0.8 | 1.2 ± 0.6 |

[a]T5, T20 and T60 are the ratios of UVA-irradiated samples to the controls at 5 min, 20 min and 60 min, respectively. The data reported here were normalized using *ldhA* as internal control (43). SD was calculated from six data points which included three independent biological samples and two technical replicates for each biological sample. Strandard curve for *recA* was used to calculate the cDNA copies.

**Gene expression profile following UVB irradiation.** The number of functional categories of up-regulated genes in response to UVB irradiation (14) was more than that of UVC (11), but less than that of UVA (16). Similar to the UVC transcriptional profile, both "hypothetical proteins" (35.1%) and "conserved hypothetical proteins" (29.2%) were dominant. In addition, the number of genes in "DNA metabolism" and "other categories" decreased slightly whereas the number of genes in "cellular processes", "transporter and binding proteins" and "regulatory function" increased slightly compared to the UVC profile (Figure 3.2, UVB). Those changes indicated a shift in response to damage induced by short wavelength UVR to long wavelength UVR: from direct DNA damage and activation of prophages to global photo-oxidative damage.

Genes induced by UVB could be roughly divided into "UVC-pattern" genes and "UVA-pattern" genes. "UVC-pattern" genes were mainly distributed in cluster I, III and IV whereas "UVA-pattern" genes were mainly distributed in the cluster II (Supplemental Figure 3.1, UVB; Supplemental Table 3.3). A strong SOS induction was observed following UVB irradiation, which indicated that, similar to UVC, photons at UVB

wavelengths can cause direct DNA damage in MR-1 (Table 3.2). Similar to UVA, I observed the induction of genes encoding for an antioxidant enzyme (SO3349: 9.3 fold), iron sequestration (SO3348: 10.7 fold), multidrug efflux pumps (SO4328: 4.9 fold), and production of toxin and resistance traits (SO4170: 6.9 fold and SO4327: 4.3 fold) although the number of induced genes in each category was less than that for UVA (Supplemental Table 3.3). This result confirmed my previous observation that the UVB induced stress response was more similar to that of UVC (38).

**Discussion**

Possession of efficient DNA repair capacity is essential for the survival of all organisms following UVR irradiation. Although strong induction of several genes that are subject to SOS regulation was observed in MR-1, some DNA damage repair genes were not damage-inducible by UVR. For example, expression of the NER component genes *uvrA*, *uvrB* and *uvrD*, and the *E. coli* SOS regulon genes *ruvAB* and *recF* were not induced in any of my experimental conditions. Similarly, genes involved in response to oxidative DNA damage such as *nfo*, *xthA* and *mutM* that are damage-inducible in *E. coli* (6, 20, 22), were not induced in MR-1 following UVA exposure. Although MR-1 encodes most DNA damage repair genes in common with *E. coli*, several genes that have demonstrated importance in DNA repair in *E. coli* are absent from the MR-1 genome. For example, both *dinI* and *dinD*, which are highly induced following UVC irradiation in *E. coli* (8); both *nfo* and *nfi*, which are important genes in BER in *E. coli* (14) and *sodA*, which encodes an inducible superoxide dismutase (MnSOD), are not present in the MR-1 genome. In addition, genes involved in very short patch mismatch repair, e.g. *vsr*, which

encodes a DNA mismatch endonuclease and genes involved in the adaptive response, e.g. *alkA*, which encodes a 3-methyl-adenine DNA glycosylase II, are not present on the MR-1 genome (17). Thus alteration in gene regulation and lack of certain genes may contribute to the difference in radiation resistance between *E. coli* and *S. oneidensis* (38).

The cellular response to oxidative stress is important since ROS can damage a variety of molecules in the cell including DNA, membrane lipids and proteins. ROS can be produced by the incomplete reduction of oxygen during respiration, by exposure to radiation or to oxidation-reduction (redox) active drugs or by release from macrophages in response to bacterial invasion. The induction of more than 280 genes following UVA irradiation indicated MR-1 possesses an active regulation network in response to oxidative stress. The gene product of SO1328 has 34% identity with OxyR of *E. coli* at the amino acid level. The conservation of the two cysteine residues (Cys-203 and Cys-212 in MR-1) that are required for activation of OxyR in *E. coli* (Cys-199 and Cys-208 in *E. coli*) (52) may suggest a similar regulatory mechanism in MR-1. However, no putative *soxR* or *soxS* is found in the MR-1 genome, although there are ten transcriptional regulators of the MerR family, to which SoxR belongs and five transcriptional regulators of the Ara/Xyls family, to which SoxS belongs. I observed the induction of two transcriptional regulators of Ara/Xyls family proteins (SO1762: 4.8 fold and SO0317: 2.4 fold) following UVA irradiation. The potential roles of these regulators in response to oxidative stress in *S. oneidensis* MR-1 needs further investigation.

Many sequenced bacterial genomes harbor prophages or phage-like elements, which have been implicated in pathogenesis and in shaping bacterial as well as viral genomes (5). Although the genome of MuSo1 (SO0641-SO0683) is interrupted by the

insertion elements ISSod1-3, MuSo2 (SO2652-SO2704) is almost intact (17). My data strongly suggested that all three prophages in the MR-1 genome were potentially active following UVR irradiation. The activation of prophages in MR-1 can be the major lethal factor following short wavelength UVR exposure. I previously demonstrated that there is a difference in UVC sensitivity within *S. oneidensis* strains and among other species of the *Shewanella* genus (38). No MR-1 type prophages were identified in any other *Shewanella* strains that have been sequenced including *S.* sp. PV-4, *S. denitrificans* OS220, *S. frigidimarina* NCIMB 400 and *S. putrefaciens* CN-32 (K. Konstantinidis pers. comm.). All of those strains showed higher UVC resistance than MR-1 (data not shown). These observations suggest that activation of prophages in MR-1 contributes greatly to its high UVC sensitivity.

Approximately 40% of annotated ORFs in MR-1 belong to either conserved hypothetical protein (871 ORFs) or hypothetical protein (1161 ORFs). A total of 181 of those ORFs were induced under my experimental conditions, among which 18 were induced in response to all three wavelength groups of UVR, 61 were induced in response to both UVB and UVC, and 19 were induced in response to both UVB and UVA (Supplemental Figure 3.2). There are 8, 12 and 64 ORFs induced specifically by UVC, UVB or UVA irradiation, respectively (Supplemental Figure 3.2; Supplemental Table 3.4). The potential biological function of these ORFs can be inferred based on their induction pattern among the three treatments. For example, eighteen of hypothetical and conserved hypothetical proteins that were induced in all three treatments were grouped either with IS elements, indicating their potential function in transposition, or with the SOS regulon, indicating their potential function in DNA damage repair or cell division,

or with prophage genes, indicating their potential functions in prophage replication, transcription or transposition and maturation (Table 3.2). One of particular interesting hypothetical genes is SO4604, which is located in the same operon as *lexA*. Strong induction of this gene was observed in MR-1 following UVC (12.5 fold), UVB (20.0 fold) and UVA (6.9 fold) exposure. The new annotation indicated that the product of this ORF is similar to SulA of *E. coli* (M. Romine, pers. comm.), which is an inhibitor of cell division in *E. coli*. *sulA* is subject to the SOS regulation (8). Induction of SO4604 after UVC exposure correlated well with my observation of the occurrence of filamentous cells of MR-1 (Figure 3.3B). Detailed information on each hypothetical or conserved hypothetical ORF is summarized in Supplemental Table 3.4. Such results provide clues about the potential biological function of conserved hypothetical and hypothetical proteins. In conjunction with other approaches, we will gain a better understanding of those genes in MR-1 as well as in other organisms.

This study systematically investigated and compared the genomic response to the three important wavelength groups of UV radiation: UVC, UVB and UVA. Living organisms have to face the toxic insults from solar UV radiation since life began on the earth. Although current solar UV radiation reaching to earth's surface contains mainly UVA (95%) and UVB (5%) and no UVC due to ozone filtration, unattenuated UV radiation prior to accumulation of oxygen in the earth's atmosphere would have provided strong selective pressures for the evolution of mechanisms in DNA repair and damage tolerance. Continued selection for UVA resistance is obvious, but selection for UVC resistance may occur from the exposure to UVB, from other DNA damaging agents or from nonstatic environments. Alteration in gene regulation and loss of certain genes

important in DNA repair as well as in defending against oxidative stress appears to have occurred in *S. oneidensis* MR-1, an organism not known so far to live currently in environments exposed to solar light.

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# CHAPTER 4

# GENOME-WIDE EXAMINATION OF NATURAL SOLAR RADIATION RESPONSE IN SHEWANELLA ONEIDENSIS MR-1

**Abstract**

I delineated the cellular response of *Shewanella oneidensis* MR-1 to natural solar radiation by analyzing the transcriptional profile following exposure to ambient solar light at a dose which yields about 20% survival rate. More than one thousand genes showed significant differential expression ($P<0.01$) of at least a two-fold change in magnitude during a 1 h recovery period. This genomic response is much greater than that observed after exposure to UVB or UVA, of which a total of 195 and 403 genes showed differential expression, respectively. I observed the induction of DNA damage repair genes, the SOS response as well as a detoxification strategy previously observed for UVA irradiation. Few prophage-related genes were induced by solar radiation, however, in contrast to what was observed following UVB or UVC irradiation. Overall, the cellular response to solar radiation in MR-1 was more similar to that of UVA than that of UVB, but with more genes involved in detoxification induced compared to either UVB or UVA or their sum. Hence oxidative stress appeared to contribute greatly to the solar radiation induced cytotoxic effects in MR-1. The number of differentially expressed genes from most functional categories (15 up-regulated and 19 down-regulated) increased compared to either of UVB or UVA or their sum, which indicates that natural sunlight impacts biological processes in a much more complex way than previously thought.

**Introduction**

The deleterious effects of solar light on biological systems are thought to be due primarily to solar ultraviolet radiation (UVR) (Diffey 1991). Solar UVR reaching earth's surface contains mainly (95%) of UVA (320 to 400 nm) and a small portion (about 5%) of UVB (290 to 320 nm), which is collectively called near UV. UV radiation with wavelengths less than 290 nm (far UV) is attenuated by the stratospheric ozone layer. The biological effects induced by near UV are much more complex than for far UV due to the complex damage processes (Jagger 1983; Webb 1977). First, near UVR damages the cell through a process called "photosensitization", in which DNA along with many other molecules (photoreceptors or chromophores) can absorb the photons in wavelengths of 290-400 nm, being damaged directly or transfer the energy to other molecules, causing secondary damage to the cell. Typical photoreceptors include amino acids such as tryptophan and cysteine; components in respiratory chain such as porphyrins, flavins and quinones (Jagger 1983), and components of protein synthesis machinery such as tRNA (Eisenstark 1987). Near UV can also induce the formation of reactive oxygen species (ROS) including hydrogen peroxide, superoxide anion, hydroxyl radical and singlet oxygen (Eisenstark 1989), which can damage a variety of cell components as well as physiological processes. Absorption of near-UV photons by DNA results in formation of characteristic lesions including pyrimidine dimers (CPD), pyrimidine pyrimidone (6-4) photoproducts ((6-4) PD) as well as other minor photoproducts such as thymine glycols and pyrimidine hydrates (Friedberg et al. 1995). The Dewar isomer of (6-4) PD is a significant photoproduct after solar light exposure (Perdiz et al. 2000). In the presence of ROS, more DNA lesions are generated due to oxidative DNA damage including single

strand breaks, double strand breaks, DNA cross links and DNA protein cross links (Tyrrell 1991).

Solar UV radiation is perhaps one of the most mutagenic agents to life on earth. Unattenuated UV radiation prior to accumulation of oxygen in the earth's atmosphere would have provided strong selective pressures for the evolution of mechanisms for DNA repair and damage tolerance. Tolerance to solar UVR may arise from different mechanisms such as physical screening of the radiation by near UV radiation absorbing compounds; by interference with the action of deleterious photoproducts, e.g. quenching of singlet oxygen by carotenoid pigments; and repair of DNA damage (Jagger 1983). Bacteria are particularly vulnerable to UVR damage due to their small size and unicellular structure. Thus, the possession of mechanisms to repair UVR-induced damage as well as other sheltering strategies are essential contributors to the ecological fitness of organisms that are regularly exposed to solar UVR.

*Shewanella oneidensis* MR-1, a facultative anaerobic Gamma proteobacterium, possesses remarkable respiratory versatility and is widely distributed in nature, with aquatic environments and sediments as its primary habitat (Venkateswaran et al. 1999). My previous study demonstrated that this bacterium is extremely sensitive to solar light (Qiu et al. 2004). For example, more than 80% of cells died after exposure to Michigan summer sun light for 10 to 15 min. Genomic responses of MR-1 to each component of solar radiation (UVB and UVA) have been characterized. DNA damage and activation of prophages in MR-1 are the major lethal factors induced by UVB whereas global photo-oxidative damage is the primary lethal factor induced by UVA. MR-1 possesses active detoxifying mechanisms including the activation of antioxidant enzymes and proteins;

production of toxins, and activation of multidrug and heavy metal efflux pumps. Here I reported my examination of genomic response of MR-1 to natural solar light. Surprisingly, the genomic response to solar light is much more global than to either UVB or UVA. Approximately 10% of the genome was up-regulated and 18% of the genome was down-regulated. This unique gene expression profile suggests that natural solar radiation induced biological effects is much more complex than previously thought.

**Materials and methods**

***S. oneidensis* MR-1 whole genome cDNA array.** *S. oneidensis* MR-1 whole genome cDNA arrays containing about 95% of total ORFs were produced at Oak Ridge National laboratory (Gao et al. 2004). In brief, a total of 4,197 PCR amplicons and 451 oligonucleotides were deposited onto Corning Ultra GAPS slides (Corning, Corning, NY) using a Microgrid II arrayer (Matrix, Hudson, NH) with 16 (4 x 4) SMP2.5 pins (Telechem, Inc., Sunnyvale, CA). The arrays were printed with two replicates, each containing a 4 x 4 subgrid with the spot distance of 210 microns and the spot diameter of 140-180 microns. A total of 276 control spots including black (no DNA deposited) and 10 different *Arabadopis* genes (Strategene, La Jolla, CA) and four genomic DNA at each subgrid were also included on the array. After UV-crosslinking (250 mJ) using a Stratalinker (Strategene), arrays were stored in a desiccator.

**Microarray hybridization and data analysis.** MR-1 was grown in Davis medium with 15 mM lactate as the carbon source until the $OD_{600}$ reached 0.2-0.3 at which time the culture was split into two samples. One sample was used for ambient solar light irradiation and the other was used as a control. Solar irradiation was performed as described previously (Qiu et al. 2004). Briefly, 50 ml cell suspensions were transferred

into sterile boxes constructed of Acrolyte OP-4 plastic (Professional Plastics, Austin, TX), which transmits greater than 90% of the total radiation throughout the UVA and UVB wavelengths (Acrolyte OP-4 technical data sheet; Cyro Industries, Arlington, NJ). Two boxes were maintained on ice on a rocking platform during the exposures with control samples covered with aluminum foil. Solar UVB radiation was measured with a UVB detector (SED240/UVB-1/W) attached to an IL-1700 research radiometer (International Light, Newburyport, Mass.). After exposure to 558 J $m^{-2}$ of solar UVB, which yields about a 20% survival rate for MR-1 (Qiu et al. 2004), both irradiated and control samples were shaded and placed in a shaker at 30°C.

Cells were collected after 5 min, 20 min and 60 min of incubation for RNA extraction. Prehybridization and RNA labeling were performed as described by Schroeder (Schroeder et al. 2002) with a 2:3 ratio of 5-(3-aminoallyl)-dUTP and dTTP. Hybridization and washing were carried out as described by Hegde et al. (Hegde et al. 2000). At each time point of each treatment, eight hybridizations from four biological replicates and two technical replicates (dye-swap) were performed. GENESPRING 6.0 software (Silicon Genetics, Redwood City, CA) was used to analyze all microarray hybridization data. Only those spots with more than 80% of pixels greater than background plus 2SD in either cy5 or cy3 channel were used for analysis. Data were normalized both per chip and per gene (Lowess method). Those genes that showed a statistically significant change in gene expression ($P<0.01$) and a > 2-fold change in magnitude were regarded as significant.

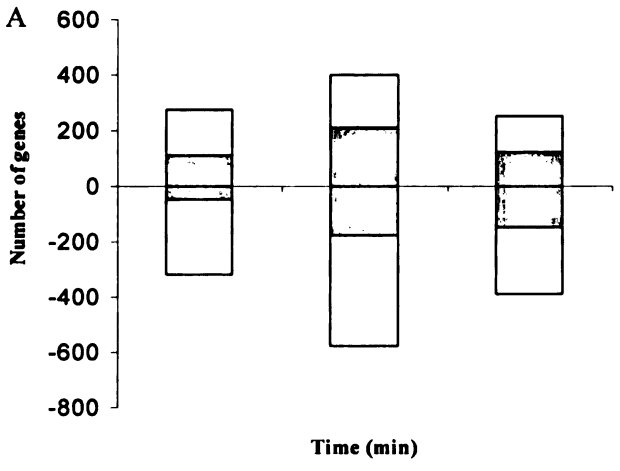
**Quantitative real time reverse transcription PCR (Q RT-PCR).** Q RT-PCR analysis was performed for 12 selected genes (Table 4.3) as described previously. Briefly,

two microgram of total RNA from each sample was converted to cDNA in the same condition as used for the microarray experiment except that dTTP instead of aa-dUTP and dTTP mixture was used. After hydrolyzing total RNA, total cDNA was purified using Qiagen PCR purification kit (Qiagen, Valencia, CA) and quantified using a spectrophotometer. Gene specific primers were designed using Primer Express® 1.0 software (Applied Biosystems, Foster City, CA). All amplicons were in the range of 90-100 bp. The specificity was first checked by blasting the primer sequences against the MR-1 genome. Both primer and template concentration for each gene were optimized in 1X SYBR Master Mixture (Applied Biosystems) using an ABI 7900HT Sequence Detection System (Applied Biosystems). The reaction specificity was further confirmed by dissociation curves after each PCR run. A standard curve for *recA* was constructed using purified PCR product. Since the *uvrB* gene of MR-1 was not damage inducible (Qiu et al. 2004), it was used as an internal control to normalize the difference in reverse transcription efficiency (Thellin et al. 1999). Duplicate runs were performed for each sample. Images in this dissertation are presented in color.

**Results and discussion**

**Global gene expression trend in response to solar radiation**. The genomic response of MR-1 to natural solar radiation was examined during a 1 h recovery period following solar light exposure using a microarray containing almost 95% of the predicted open reading frames (ORFs). A total of 595 (276 induced and 319 repressed) at 5 min, 973 (399 induced and 574 repressed) at 20 min and 645 (254 induced and 391 repressed) at 60 min showed significant differential expression ($P<0.01$) (Figure 4.1A). Of the total

differentially expressed genes, about 73-, 60- and 57% showed less than 3-fold change in gene expression at 5min, 20 min and 60min, respectively (Figure 4.1A). Approximately 10% of the genome (553 genes) was induced whereas about 18% of the genome (884 genes) was repressed during the 1 h recovery period, which is the most extensive response among all the radiation stress responses examined previously. In addition, more genes were repressed than induced, which is in contrast to the global expression trend of either UVB or UVA or their sum (Figure 4.1B). This result suggests that solar radiation induced stress response in MR-1 is much more complex than that of UVB or of UVA. More cellular processes appear impacted by solar radiation exposure in MR-1 although most of genes responded in a subtle way.



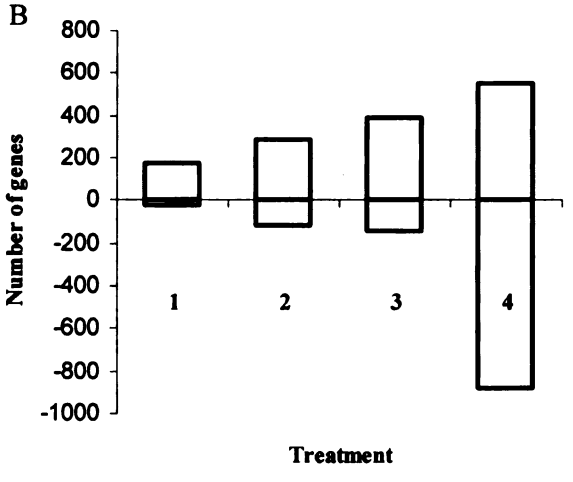



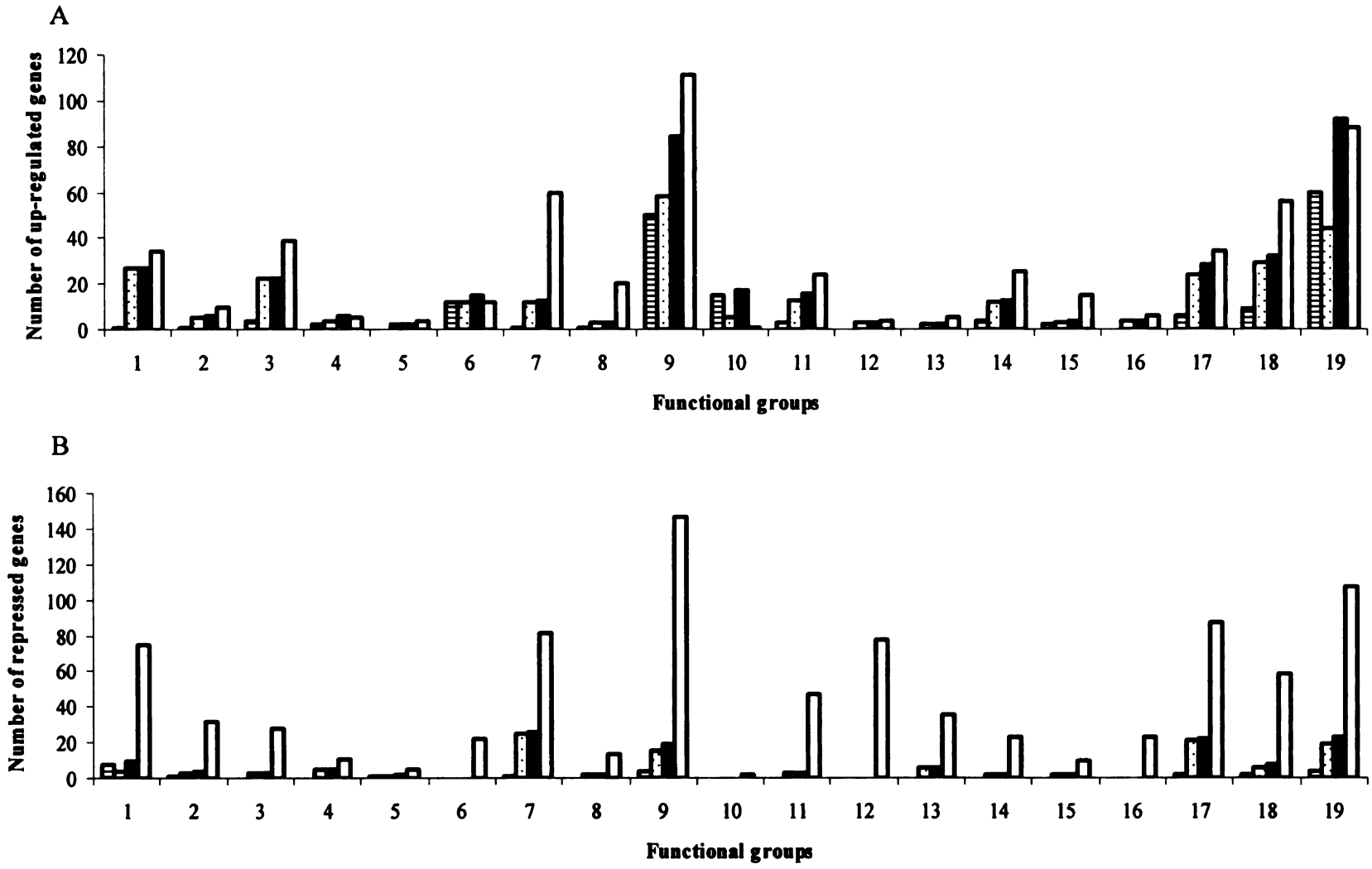

Figure 4.1. A: the global gene expression trend in response to solar radiation during a 1 h recovery period after exposure. Gray bars indicate the number of differentially expressed genes with change greater than 3 fold; B: comparison of differential expression following UVB (1), UVA (2) and solar radiation (4) exposure. 3 is the sum of UVB and UVA responses. Plus number indicates the number of induced genes and minus number indicates the number of repressed genes.

**Functional distribution of differentially expressed genes in response to solar radiation.** Based on the TIGR annotation (Heidelberg et al. 2002), five hundred and fifty three up-regulated genes after solar radiation exposure were distributed into 19 functional groups (Figure 4.2A). Besides "hypothetical proteins" and "conserved hypothetical

proteins", the five largest groups are "energy metabolism" (60 genes), "unknown function" (56 genes), "cellular process" (39 genes), "biosynthesis" (34 genes) and "transport and binding proteins" (34 genes), which is very similar to that of UVA (Figure 4.2A, group 7, 18, 3, 1 and 17). Since solar UV radiation contains both UVA and UVB, I compared the solar light induced differential expression profile to the sum of UVB and UVA responses in MR-1. More genes in 15 functional groups were induced, among which "energy metabolism" increased most (47 genes) (Figure 4.2A, group 7). Four functional categories showed a decrease in the number of up-regulated genes following solar light exposure, of which genes from "other categories" dropped most extensively. Only one prophage-related gene was induced following solar light exposure (Figure 4.2A, group 10). This result suggests that, unlike UVB, solar radiation appeared unable to activate the prophages in MR-1 at the dose examined.

A total of 884 genes were repressed in MR-1 following solar radiation exposure, which is much higher than for UVB (23 genes) or UVA (117 genes). The number of repressed genes in all 19 functional categories increased (Figure 4.2B), of which the greatest increase was observed for "conserved hypothetical proteins" (128 genes), "hypothetical protein" (84 genes), "protein synthesis" (78 genes), "biosynthesis" (65 genes) and "transport and binding proteins" (65 genes) (Figure 4.2B, group 9, 19, 12, 1 and 17).

Figure 4.2. Comparison of the distribution of the up-regulated (A) and down-regulated (B) genes in various functional categories following UVB (filled with lines), UVA (filled with dots) and solar radiation (empty bars) exposure. The solid black column represents the sum of the differentially expressed genes following UVB and UVA exposure. The functional category of each number stands for: 1: biosynthesis; 2: cell envelope; 3: cellular processes; 4: central intermediary metabolism; 5: disrupted reading frame; 6: DNA metabolism; 7: energy metabolism; 8: fatty acid and phospholipid metabolism; 9: conserved hypothetical proteins; 10: other categories; 11: protein fate; 12: protein synthesis; 13: purines, pyrimidines, nucleosides, and nucleotides; 14: regulatory functions; 15: signal transduction; 16: transcription; 17: transport and binding proteins; 18: unknown function; 19: hypothetical proteins.


A
Number of up-regulated genes
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Functional groups
B
Number of repressed genes
160
140
120
100
80
60
40
20
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Functional groups

I observed some common cellular responses to solar radiation, UVB and UVA, e.g. induction of DNA repair genes; however, the functional distribution of differentially expressed genes in response to solar radiation is more similar to that of UVA than UVB. This is not surprising since the majority of solar UV (95%) is in the UVA wavelength range. Indeed, about 28% of solar radiation induced genes were induced by UVA alone whereas less than 10% of solar radiation induced genes were induced by UVB alone (Figure 4.3A). The great number of unique genes (390 induced and 788 repressed) that showed differential expression only following solar radiation exposure indicates the other portion of solar radiation, e.g. visible and infrared light, may impact cellular processes in MR-1 (Figure 4.3).

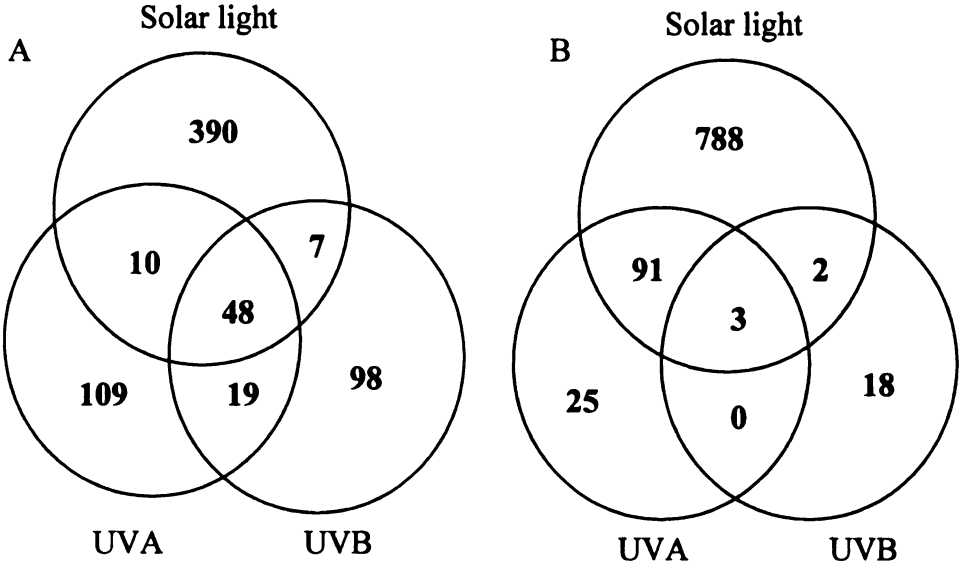



Figure 4.3. Venn diagram of up-regulated genes (A) and down-regulated genes (B) in response to UVA, UVB and natural solar radiation.

**Common cellular response to UVB, UVA and solar radiation**. Similar to both UVB and UVA, I observed the induction of several DNA damage repair genes as well as the SOS response in MR-1 following solar radiation exposure although the induction was less substantial compared to either UVB or UVA (Table 4.1). Two hypothetical genes (SO3367 and SO4605) and four conserved hypothetical genes (SO1757, SO2603, SO2604 and SO4604), whose response clustered with DNA damage genes in a previous study (Chapter 3), showed a slight induction following solar radiation exposure (Table 4.1). These data support our previous report on the potential biological function of those hypothetical ORFs (Supplemental Table 3.4). Induction of *phrB* correlated well with our previous observation of increased survival rate in solar radiation irradiated MR-1 after photoreactivation (Qiu et al. 2004). Similar to UVA induced stress response, I not only observed the induction of genes involved in scavenging reactive oxygen species, in sequestering iron, in degradative pathways as well as genes encoding multidrug and heavy metal efflux pumps, but more genes in each group were induced (Table 4.1). In addition, more genes involved in general stress response, e.g. heat shock proteins, and in cell motility were induced following solar radiation exposure (Supplemental Table 4.1). I also observed the induction of several genes for production of toxin and antibiotic resistance as I did for UVA (Table 4.1). Strong induction of antioxidant proteins and enzymes suggested that solar radiation can induce the formation of reactive oxygen species at the dose examined. Hence, global photo-oxidative damage is the most important cytotoxic effect induced by solar radiation in MR-1. Similar to UVA, I observed active detoxification mechanisms in MR-1 following solar radiation exposure,

Table 4.1. Induction of DNA damage genes, the SOS response and genes involved in defending against oxidative stress in MR-1 after exposure to natural solar radiation.

| SO number | Gene | Product | Function | Solar radiation | | |
|---|---|---|---|---|---|---|
| | | | | 5 min | 20 min | 60 min |
| SO3061 | *topB* | DNA topoisomerase III | DNA metabolism | 1.5 ± 0.2[a] | 2.7 ± 0.3 | 4.8 ± 1.0 |
| SO3429 | *recX* | Regulatory protein RecX | - | 1.2 ± 0.2 | 1.3 ± 0.1 | 3.0 ± 0.6 |
| SO3430 | *recA* | RecA protein | - | 2.3 ± 0.4 | 2.1 ± 0.3 | 4.4 ± 0.4 |
| SO4603 | *lexA* | LexA repressor | Regulatory functions | 3.3 ± 0.7 | 5.0 ± 1.4 | 7.5 ± 1.6 |
| SO4604 | | Conserved hypothetical protein | Induced by UVA and UVB | 3.2 ± 1.0 | 6.0 ± 1.0 | 8.7 ± 2.3 |
| SO3462 | *recN* | DNA repair protein RecN | DNA metabolism | 3.1 ± 0.9 | 5.2 ± 1.4 | 8.0 ± 1.7 |
| SO4364 | *recG* | ATP-dependent DNA helicase RecG | - | 1.7 ± 0.3 | 2.1 ± 0.6 | 2.2 ± 0.3 |
| SO1114 | *dinP* | DNA-damage-inducible protein P | Unknown function | 2.4 ± 1.0 | 3.5 ± 0.6 | 4.4 ± 0.7 |
| SOA0013 | *umuD* | UmuD protein | DNA metabolism | 1.5 ± 1.4 | 4.0 ± 0.9 | 6.2 ± 2.2 |
| SOA0012 | *umuC* | UmuC protein | - | 0.7 ± 0.7 | 1.5 ± 0.1 | 2.9 ± 0.6 |
| SO3384 | *phrB* | deoxyribodipyrimidine photolyase | - | 7.3 ± 1.9 | 5.0 ± 0.8 | 15.2 ± 4.5 |
| SO1331 | | MutT/nudix family protein | - | 2.3 ± 0.3 | 1.6 ± 0.2 | 1.3 ± 0.1 |
| SO1757 | | Conserved hypothetical protein | Induced by UVA and UVB | 13.9 ± 3.2 | 10.3 ± 1.1 | 6.2 ± 1.2 |
| SO2603 | | Conserved hypothetical proteins | - | 2.7 ± 0.4 | 2.2 ± 0.5 | 2.9 ± 0.4 |
| SO2604 | | Conserved hypothetical proteins | - | 3.8 ± 0.7 | 3.4 ± 1.0 | 3.6 ± 0.5 |
| SO3327 | | Hypothetical protein | - | 1.8 ± 0.4 | 3.2 ± 2.2 | 2.3 ± 0.8 |
| SO4605 | | Hypothetical protein | - | 0.5 ± 0.7 | 3.1 ± 1.0 | 2.2 ± 1.3 |
| SO0956 | *ahpF* | alkyl hydroperoxide reductase, F subunit | Cellular processes | 8.1 ± 3.8 | 10.0 ± 6.4 | 4.3 ± 1.0 |
| SO0958 | *ahpC* | alkyl hydroperoxide reductase, C subunit | - | 20.7 ± 6.0 | 18.4 ± 5.0 | 9.5 ± 2.8 |
| SO1070 | *katB* | catalase | - | 5.8 ± 3.3 | 7.0 ± 3.5 | 1.8 ± 1.1 |
| SO0725 | *katG*-1 | catalase/peroxidase HPI | - | 3.2 ± 1.1 | 4.6 ± 2.5 | 1.5 ± 0.3 |
| SO4405 | *katG*-2 | catalase/peroxidase HPI | - | 1.7 ± 0.4 | 3.0 ± 0.7 | 0.9 ± 0.1 |
| SO1773 | | catalase-related protein, authentic point mutation | - | 3.5 ± 1.3 | 6.3 ± 1.3 | 6.3 ± 2.9 |
| SO2178 | *ccpA* | cytochrome c551 peroxidase | - | 10.6 ± 7.4 | 7.0 ± 3.2 | 2.0 ± 0.4 |
| SO1563 | | glutathione peroxidase, putative | - | 4.0 ± 1.2 | 4.0 ± 1.1 | 1.9 ± 0.6 |
| SO3349 | | glutathione peroxidase, putative | - | 8.0 ± 1.0 | 7.9 ± 1.6 | 11.9 ± 3.3 |
| SO0976 | *ohr* | organic hydroperoxide resistance protein | - | 4.0 ± 1.4 | 6.2 ± 1.1 | 1.1 ± 0.2 |
| SO2881 | *sodB* | superoxide dismutase, Fe | - | 2.8 ± 0.7 | 1.4 ± 0.4 | 1.6 ± 0.2 |
| SO1158 | | Dps family protein | - | 19.2 ± 9.8 | 26.2 ± 11.9 | 5.2 ± 2.9 |
| SO1111 | *bfr*2 | bacterioferritin subunit 2 | Transport and binding protein | 0.5 ± 0.7 | 3.1 ± 1.0 | 2.2 ± 1.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SO1112 | *bfr*1 | bacterioferritin subunit 1 | Transport and binding protein | 6.1 ± 2.5 | 10.6 ± 3.3 | 5.0 ± 1.6 |
| SO1877 | *bcp* | bacterioferritin comigratory protein | - | 1.4 ± 0.2 | 1.0 ± 0.1 | 2.2 ± 0.3 |
| SO0518 | | outer membrane efflux family protein, putative | - | 2.2 ± 2.1 | 6.9 ± 1.2 | 2.5 ± 2.1 |
| SO0519 | | cation efflux protein, putative | - | 2.0 ± 1.5 | 6.6 ± 5.7 | 3.2 ± 2.6 |
| SO0520 | | heavy metal efflux pump, CzcA family | - | 1.3 ± 0.4 | 3.4 ± 2.0 | 2.2 ± 1.4 |
| SO4597 | | heavy metal efflux system protein, putative | - | 1.3 ± 0.3 | 11.0 ± 3.1 | 5.3 ± 4.2 |
| SO4598 | | heavy metal efflux pump, CzcA family | - | 0.9 ± 0.1 | 5.6 ± 1.4 | 6.2 ± 4.4 |
| SOA0153 | | heavy metal efflux pump, CzcA family | - | 1.1 ± 0.1 | 10.4 ± 3.8 | 4.5 ± 2.9 |
| SOA0154 | | heavy metal efflux protein, putative | - | 2.1 ± 0.6 | 20.0 ± 6.9 | 4.2 ± 2.7 |
| SO0525 | | drug resistance transporter, EmrB/QacA family protein | - | 1.9 ± 0.5 | 3.5 ± 1.2 | 3.3 ± 1.2 |
| SO1923 | | AcrB/AcrD/AcrF family protein | - | 2.1 ± 0.9 | 1.9 ± 0.6 | 1.9 ± 0.4 |
| SO1924 | | AcrB/AcrD/AcrF family protein | - | 0.5 ± 0.1 | 5.2 ± 0.5 | 4.1 ± 1.5 |
| SO4328 | | AcrB/AcrD/AcrF family protein, authentic frameshift | - | 3.6 ± 1.5 | 4.8 ± 0.4 | 3.4 ± 0.5 |
| SOA0159 | | multidrug efflux transporter | - | 1.0 ± 0.2 | 4.3 ± 2.8 | 3.2 ± 1.4 |
| SO4148 | | HlyD family secretion protein | - | 1.1 ± 0.8 | 2.3 ± 0.6 | 2.4 ± 1.1 |
| SO4149 | | RTX toxin, putative | Toxin and resistance production | 2.0 ± 1.1 | 2.8 ± 1.9 | 1.9 ± 0.8 |
| SO1925 | | HlyD family secretion protein | Transport and binding protein | 0.6 ± 0.1 | 3.6 ± 0.9 | 2.9 ± 1.0 |
| SO4327 | | HlyD family secretion domain protein | Unknown function | 4.1 ± 1.2 | 4.8 ± 0.5 | 3.7 ± 0.8 |
| SO0918 | *aac* | aculeacin A acylase | Toxin and resistance production | 2.3 ± 0.6 | 2.0 ± 0.4 | 1.1 ± 0.1 |
| SO0837 | | beta-lactamase, putative | - | 2.2 ± 0.7 | 2.7 ± 0.4 | 1.8 ± 0.7 |
| SO1796 | *lon* | ATP-dependent protease La | Degradation | 1.7 ± 0.4 | 3.8 ± 0.7 | 2.6 ± 0.5 |
| SO1987 | | ATP-dependent protease La (LON) domain protein | - | 7.7 ± 1.1 | 9.3 ± 1.2 | 9.9 ± 2.6 |
| SO2626 | *clp*A | ATP-dependent Clp protease, ATP-binding subunit | - | 1.2 ± 0.2 | 2.6 ± 0.2 | 2.6 ± 0.6 |
| SO3577 | *clp*B | clpB protein | - | 0.6 ± 0.1 | 4.7 ± 1.6 | 3.1 ± 0.7 |
| SO3142 | *dcp*-1 | peptidyl-dipeptidase Dcp | - | 2.0 ± 0.3 | 1.2 ± 0.2 | 3.1 ± 0.5 |
| SO0022 | *pep*Q | prolidase | - | 1.9 ± 0.2 | 2.1 ± 0.6 | 2.3 ± 0.3 |
| SO1915 | | serine protease, subtilase family | - | 1.8 ± 0.2 | 2.3 ± 0.2 | 1.3 ± 0.2 |
| SO3800 | | serine protease, subtilase family | - | 2.1 ± 0.5 | 2.2 ± 0.3 | 1.3 ± 0.2 |
| SO3942 | | serine protease, HtrA/DegQ/DegS family | - | 0.9 ± 0.1 | 4.8 ± 1.3 | 3.9 ± 0.4 |
| SO3083 | | peptidase, M16 family | - | 1.3 ± 0.4 | 1.4 ± 0.3 | 2.3 ± 0.6 |
| SO3560 | | peptidase, M16 family | - | 2.0 ± 0.3 | 1.4 ± 0.4 | 2.0 ± 0.2 |
| SO1561 | | peptidase, M1 family | - | 1.6 ± 0.2 | 4.3 ± 0.9 | 2.4 ± 0.2 |

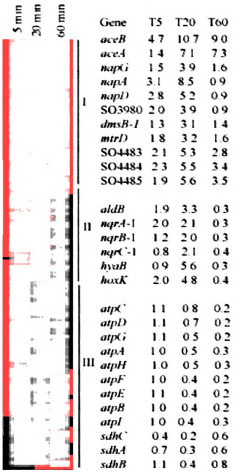
[a] standard deviation.

which included the activation of antioxidant proteins and enzymes, fine tuning of internal iron concentration, activation of multidrug and heavy metal efflux pumps as well as global cellular regulation to overcome solar radiation induced cytotoxic effects.

**Response of energy metabolic genes.** As discussed above, genes in "energy metabolism" represent a large portion of total differentially expressed genes following solar radiation exposure (Figure 4.2). Three distinct response patterns were revealed by hierarchical cluster analysis (Figure 4.4). The first cluster contained genes that were induced at least at one time point examined. I observed a strong induction for genes encoding the components of glyoxylate bypass (*aceB* and *aceA*) and for genes involved in anaerobic respiration (*napG*, *napA*, *napD* and *dmsB*-1) (Figure 4.4; Supplemental Table 4.2). In addition, I observed induction of genes involved in degradation of amino acids including phenylalanine, valine, histidine, serine, leucine and methionine and of genes involved in electron transport such as cytochrome *c*, cytochrome *b*, iron-sulfur cluster-binding protein, electron transfer flavoprotein-ubiquinone oxidoreductase and ferredoxin-NADP reductase (Supplemental Table 4.2). The second cluster contains genes that were induced slightly at 20 min, but repressed at 60 min (Figure 4.4). Genes in this group included those involved in anaerobic respiration (*fdrCAB*), fermentation (*adhB*) and electron transport (*hyaB* and *hoxK*) (Figure 4.4 and Supplemental Table 4.2). The third cluster contains genes that were repressed at least at one time point examined. I observed a strong repression of genes encoding NADH:ubiquinone oxidoreductase (SO1103-SO1108), enzymes in the TCA cycle (SO1927-SO1929: succinate dehydrogenase; SO1930-SO1931: 2-oxoglutarate dehydrogenase and SO1932-SO1933:

succinyl-CoA synthase) as well as in glycolysis (*tpiA*, *gapA-3* and *ppsA*) and for genes encoding the ATP synthase (SO4746-SO4754). (Figure 4.4; Supplemental Table 4.2).

Regulation of metabolic gene expression suggests a fine tuning in MR-1 to meet the energy requirement for recovering from solar radiation induced stress. Repression of several TCA cycle genes and glycolysis genes and ATP synthase genes indicates a transient inhibition of energy production, which correlates well with the inhibition of cell division due to solar radiation induced DNA damage. Interestingly, MR-1 carries two operons (SO0902-SO0907 and SO1103-SO1108) encoding NADH: ubiquinone oxidoreductase, which is a membrane complex of six subunits (*nqrA*, *nqrB*, *nqrC*, *nqrD*, *nqrE* and *nqrF*) that accommodates a 2Fe-2S center and several flavins. This enzyme oxidizes NADH and reduces ubiquinone and uses the energy of this redox reaction to translocate sodium across the cell membrane. The first operon (SO0902-SO0907) showed a slight induction at 20 min and a repression at 60 min whereas the second operon (SO1103-SO1108) was repressed at 20 min and returned to basal expression at 60 min. The difference in expression of these two operons may indicate changes in production of energy following solar radiation exposure since this complex is the entry point of electrons into the respiratory chain. A shift from aerobic respiration to anaerobic respiration and activation of the glyoxylate shunt not only reduces the energy production, but also minimizes chances of generating additional ROS in the cell due to aerobic respiration. A similar observation has been reported for *Deinococcus radiodurans* during the early recovery stage after exposure to ionizing radiation (Liu et al. 2003).




| | Gene | T5 | T20 | T60 |
|---|---|---|---|---|
| I | *aceB* | 4.7 | 10.7 | 9.0 |
| | *aceA* | 1.4 | 7.1 | 7.3 |
| | *napG* | 1.5 | 3.9 | 1.6 |
| | *napA* | 3.1 | 8.5 | 0.9 |
| | *napD* | 2.8 | 5.2 | 0.9 |
| | SO3980 | 2.0 | 3.9 | 0.9 |
| | *dmsB-1* | 1.3 | 3.1 | 1.4 |
| | *mtrD* | 1.8 | 3.2 | 1.6 |
| | SO4483 | 2.1 | 5.3 | 2.8 |
| | SO4484 | 2.3 | 5.5 | 3.4 |
| | SO4485 | 1.9 | 5.6 | 3.5 |
| II | *aldB* | 1.9 | 3.3 | 0.3 |
| | *nqrA-1* | 2.0 | 2.1 | 0.3 |
| | *nqrB-1* | 1.2 | 2.0 | 0.3 |
| | *nqrC-1* | 0.8 | 2.1 | 0.4 |
| | *hyaB* | 0.9 | 5.6 | 0.3 |
| | *hoxK* | 2.0 | 4.8 | 0.4 |
| III | *atpC* | 1.1 | 0.8 | 0.2 |
| | *atpD* | 1.1 | 0.7 | 0.2 |
| | *atpG* | 1.1 | 0.5 | 0.2 |
| | *atpA* | 1.0 | 0.5 | 0.3 |
| | *atpH* | 1.0 | 0.5 | 0.3 |
| | *atpF* | 1.0 | 0.4 | 0.2 |
| | *atpE* | 1.1 | 0.4 | 0.2 |
| | *atpB* | 1.0 | 0.4 | 0.2 |
| | *atpI* | 1.0 | 0.4 | 0.3 |
| | *sdhC* | 0.4 | 0.2 | 0.6 |
| | *sdhA* | 0.7 | 0.3 | 0.6 |
| | *sdhB* | 1.1 | 0.4 | 0.8 |

Figure 4.4. Hierarchical cluster analysis of differentially expressed energy metabolic genes in response to natural solar radiation. The distance was calculated using standard correlation. The number indicates the relative expression level for the selected genes in each cluster. SO3980 encodes a cytochrome *c*552 nitrite reductase; SO4483 encodes a putative cytochrome *b*; SO4484 encodes a cytochrome *c*-type protein Shp and SO4485 encodes a diheme cytochrome *c* (This figure is presented in color).

**Response of biosynthesis genes**. As a consequence of reduction in energy production, I observed a reduction in biosynthesis of amino acids, proteins, cofactors, prosthetic groups and carriers following solar radiation exposure (Figure 4.2). Among 153 down-regulated biosynthesis genes, strong repression was observed for genes involved in biosynthesis of amino acids in the aspartate family, e.g. SO4054-SO4056, in the glutamate family, e.g. SO0275-SO0279 and in biosynthesis of heme, porphyrin, and cobalamin, e.g. *hemH*-1 and *hemB*-2 (Table 4.2 and Supplemental Table 4.3). A total of 78 genes involved in protein synthesis were repressed, which includes 48 genes encoding ribosomal proteins, nine genes encoding translation factors and others involved in either tRNA aminoacylation or tRNA and rRNA base modification (Supplemental Table 4.3). However, I also observed the induction of more than 30 biosynthesis genes following solar radiation exposure, of which strong induction was seen for the tryptophan operon (SO3019-SO3024) and for *iscS*, which encodes a cysteine desulfurase involved in assembling iron-sulfur clusters for protein (Table 4.2). Tryptophan is a chromophore for near UV radiation (UVB and UVA) and undergoes a biochemical alternation that yields hydrogen peroxide as a photoproduct (McCormick 1976). The induction of the tryptophan operon may be a consequence of the photo-damage to tryptophan in MR-1 during solar radiation exposure. I also observed a strong induction for *hemH*-2 (SO3348), which encodes a ferrochelatase that inserts iron into protoporphrin IX to make heme. The induction of this gene was observed in MR-1 following UVB and UVA irradiation, which implicates a potential role in defending against oxidative stress. Interestingly, MR-1 carries another copy of *hemH* (SO2019: *hemH*-1). However, the expression of *hemH*-1 remained unchanged until 60 min, when it was repressed more than 3-fold (Table 4.2).

The different response of *hemH*-1 and *hemH*-2 may indicate that there is a difference in their physiological role.

Table 4.2. Relative expression of biosynthesis genes and biosynthesis related genes in MR-1 after exposure to natural solar radiation.

| ID | Gene | Porduct | Function | Solar radiation | | |
|---|---|---|---|---|---|---|
| | | | | 5 min | 20 min | 60 min |
| SO0817 | *metR* | transcriptional activator protein MetR | Regulatory functions | 0.34 ± 0.26[a] | 0.22 ± 0.09 | 0.37 ± 0.18 |
| SO0818 | *metE* | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase | Amino acid biosynthesis | 0.54 ± 0.06 | 0.20 ± 0.10 | 0.10 ± 0.12 |
| SO0929 | *metK* | S-adenosylmethionine synthetase | Central intermediary metabolism | 1.31 ± 0.37 | 0.26 ± 0.07 | 0.24 ± 0.04 |
| SO1030 | *metH* | 5-methyltetrahydrofolate--homocysteine methyltransferase | Amino acid biosynthesis | 1.36 ± 0.73 | 0.43 ± 0.16 | 0.39 ± 0.05 |
| SO1676 | *metA* | homoserine O-succinyltransferase | - | 0.96 ± 0.29 | 0.61 ± 0.35 | 0.39 ± 0.07 |
| SO2619 | *metG* | methionyl-tRNA synthetase | Protein synthesis | 0.53 ± 0.12 | 0.35 ± 0.03 | 0.78 ± 0.11 |
| SO4054 | *metF* | 5,10-methylenetetrahydrofolate reductase | Amino acid biosynthesis | 0.21 ± 0.40 | 0.12 ± 0.05 | 0.10 ± 0.03 |
| SO4055 | *metL* | aspartokinase II/homoserine dehydrogenase, methionine-sensitive | - | 0.43 ± 0.15 | 0.27 ± 0.16 | 0.11 ± 0.02 |
| SO4056 | *metB* | cystathionine gamma-synthase | - | 0.46 ± 0.53 | 0.16 ± 0.12 | 0.10 ± 0.03 |
| SO4057 | *metJ* | met repressor | Regulatory functions | 0.22 ± 0.26 | 0.34 ± 0.13 | 0.34 ± 0.20 |
| SO0275 | *argC* | N-acetyl-gamma-glutamyl-phosphate reductase | Amino acid biosynthesis | 0.44 ± 0.20 | 0.34 ± 0.08 | 0.10 ± 0.03 |
| SO0276 | *argB* | acetylglutamate kinase | - | 0.41 ± 0.12 | 0.45 ± 0.14 | 0.10 ± 0.03 |
| SO0277 | *argF* | ornithine carbamoyltransferase | - | 0.32 ± 0.08 | 0.44 ± 0.18 | 0.08 ± 0.02 |
| SO0278 | *argG* | argininosuccinate synthase | - | 0.42 ± 0.12 | 0.50 ± 0.30 | 0.07 ± 0.04 |
| SO0279 | *argH* | argininosuccinate lyase | - | 0.46 ± 0.07 | 0.96 ± 0.34 | 0.09 ± 0.02 |
| SO0769 | *argR* | arginine repressor | Regulatory functions | 0.42 ± 0.10 | 0.27 ± 0.10 | 0.24 ± 0.07 |
| SO4245 | *argA* | amino-acid acetyltransferase | Amino acid biosynthesis | 0.51 ± 0.15 | 0.33 ± 0.14 | 0.26 ± 0.10 |
| SO0435 | *hemE* | uroporphyrinogen decarboxylase | Biosynthesis of cofactors, prosthetic groups, and carriers | 1.08 ± 0.18 | 0.61 ± 0.11 | 0.33 ± 0.03 |
| SO1300 | *hemL* | glutamate-1-semialdehyde-2,1-aminomutase | - | 0.71 ± 0.12 | 0.59 ± 0.25 | 0.37 ± 0.05 |
| SO2019 | *hemH*-1 | ferrochelatase | - | 0.78 ± 0.14 | 0.75 ± 0.22 | 0.33 ± 0.05 |
| SO2587 | *hemB*-1 | delta-aminolevulinic acid dehydratase | - | 0.96 ± 0.10 | 1.22 ± 0.14 | 0.62 ± 0.12 |
| SO3348 | *hemH*-2 | ferrochelatase | - | 5.95 ± 0.63 | 5.01 ± 0.55 | 11.72 ± 1.85 |
| SO4208 | *hemB*-2 | delta-aminolevulinic acid dehydratase | - | 0.99 ± 0.21 | 0.72 ± 0.09 | 0.31 ± 0.04 |
| SO4313 | *hemC* | porphobilinogen deaminase | - | 2.37 ± 0.34 | 1.37 ± 0.43 | 0.41 ± 0.04 |
| SO4314 | *hemD* | uroporphyrinogen-III synthase | - | 1.26 ± 0.24 | 1.04 ± 0.33 | 0.45 ± 0.08 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SO4730 | *hemN* | oxygen-independent coproporphyrinogen III oxidase | - | 1.54 ± 0.52 | 1.40 ± 0.60 | 0.38 ± 0.07 |
| SO3019 | *trpE* | anthranilate synthase component I | Amino acid biosynthesis | 7.47 ± 1.95 | 2.93 ± 0.27 | 1.70 ± 0.15 |
| SO3020 | *trpG* | glutamine amido-transferase | - | 9.04 ± 3.64 | 3.67 ± 0.57 | 1.87 ± 0.20 |
| SO3021 | *trpD* | anthranilate phosphoribosyltransferase | - | 6.29 ± 3.23 | 2.77 ± 0.45 | 1.73 ± 0.20 |
| SO3022 | *trpC/F* | indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase | - | 2.60 ± 1.52 | 2.53 ± 0.57 | 1.56 ± 0.27 |
| SO3023 | *trpB* | tryptophan synthase, beta subunit | - | 1.29 ± 0.29 | 3.59 ± 0.96 | 1.66 ± 0.16 |
| SO3024 | *trpA* | tryptophan synthase, alpha subunit | - | 1.09 ± 0.24 | 3.14 ± 0.78 | 1.24 ± 0.41 |
| SO2265 | *iscS* | cysteine desulfurase | Biosynthesis of cofactors, prosthetic groups, and carriers | 0.90 ± 0.23 | 0.88 ± 0.14 | 5.73 ± 1.70 |

[a]standard deviation.

**Validation of gene expression profile by Q RT-PCR.** Q RT-PCR analysis was carried out for 12 selected genes, which included highly induced genes (*ohr*, *katB* and SOA0154), moderately induced genes (*recA*, SO1924 and SO4328), slightly induced gene (SO1923 ), highly repressed gene (SO3671) and genes with no change in expression level (*radC*, *trxC* and SO1762). I previously demonstrated that nucleotide excision repair component genes *uvrA*, *uvrB* and *uvrD* were not damage inducible (Qiu et al. 2004), thus *uvrB* was used as internal control to normalize the difference in reverse transcription efficiency. A good correlation ($R^2$: 0.7955) was obtained between microarray analysis and Q RT-PCR analysis (Table 4.3). The induction fold measured by microarray hybridization for highly induced genes (*ohr, katB* and SOA0154) were lower than that measured by Q RT-PCR (Table 4.3). This trend is consistent with a previous report on validation of cDNA array by Q RT-PCR analysis (Yuen et al. 2002). The Q RT-PCR assay confirmed that, similar to UVA, multidrug and heavy metal efflux pumps were activated by solar radiation. However, unlike UVA, there was no induction of transcriptional regulator SO1762 but a strong induction of *katB* following solar radiation exposure, which may indicate the difference between UVA- and natural solar radiation-induced oxidative damage in MR-1.

Solar UV radiation, especially UVB has been the focus of solar radiation induced biological effects because of its potential lethal and mutagenic effects. As expected, I observed the induction of the SOS response in MR-1 following exposure to 558 J $m^{-2}$ of solar UVB. However, more genes involved in detoxification were induced compared to either UVA or UVB or their sum, which may indicate that natural solar radiation induced oxidative damages are much more global and complex than that by UVA or UVB. This

could be due to the complexity of natural solar UV radiation. The great genomic response to solar light indicates that in addition to the cytotoxic effects induced by solar UV radiation, visible and infrared light may also impact cellular processes in a subtle way.

Table 4.3. Comparison of relative gene expression between microarray analysis and Q RT-PCR assay

| Gene | [a]T5 | | [a]T20 | | [a]T60 | |
|---|---|---|---|---|---|---|
| | Array | Q RT-PCR | Array | Q RT-PCR | Array | Q RT-PCR |
| *ohr* | 4.0 ± 1.4 | 20.2 ± 7.1 | 6.2 ± 1.1 | 38.3 ± 9.3 | 1.1 ± 0.2 | 2.1 ± 0.3 |
| *katB* | 5.8 ± 3.3 | 10.3 ± 5.1 | 7.0 ± 3.5 | 39.7 ± 10.6 | 1.8 ± 1.1 | 2.3 ± 0.2 |
| SOA0154 | 2.1 ± 0.6 | 2.0 ± 0.6 | 20.0 ± 6.9 | 70.6 ± 13.5 | 4.2 ± 2.7 | 2.4 ± 1.6 |
| SO1923 | 1.9 ± 0.6 | 1.0 ± 0.5 | 3.3 ± 3.5 | 6.5 ± 1.4 | 2.0± 0.4 | 4.2 ± 0.2 |
| SO1924 | 0.5 ± 0.1 | 0.2 ± 0.1 | 5.2 ± 0.5 | 8.3 ± 1.0 | 4.1 ± 1.5 | 8.7 ± 2.5 |
| SO4328 | 3.6 ± 1.5 | 4.5 ± 2.1 | 4.8 ± 0.4 | 6.9 ± 1.8 | 3.4 ± 0.5 | 2.5 ± 0.3 |
| *recA* | 2.3 ± 0.4 | 1.9 ± 0.3 | 2.1 ± 0.3 | 2.4 ± 0.4 | 4.4 ± 0.4 | 3.4 ± 0.4 |
| SO3671 | 1.0 ± 0.2 | 0.4 ± 0.1 | 0.3 ± 0.3 | 0.1 ± 0.0 | 0.3 ± 0.2 | 0.1 ± 0.1 |
| *radC* | 1.3 ± 0.2 | 0.8 ± 0.0 | 0.6 ± 0.1 | 2.2 ± 1.4 | 0.7 ± 0.1 | 1.9 ± 0.2 |
| *trxC* | 0.6 ± 0.1 | 0.3 ± 0.1 | 1.6 ± 0.3 | 1.6 ± 0.7 | 1.9 ± 0.4 | 1.5 ± 0.2 |
| SO1762 | 1.2 ± 0.2 | 1.1 ± 0.2 | 1.7 ± 0.3 | 1.7 ± 0.1 | 1.4 ± 0.3 | 0.9 ± 0.1 |

[a]T5, T20 and T60 are the ratios of solar radiation-irradiated samples to the controls at 5 min, 20 min and 60 min, respectively. The data reported here was normalized using *uvrB* as internal control (Thellin et al. 1999). SD was calculated from eight data points which included four independent biological samples and two technical replicates for each biological sample. The strandard curve for *recA* was used to calculate the cDNA copies.

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# CHAPTER 5

# TRANSCRIPTOME ANALYSIS OF IONIZING RADIATION RESPONSE IN SHEWANELLA ONEIDENSIS MR-1

**Abstract**

I observed the induction of 237 genes and repression of 52 genes in *Shewanella oneidensis* MR-1 during a 1 h recovery period after gamma ray exposure. The genomic response of MR-1 to ionizing radiation is very similar to UVC, which included a strong SOS induction and the activation of prophages. I also observed the induction of genes encoding antioxidant enzymes and proteins, however, no induction was observed for genes encoding multidrug or heavy metal efflux pumps or for genes involved in production of toxin and antibiotic resistance as I observed for UVA. The different detoxifying strategies used by MR-1 indicate a distinct difference between oxidative stress induced by UVA and by ionizing radiation.

**Introduction**

Ionizing radiation is potentially lethal and mutagenic to all organisms. The cellular response to ionizing radiation is complex due to the complexity of the damage. Ionizing radiation can randomly damage all cellular components through direct deposit of radiation energy into target molecules. Meanwhile, it causes damage indirectly by generating a variety of reactive species, e.g. through radiolysis of water, which further damages a variety of molecules as well as biological processes in the cell (Rilay 1994; Tolence 1987).

The cytotoxic and mutagenic effects induced by ionizing radiation are thought to be primarily the result of DNA damage, which includes single strand breaks (SSB), double strand breaks (DSB), base modification, abasic sites and sugar modification (Goodhead 1994; Teoule 1987). Bacteria have evolved several mechanisms to remove gamma ray induced DNA lesions and restore the integrity of the genome. RecA mediated homologous recombinational repair is very important because it can repair DSBs, which are considered the most lethal effect due to the difficulty of their repair. Base excision repair (BER), which is involved in the sequential action of a DNA glycosylase and an AP (*apurinic/apyrimidinic*) endonuclease followed by repair synthesis of DNA and DNA ligation (Friedberg et al. 1995) is important in protecting DNA from oxidative damage. In addition, LexA-RecA mediated SOS response, a global cellular response to DNA damage involving the induction of more than 30 unlinked genes, nucleotide excision repair (NER), which is efficient in removing clustered DNA damage, and mismatch repair, particularly very short patch repair and MutY dependent mismatch repair play very important roles in bacterial survival following gamma ray irradiation (Friedberg et al. 1995; Teoule 1987).

Extensive studies have focused on *Deinococcus* radiodurans, an extremely radiation resistant bacterium. A RecA-independent, single stranded DNA annealing repair pathway was reported to be important in *D. radiodurans* to recover from a low dose of gamma ray exposure (Daly et al. 1996). Recently, Levin-Zaidman et al. proposed that an ATP-dependent ligase mediated non-homologous end-joining pathway (NHEJ) as well as the presence of unusual ring-like nucleoid conformation may facilitate the repair of DSBs in *D. radiodurans* (Levin-Zaidman et al. 2003).

The ionizing radiation-induced reactive oxygen species (ROS) include superoxide ($O_2^{-\cdot}$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($HO\cdot$), peroxyl ($ROO\cdot$) and alkoxyl ($RO\cdot$), of which the hydroxyl radical is the most reactive species and therefore the most hazardous. Bacteria have involved various mechanisms to defend against oxidative stress. For example, elimination of ROS by antioxidant enzymes, disruption of perpetuating chain reaction by cellular thiols or other antioxidants, sequestration of transition metals otherwise available for Fenton-like reactions and coordination of cellular processes such as inhibition of cell division and activation of degradative processes to replace damaged molecules (Riley 1994).

*Shewanella oneidensis* MR-1, an environmental Gamma proteobacterium, can reduce a variety of compounds including toxic metals and radionuclides (Myers and Nealson 1988; Middleton et al. 2003). Hence, it has great potential in use for bioremediation. Previous study indicates that MR-1 is sensitive to UV radiation and ionizing radiation. The molecular basis of this high sensitivity has been investigated for UV radiation. Here, I examined the genomic response to ionizing radiation in *S. oneidensis* MR-1 using a microarray containing almost 95% of total ORFs. By comparing

the major DNA repair pathways and mechanisms used to defend against oxidative stress induced by gamma ray to *E. coli* and to *D. radiodurans*, I hope to gain a better understanding of what are the important traits in determining the bacterial radiation resistance and sensitivity.

**Results and discussion**

**Survival of *S. oneidensis* MR-1 after ionizing radiation exposure.** MR-1 was grown in Davis medium with 15 mM lactate as carbon source to an $OD_{600}$ of about 0.2. The survival rate following gamma ray exposure was determined using a $^{60}Co$ gamma cell irradiation unit (J. L. Shepherd and Associates, San Fernando, CA). The CFU was determined on LB plates. A 20% survival rate was obtained at a dose of 40 Gy. The $D_{10}$ and $D_{37}$ of MR-1 was 49.7 and 33.1 Gy, respectively, which are more than two orders of magnitude lower than for *D. radiodurans* (Moseley 1983) and about 5 fold less than for *E. coli* (Smith 1976). This result is consistent with the high UV sensitivity of MR-1 reported previously (Qiu et al. 2004).

**Gene expression trend in response to ionizing radiation.** The gene expression profile was examined at 5, 20 and 60 min after irradiation with 40 Gy of gamma rays using a microarray containing approximately 95% of total ORFs. RNA extraction, labeling and hybridization were carried out as described previously (Qiu et al. 2004). Three independent culture and irradiation treatments were performed and served as biological replicates. At each time point, two technical replicates in hybridization (Dye-swap) were carried out for each biological replicate. Genes that showed a statistically significant change in gene expression ($P<0.05$) and a > 2-fold change in magnitude were

regarded as significant. The total number of differentially expressed genes increased during the 1 h recovery period. After 5 and 20 min, only 50-60 genes were differentially expressed whereas at 60 min, a total of 210 genes were induced and 47 genes were repressed (Figure 5.1). About 6% of the genome responded to ionizing radiation stress, which is higher than for UVC (4%) but lower than for UVA (8%). The gene expression trend following gamma ray exposure was very similar to that observed for UVC except that the increase in the total number of differentially expressed genes was slower from 5 min to 20 min for ionizing radiation.

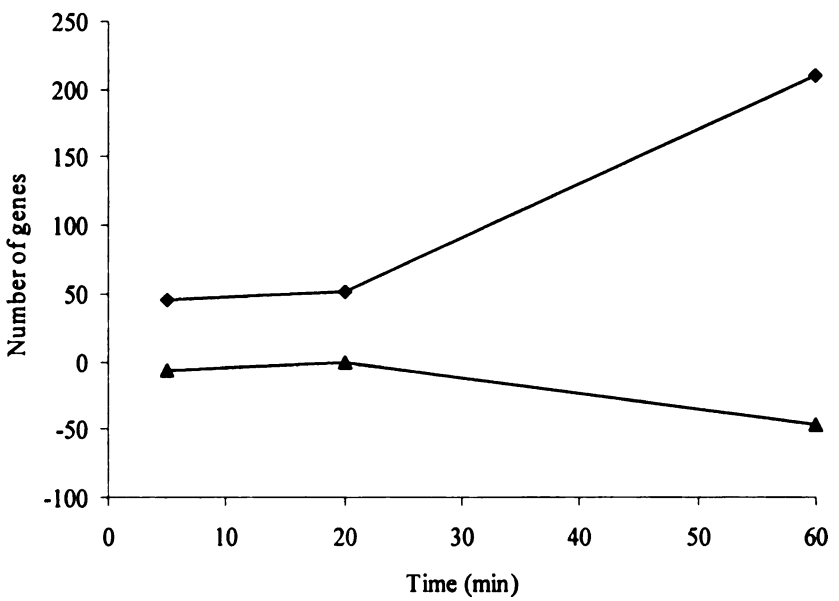


Figure 5.1. The global gene expression trend in response to ionizing radiation during a 1h recovering period after gamma ray exposure. Diamond (◆) represents up-regulated genes and triangle (▲) represents down-regulated genes. The positive number of Y axis represents the number of up-regulated genes where as the negative number indicates the number of down-regulated genes.

**Global gene expression profile in response to ionizing radiation.** Based on the TIGR annotation (Heidelberg et al. 2002), the total of 237 up-regulated genes were distributed into 16 functional categories (Figure 5.2). Similar to the global gene expression profile of UVC, the four largest groups were "hypothetical proteins" (37.6%), "conserved hypothetical proteins" (20.7%), "other categories" (11.0%), which mainly are prophage-related genes and transposases and "DNA metabolism" (8.0%). Unlike the UVC gene expression profile, more genes categorized in "cellular processes", "energy metabolism" and "transport and binding proteins" were induced, which is a characteristic of the UVA gene expression profile. Consistent with this trend, about 48% of the up-regulated genes in response to ionizing radiation were induced by UVC whereas only about 17% of the up-regulated genes in response to ionizing radiation were activated by UVA (Figure 5.3). Similar to UVC, ionizing radiation can cause DNA damage as well as activate of prophages in MR-1. In addition, ionizing radiation can cause oxidative stress in MR-1 as I observed for UVA previously (Chapter 3).

A total of 52 down-regulated genes were distributed into 13 functional categories (Figure 5.2). Similar to both UVC and UVA gene expression profiles, a great number of genes in the "hypothetical proteins" (19.2%), "conserved hypothetical proteins" (15.4%) and "energy metabolism" (13.5%) categories were repressed. However, more genes in "biosynthesis" (19.2%) were repressed than either UVC (5.5%) or UVA (3.4%) (Chapter 3), which was a characteristic of the gene expression profile in response to gamma rays in MR-1.

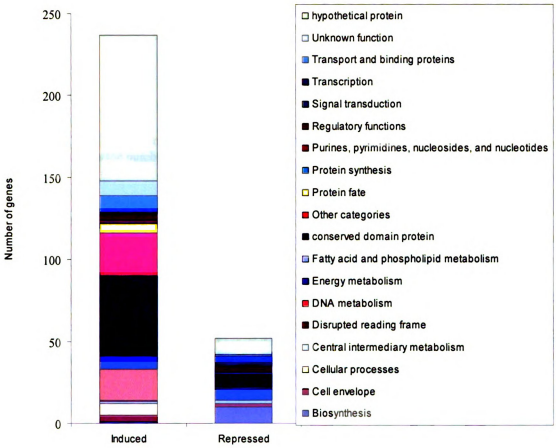


Figure 5.2. Distribution of the differentially expressed genes in various functional categories following gamma ray exposure. The total number of induced genes is 237 and of repressed genes is 52 (This figure is presented in color).

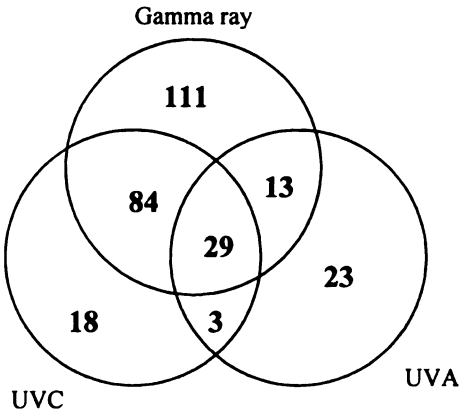


Figure 5.3. Venn diagram of up-regulated genes in response to ionizing radiation, UVC and UVA. The number represents the number of genes in each group.

**DNA damage repair capacity in *S. oneidensis* MR-1.** About 2.8% of the MR-1 genome is implicated in DNA replication, recombination and repair, which is comparable to that of *E. coli* (2.7%) (Blattner et al. 1997) and *D. radiodurans* (3.1%) (Makarova et al. 2001; White et al. 1999). Our previous studies indicated that photoreactivation, NER and the SOS response including mutagenic repair were functional in MR-1 (Qiu et al. 2004). Both RecBCD and RecF recombinational repair pathways are present in MR-1 (Table 1.1). In addition, MR-1 carries a NAD-dependent DNA ligase (*ligA*) and an ATP-dependent DNA ligase (SO2204), a complete methyl-directed mismatch repair pathway (*mutS*, *mutL*, *mutH*) and genes (*mutM*, *mutY* and *mutT*) that are important in preventing mutation due to the oxidized base 8-oxoG (Heidelberg et al. 2002). However, compared to *E. coli*, a few genes involved in DNA repair are absent in MR-1 such as genes encoding the components of adaptive response (*alkA*), for the components of very short patch mismatch repair such as *dcm* and *vsr*, and for the components of the RecE mediated recombinational repair such as *recE*, *recT* and *rus*. No putative genes of *dinI*, *dinD* or *dinJ*, which belong to the SOS regulon in *E. coli* were found in MR-1. In addition,

several genes (*mug*, *nfo*, *nfi*, *nei* and *xseB*) involved in base excision repair in *E. coli* are absent from the MR-1 genome (Table1.1).

**Induction of DNA damage repair genes after ionizing radiation exposure.** About 13.9% of genes in the DNA replication, recombination and repair category were induced in MR-1 during the 1 h recovery period following gamma ray irradiation (Supplemental Table 6.1). Similar to cellular response to UVC, a strong SOS induction was observed (Supplemental Table 6.1), which included the induction of *recA* (8.8 fold), *lexA* (12.7 fold), *recN* (13.7 fold), *recX* (8.0 fold), *dinP* (9.8 fold), *topB* (7.7 fold), *umuD* (15.6 fold), *umuC* (8.0 fold) and *recG* (2.4 fold). However, several genes that did not show any induction following UVC exposure were up-regulated after gamma ray irradiation such as *polB* (4.6 fold), *polA* (3.3 fold), *mutH* (2.4 fold), *dinG* (3.3 fold) and SO0690 (4.4 fold), which encodes a type II DNA modification methyltransferase. I also observed a slight induction of several genes that were involved in DNA replication such as *dnaG* (2.1 fold), *dnaB* (2.6 fold) and SO1817 (2.2 fold), which encodes a replication protein n. In addition, a slight induction of SO0393 (2.5 fold), which encodes a DNA binding protein Fis (Factor for inversion stimulation), was observed at 60 min. Fis is a site-specific DNA binding protein that can bend DNA and is involved in many site specific recombinations (Johnson et al. 1987). Fis can also act as a transcriptional activator, regulating the expression of many genes, including its own (Finkel and Johnson 1992). This result indicates that cellular response to gamma ray induced DNA damage is more complex and global than to UVC. It appeared that both SOS response and recombinational repair played important roles in repairing gamma ray induced DNA

damage. In contrast, I did not observe any induction of *phrB*, which may suggest that photoreactivation plays a minor role in repairing gamma ray induced DNA damage.

**Induction of prophage-related genes by ionizing radiation.** There are three prophages in the MR-1 genome (Heilderberg et al. 2002). Our previous study indicated that UVC can activate the lytic cycle of prophages, which contributes greatly to the high UVC sensitivity of MR-1. More prophage related genes were induced following exposure to gamma ray in MR-1. A total of 64, 26 and 28 ORFs of prophage LambdaSo, MuSo1 and MuSo2 were up-regulated, respectively (Table 5.1), which indicates that, similar to UVC, ionizing radiation can activate the lytic cycles of prophages in MR-1.

**Putative genes involved in defending against oxidative stress in MR-1.** MR-1 carries genes encoding for catalase, Fe-containing superoxide dismutase (FeSOD), glutathione peroxidase, glutathione S-transferase, organic hydroperoxide resistance protein and a Dps protein, which plays an important role in sequestration of iron and stabilizing DNA (Ilari et al. 2002). In addition, MR-1 carries genes encoding ferritin, an iron storage protein and antioxidant which is potentially important in defending against oxidative stress (Supplemental Table 5.2). MR-1 also carries a complete glutaredoxin system and a complete thioredoxin system (Supplemental Table 5.2), which are known hydrogen donors for ribonucleotide reductase (RNase), the essential enzyme for deoxyribonucleotide and DNA biosynthesis (Prieto-Alamo et al. 2000). Regarding regulatory genes, MR-1 has a putative *oxyR* (SO1328), but no homologs of SoxRS are found.

Table 5.1. Induction of prophage-related genes by ionizing radiation

| Prophage | [a]Length (bp) | Total No. of ORFs | Total No. of induced ORFs | Hypothetical ORF | | Conserved hypothetical ORF | | Others | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Total | Induced | Total | Induced | Total | Induced |
| LambdaSo | 51,857 | 75 | 64 | 38 | 32 | 16 | 16 | 21 | 16 |
| MuSo1 | 34,551 | 42 | 26 | 21 | 14 | 8 | 4 | 14 | 8 |
| MuSo2 | 35,666 | 53 | 28 | 20 | 16 | 17 | 5 | 16 | 7 |

[a]according to Heidelberg et al. 2002.

**Gamma ray induced oxidative stress response in MR-1.** Several genes that are directly involved in scavenging ROS were up-regulated in MR-1 during a 1 h recovery period following gamma ray irradiation, which included *katB* (12.6 fold), *katG*-1 (5.5 fold), *ahpFC* (3.9- 11.0 fold), *ccpA* (3.8 fold), *dps* (3.0 fold) and *gst* (3.6 fold) (Supplemental Table 5.2). In addition, SO1762, a putative transcriptional regulator that was induced almost 5-fold in MR-1 following UVA exposure, showed an induction of 3.6 fold at 5 min. Both *trxA* and *trxC* were induced slightly at 60 min (Supplemental Table 5.2). I also observed induction of some genes that are involved in degradative process such as SO2964 (25.0 fold), which encodes a ClpP protease, SO1115 (4.0 fold), which encodes an aminoacyl-histidine dipeptidase, SO4162 (3.8 fold), which encodes an ATP-dependent protease HslV, SO3577 (2.9 fold), which encodes a ClpB protein and SO4699 (2.9 fold), which encodes an oligopeptidase. This result indicates that MR-1 actively coordinated various biological processes to overcome gamma ray induced oxidative stress.

Although both UVA and ionizing radiation can induce the formation of ROS, of which hydroxyl radical is the major oxygen species of concern (Perk et al. 1990), I observed a distinct difference between the responses to oxidative stress induced by the two. For example, I did not observe any induction of genes encoding glutathione peroxidase (SO1563 and SO1773) following gamma ray irradiation: those were highly up-regulated in response to UVA. A similar case was observed for *ohr*, which encodes an organic hydroperoxide resistance protein. I did not observe any induction of genes encoding multidrug and heavy metal efflux pumps, genes involved in production and secretion of toxin and production of antibiotic resistance, and genes involved in cell

motility, all of which were induced in MR-1 following UVA exposure. The cellular response to gamma ray induced oxidative stress was much less global than for UVA. MR-1 appears using a different detoxifying strategy to defend against oxidative stress induced by gamma ray from what I observed previously for UVA-induced oxidative stress (Chapter 3).

**Validation of the gene expression profile by quantitative real time reverse transcription PCR (Q RT-PCR).** Q RT-PCR analysis was carried out for seven selected genes, which included highly induced genes (*recA* and *katB*), moderately induced genes (SO1762), slightly induced genes (*trxC*), slightly repressed gene (SO1490) and genes with no change in expression level (SO1924 and SOA0154) as described previously (Chapter 3). *ldhA* was used as internal control to normalize the difference in reverse transcription efficiency. A high correlation ($R^2$: 0.9115) was obtained between microarray analysis and Q RT-PCR analysis (Table 5.2). The induction fold measured by microarray hybridization for highly induced genes (*recA* and *katB*) were lower than that measued by Q RT-PCR. This trend is consistent with previous report (Yuen et al. 2002) and our previous analysis (Chapter3 and Chapter 4). Q RT-PCR assay confirmed that there was no induction of multidrug (SO1924) or heavy metal (SOA0154) efflux pump in MR-1 after exposure to gamma ray.

Table 5.2. Comparison of relative gene expression between microarray analysis and Q RT-PCR assay

| Gene | [a]T5 | | [a]T20 | | [a]T60 | |
|---|---|---|---|---|---|---|
| | Array | Q RT-PCR | Array | Q RT-PCR | Array | Q RT-PCR |
| *recA* | 7.0 ± 1.4 | 14.2 ± 5.8 | 9.0 ± 2.2 | 15.0 ± 3.1 | 6.8 ± 2.3 | 13.0 ± 2.4 |
| *katB* | 14.9 ± 9.6 | 236.5 ± 130.9 | 1.9 ± 1.2 | 2.7 ± 0.7 | 1.5 ± 0.9 | 2.1 ± 0.5 |
| SO1762 | 4.2 ± 2.4 | 9.4 ± 8.3 | 3.7 ± 1.6 | 5.0 ± 4.0 | 3.4 ± 2.1 | 3.7 ± 1.8 |
| *trxC* | 2.1 ± 0.8 | 3.1 ± 1.7 | 1.7 ± 0.2 | 2.3 ± 0.5 | 2.4± 0.4 | 4.3 ± 1.1 |
| SO1924 | 1.5 ± 0.3 | 2.3 ± 0.9 | 1.1 ± 0.1 | 1.4 ± 0.5 | 1.1 ± 0.2 | 1.1 ± 0.2 |
| SOA0154 | 0.8 ± 0.1 | 1.0 ± 0.2 | 1.1 ± 0.3 | 1.3 ± 0.6 | 1.1 ± 0.4 | 1.1 ± 0.2 |
| SO1490 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.9 ± 0.3 | 0.8 ± 0.2 | 0.5 ± 0.2 | 0.6 ± 0.2 |

[a]T5, T20 and T60 are the ratios of ionizing radiation-irradiated samples to the controls at 5 min, 20 min and 60 min, respectively. The data reported here was normalized using *ldhA* as internal control (Thellin et al. 1999). SD was calculated from six data points which included three independent biological samples and two technical replicates for each biological sample. The strandard curve for *recA* was used to calculate the cDNA copies. Primers are designed as described previously (Chapter 3) for quantification of genes SO1762 (F: 5'CCAAATCCCGTGGTGTACG3'; R: 5'CGCGCAGTAAGCGATTATCC3'), *trxC* (F: 5'CAGCCCCCATCGAGCTC3'; R: 5'CACCAACTGGCCCAAAAATC3') and SO1490 (F: 5'TAAAGGTATCGCCCTGGTGG3'; R: 5'CACTAACGGCAGTTGTGGCTTAG3').

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# CHAPTER 6

# SUMMARY AND FUTURE PERSPECTIVE

**Comparison of transcriptional profiles of MR-1 in response to five radiation stress conditions**

I delineated the genomic response of *Shewanella oneidensis* MR-1 to five environmentally relevant stress conditions: UVC, UVB, UVA, solar radiation and ionizing radiation at doses that yielded 20% survival rates. A total of 4.2-, 3.9-, 8.1-, 28.0-, and 5.9% of the MR-1 genome showed differential expression following UVC, UVB, UVA, natural solar light, and ionizing radiation exposure, respectively (Table 6.1). In all five treatments, both conserved hypothetical and hypothetical are the dominant gene groups that are differentially expressed (Figure 6.1 and Figure 6.2). The gene expression profile of MR-1 in response to ionizing radiation is more similar to that of UVC, which is characterized by a strong induction of the SOS response and of many prophage related genes, but with some oxidative stress response (Table 6.1). Genomic response to UVB is a combination of the UVC and UVA responses, which supports previous reports that photons of UVB wavelengths can damage DNA directly as well as can induce the formation of ROS species (He and Häder 2002). However, induction of heavy metal and multidrug efflux pumps in MR-1 following UVA exposure is a previously unknown phenotype for this stress although the traditional UVA-induced stress responses occur in MR-1 such as induction of antioxidant proteins and enzymes and activation of genes involved in degradative pathways. Consistent with natural solar UVR composition (95% of UVA and 5% of UVB), the genomic response of MR-1 to natural solar light is more similar to that of UVA but with more genes involved in detoxification induced (Table 6.1). In addition, the number of differentially expressed genes from most functional categories increased compared to either UVB or UVA or

their sum. "DNA metabolism" (similar to COGs L group which contains genes involved in DNA replication, repair and recombination) is the only category where the number of induced genes remained the same compared to UVA whereas "other category", which contains mainly transposases and prophage-related genes, is the only category where the number of up-regulated genes decreased compared to UVA. In addition, more genes were repressed than induced following solar light exposure, which is in contrast to any of the other four treatments (Figure 6.1 and Figure 6.2). The unique transcriptional profile of MR-1 in response to solar radiation suggests that natural solar light impacts MR-1 in a complicated way rather than a simple sum of UVA and UVB responses. Currently, the cellular response of MR-1 to solar light is under investigation by proteomics at The Environmental Molecular Sciences Laboratory at Pacific Northwest National Laboratory using the accurate mass tag (AMTs) technology as described previously for *D. radiodurans* (Lipton et al. 2002). The comparison of the transcriptional and proteomic profiles will enhance our understanding of this complicated biological process in MR-1, and especially aid in the understanding of the importance of the large number of hypothetical genes in this process.

Table 6.1. Summary of stress response in MR-1 following irradiation

| Stress | DNA damage | Oxidative stress | Induction of prophages genes | Genome response |
|---|---|---|---|---|
| UVC | [a]+++ | - | ++ | 4.2% |
| UVB | +++ | + | + | 3.9% |
| UVA | ++ | ++ | - | 8.0% |
| Solar radiation | + | +++ | - | 28.0% |
| Gamma ray | +++ | + | +++ | 5.9% |

[a]Number of + indicates the relative degree of stress response observed in MR-1, which was based on the number of induced genes and their induction fold.

Figure 6.1. Functional distribution of up-regulated genes in response to ionizing radiation, UVC, UVB, UVA and natural solar radiation (This figure is presented in color).

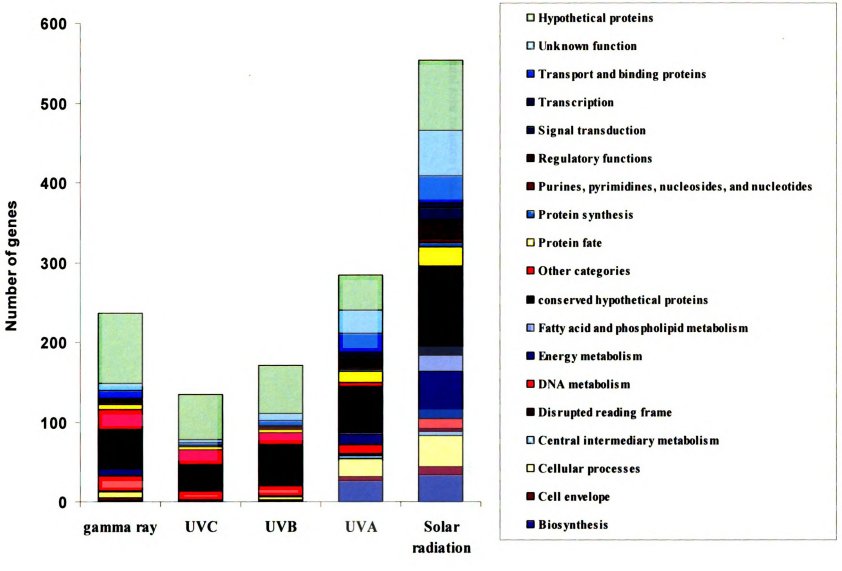
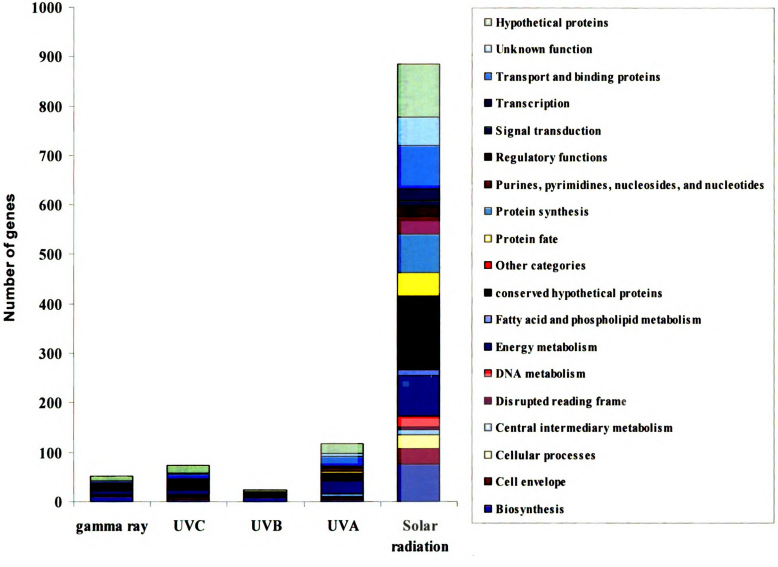

600
500
400
300
200
100
0
Number of genes
gamma ray
UVC
UVB
UVA
Solar radiation
Hypothetical proteins
Unknown function
Transport and binding proteins
Transcription
Signal transduction
Regulatory functions
Purines, pyrimidines, nucleosides, and nucleotides
Protein synthesis
Protein fate
Other categories
conserved hypothetical proteins
Fatty acid and phospholipid metabolism
Energy metabolism
DNA metabolism
Disrupted reading frame
Central intermediary metabolism
Cellular processes
Cell envelope
Biosynthesis

Figure 6.2. Functional distribution of down-regulated genes in response to ionizing radiation, UVC, UVB, UVA and natural solar radiation (This figure is presented in color).


1000
900
800
700
600
500
400
300
200
100
0
Number of genes
gamma ray
UVC
UVB
UVA
Solar radiation
Hypothetical proteins
Unknown function
Transport and binding proteins
Transcription
Signal transduction
Regulatory functions
Purines, pyrimidines, nucleosides, and nucleotides
Protein synthesis
Protein fate
Other categories
conserved hypothetical proteins
Fatty acid and phospholipid metabolism
Energy metabolism
DNA metabolism
Disrupted reading frame
Central intermediary metabolism
Cellular processes
Cell envelope
Biosynthesis

**Comparison of transcriptional profiles in response to UVC between *E. coli* and *S. oneidensis* MR-1**

Genomic response to UVC has been investigated in *E. coli* strain MG1655 (a K-12 strain having been cured of the temperate bacteriophage lambda and F plasmid) by Courcelle et al. (2001) under experimental conditions similar to mine. Although I observed similar responses to those found with MG1655 such as the induction of the SOS regulon, heat shock genes and transporter genes, I also observed a distinct difference in response to UVC stress. Among 134 UVC-induced genes in MR-1, 15 are located in the genome of prophage MuSo1, 16 are located in the genome of prophage MuSo2 and 56 are located in the genome of prophage LambdaSo, which accounts for 65% of total up-regulated genes in MR-1. In *E. coli* MG1655, however, among 165 up-regulated genes, only 6 are related to prophage (3.6%) (Courcelle et al. 2001).

Although the percentage of the genome dedicated to DNA replication, repair and recombination is very similar between *S. oneidensis* MR-1 (2.8%) and *E. coli* MG1655 (2.7%), the number of UVC induced genes is much higher in MG1655 than in MR-1 (Table 6.2). I only observed the induction for 8 out of 137 genes (5.8%) implicated in "DNA replication, repair and recombination" in MR-1 following UVC exposure whereas 18 out of 115 genes (15.7%) showed induction in MG1655. These data suggest that many DNA repair genes in MR-1 are losing their damage-inducibility. Alteration in some DNA repair gene content and regulation may be the consequence of lack of natural selection for this response in MR-1.

Table 6.2 Comparison of genome and the induction of DNA repair genes among *S. oneidensis* MR-1, *E. coli* MG1655 and *D. radiodurans* R1.

| Strain | Totoal ORF | Genes in GOGs L group (% of total) | Induced by UVC (% of L group) | Induced by gamma ray (% of L group) |
|---|---|---|---|---|
| [a]*S. oneidensis* MR-1 | 4931 | 137 (2.8%) | 8 (5.8%) | 19 (13.9%) |
| [b]*E. coli* MG1655 | 4288 | 115 (2.7%) | [d]18 (15.7%) | NA |
| [c]*D. Radiodurans* R1 | 3187 | 100 (3.1%) | NA | [e]22 (22%) |

[a]according to Heidelberg et al. 2002; [b]according to Blattner et al. 1997; [c]according to White et al. 1999. [d] according to Courcelle et al. 2001; [e]according to Liu et al. 2003.

**Comparison of transcriptional profiles in response to ionizing radiation between *D. radiodurans* and *S. oneidensis* MR-1**

The genomic response to ionizing radiation has been reported in the extremely radiation resistant strain *Deincoccus radiodurans* R1 during the 24 h of recovery period after exposure to 15 KGy of gamma rays (Liu et al. 2003). More than 40% of its genome showed differential expression with 832 genes induced and 451 genes repressed. This genomic response is much more global than in MR-1. However, the experimental condition for *D. radiodurans* R1 differed greatly from mine, a point to remember when comparing the transcriptional profiles between *D. radiodurans* R1 and MR-1. First, *D. radiodurans* R1 was grown in nutrient-rich medium (TGY) before irradiation whereas MR-1 was grown in Davis medium; after irradiation, the *D. radiodurans* R1 were diluted 20 fold using fresh TGY medium whereas MR-1 was returned to the shaker without changing the culture medium; the genomic response of *D. radiodurans* R1 was examined during a 24 h recovery period whereas the genomic response of MR-1 was examined during a 1 h recovery period; The *D. radiodurans* R1 experiment used a sample before

irradiation (T0) as the control whereas MR-1 experiment used a parellal control at each corresponding time point (Qiu et al. 2004).

To minimize the complication in comparison, I only looked at those genes that are annotated with a function in the DNA replication, repair and recombination category. Twenty two percent of genes implicated in "DNA replication, repair and recombination" showed induction in *D. radiodurans* R1 following irradiation with 15 KGy of gamma ray whereas only 19 out of 137 genes were induced in MR-1 (13.9%) (Table 6.2). Similar to *E. coli*, the NER component genes *uvrA*, *uvrB* and *uvrD* were induced in *D. radiodurans* R1 (Liu et al. 2003). This comparison supports my previous speculation that loss of DNA damage inducibility of some DNA repair genes may contribute to the high radiation sensitivity in MR-1.

Similar to the UVC-induced gene expression profile, almost 50% of gamma ray-induced genes in MR-1 are prophage related genes. A total of 64, 26 and 28 genes were induced from genomes of LambdaSo, MuSo1 and MuSo2, respectively. In contrast, only five phage-related genes showed induction in *D. radiodurans* R1 (Liu et al. 2003).

**Future perspective**

*S. oneidensis* MR-1 is one of most radiation sensitive bacteria now known. Activation of prophages in MR-1 by short wavelength UV radiation (UVC and UVB) and ionizing radiation appears to be the major lethal factor. Based on gene expression, the phage particles I observed by TEM is more like LambdaSo since the highest expression percentage was from the LambdaSo genome. Further identification and characterization of the phage particles may provide us with useful information. For example, will this

phage(s) infect other *Shewanella* strains? If it is or one of them is the LambdaSo, how much of LambdaSo is different from *E. coli* Lambda phage? What are the function of those 70% of unknown proteins (hypothetical and conserved hypothetical proteins) in the phage genome(s)? Prophages are found in most of sequenced bacteria. The impact of prophages on shaping of both bacterial and viral genomes is becoming more widely recognized (Canchaya et al. 2004). Currently, sequencing of other *Shewanella* strains is ongoing. Preliminary data suggest that there are no MR-1 like prophages present on the genomes of *S.* sp. PV-4, *S. denitrificans* OS220, *S. frigidimarina* NCIMB 400 and *S. putrefaciens* CN-32 (K. Konstantinidis pers. comm.). Thus, those strains are good candidates to address above questions.

The technology I used in this study could not resolve whether one or two or even all three prophages were activated under my experimental conditions. By deleting the prophage genome, one should be able to find which prophage(s) were actually being activated in my experimental conditions. For example, if both LambdaSo and MuSo1 were deleted and the phage particles were still seen in the UVC irradiated sample, then one could conclude that prophage Mu So2 can be activated by UVC.

I observed a good correlation between the UVC survival and natural solar radiation exposure among *Shewanella* strains tested for their UVC sensitivity, which may suggest that the lack of natural selection has impacted the DNA repair and damage tolerance mechanism in MR-1. But, what specifically is this impact? Comparison of *E. coli* K12 and *S. oneidensis* MR-1 genomes indicates that there is an alteration in DNA repair genes content as well as regulation in MR-1. For example, only a subset of the *E. coli* SOS regulon is damage inducible in MR-1. Similar cases were observed for OxyR,

which are very important in defending against oxidative stress in *E. coli*. Thus identification of those regulons will enhance our understanding of which genes in MR-1 might evolve faster. Furthermore, examination of those regulons in other strains that have similar evolution history as MR-1, e.g. strain DLM7, will provide additional evidence on whether alteration in gene content and regulation is the consequence of natural selection. Interestingly, the survival curves of MR-1 and DLM-7 are very similar (Figure 2.1).

By harboring prophages, MR-1 is extremely sensitive to UV radiation, which hinders the study of DNA repair and damage tolerance mechanisms. Isolation of a derivative strain of MR-1 that is cured of prophages would provide a valuable strain for such studies. The comparison of DNA repair and damage tolerance mechanisms between the MR-1 derivative strain (no prophages strain) with other strains for which one knows their natural habitat (evolution history) will be more comparable than using MR-1. If one constantly see similar traits in other non-radiation exposed strains as I saw in MR-1, it would be strong evidence that loss of genes and/or gene regulation is underway in lineages no longer exposed to these stresses.

Microarray based transcriptional profiling has become a powerful tool to examine the coordinate cellular response to perturbations. However, since the microarray method measures the steady state of mRNA, I was unable to see the dynamic picture following perturbation such as the stability of mRNA, changes of RNA synthesis and degradation rates, etc. No one should ever neglect the regulations that occurrs at those steps. Thus complementary approaches should always be used based upon the questions to be addressed.

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# APPENDIX A

# SUPPLEMENTAL TABLES

Supplemental Table 3.1. K-means analysis of up-regulated genes in response to UVC radiation

| SO Number | Cluster | Gene | Gene Product | Functional group | [a]Induction fold | | |
|---|---|---|---|---|---|---|---|
| | | | | | T5 | T20 | T60 |
| SO1751 | I | | membrane protein, putative | Cell envelope | 1.6 | 2.8 | 2.1 |
| SO3588 | I | | gpr1/fun34/yaaH family protein | - | 2.0 | 1.4 | 0.9 |
| SO4568 | I | *nrfD*-2 | formate-dependent nitrite reductase, nrfD protein | Central intermediary metabolism | 1.9 | 2.9 | 2.0 |
| SO2037 | I | | site-specific recombinase, phage integrase family | DNA metabolism | 1.5 | 1.8 | 2.4 |
| SO3061 | I | *topB* | DNA topoisomerase III | - | 4.0 | 7.7 | 9.5 |
| SO3429 | I | *recX* | regulatory protein RecX | - | 4.6 | 7.3 | 7.3 |
| SO3430 | I | *recA* | recA protein | - | 4.9 | 7.4 | 9.2 |
| SO3462 | I | *recN* | DNA repair protein RecN | - | 7.7 | 14.2 | 14.9 |
| SO4364 | I | *recG* | ATP-dependent DNA helicase RecG | - | 2.0 | 2.5 | 2.2 |
| SOA0012 | I | *umuC* | umuC protein | - | 3.4 | 7.7 | 8.1 |
| SOA0013 | I | *umuD* | umuD protein | - | 5.1 | 14.2 | 15.9 |
| SO1812 | I | *mdeA* | methionine gamma-lyase | Energy metabolism | 2.6 | 4.0 | 2.8 |
| SO0086 | I | | hypothetical protein | Unknown function | 1.0 | 2.6 | 1.1 |
| SO0089 | I | | hypothetical protein | - | 3.0 | 6.3 | 6.8 |
| SO1116 | I | | hypothetical protein | - | 1.2 | 2.3 | 2.2 |
| SO1753 | I | | hypothetical protein | - | 1.4 | 2.0 | 1.9 |
| SO1761 | I | | hypothetical protein | - | 2.5 | 3.0 | 2.1 |
| SO2038 | I | | hypothetical protein | - | 1.2 | 2.0 | 2.0 |
| SO3327 | I | | hypothetical protein | - | 1.8 | 4.5 | 4.4 |
| SO4363 | I | | hypothetical protein | - | 1.8 | 2.5 | 2.4 |
| SO4605 | I | | hypothetical protein | - | 5.5 | 7.4 | 6.2 |
| SO0179 | I | | conserved hypothetical protein | - | 3.3 | 2.6 | 1.9 |
| SO1757 | I | | conserved hypothetical protein | - | 13.3 | 19.9 | 16.4 |
| SO1759 | I | | conserved hypothetical protein | - | 9.3 | 10.2 | 6.6 |
| SO1810 | I | | conserved hypothetical protein | - | 1.9 | 2.2 | 1.5 |
| SO1816 | I | | conserved hypothetical protein | - | 6.4 | 27.7 | 14.8 |
| SO2603 | I | | conserved hypothetical protein | - | 3.2 | 5.0 | 5.7 |
| SO2604 | I | | conserved hypothetical protein | - | 3.7 | 6.1 | 6.7 |
| SO3849 | I | | conserved domain protein | - | 9.2 | 9.2 | 5.2 |
| SO4604 | I | | conserved hypothetical protein | - | 6.9 | 11.1 | 12.5 |
| SO3854 | I | | ISSo12, transposase | Other categories | 7.3 | 7.3 | 3.6 |
| SO1115 | I | *pepD* | aminoacyl-histidine dipeptidase | Protein fate | 1.4 | 2.1 | 2.3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO4603 | I | *lexA* | LexA repressor | Regulatory functions | 6.7 | 11.5 | 11.6 |
| SO1750 | I | | ABC transporter, ATP-binding protein | Transport and binding proteins | 1.8 | 3.5 | 2.7 |
| SO3060 | I | | outer membrane porin, putative | - | 1.4 | 2.4 | 2.9 |
| SO1114 | I | *dinP* | DNA-damage-inducible protein P | Unknown function | 3.6 | 6.6 | 6.1 |
| SO1813 | I | | DNA-binding protein, putative | - | 4.0 | 11.3 | 7.8 |
| SO1815 | I | | histone deacetylase/AcuC/AphA family protein | - | 4.8 | 13.5 | 7.5 |
| SO2260 | I | *suhB* | extragenic suppressor protein SuhB | - | 2.5 | 1.6 | 1.4 |
| SO2993 | II | | prophage LambdaSo, type II DNA modification methyltransferase, putative, truncation | DNA metabolism | 0.9 | 3.0 | 25.8 |
| SO3004 | II | | prophage LambdaSo, DNA modification methyltransferase, putative | - | 0.9 | 1.9 | 13.2 |
| SO4480 | II | *aldA* | aldehyde dehydrogenase | Energy metabolism | 1.2 | 1.2 | 2.3 |
| SO0645 | II | | hypothetical protein | Unknown function | ND | 2.0 | 10.5 |
| SO0646 | II | | hypothetical protein | - | 1.0 | 1.6 | 8.4 |
| SO0647 | II | | hypothetical protein | - | ND | 1.5 | 8.8 |
| SO0649 | II | | hypothetical protein | - | 0.6 | 1.6 | 8.3 |
| SO2656 | II | | hypothetical protein | - | 0.9 | 3.4 | 16.1 |
| SO2657 | II | | hypothetical protein | - | 1.1 | 2.5 | 11.5 |
| SO2658 | II | | hypothetical protein | - | 0.7 | 2.6 | 13.0 |
| SO2659 | II | | hypothetical protein | - | 0.7 | 2.4 | 10.3 |
| SO2661 | II | | hypothetical protein | - | 0.7 | 2.0 | 12.3 |
| SO2662 | II | | hypothetical protein | - | 1.4 | 2.5 | 7.7 |
| SO2664 | II | | hypothetical protein | - | 0.8 | 1.4 | 9.5 |
| SO2954 | II | | hypothetical protein | - | 1.3 | 1.4 | 3.1 |
| SO2983 | II | | hypothetical protein | - | 1.1 | 1.7 | 15.6 |
| SO2986 | II | | hypothetical protein | - | 1.1 | 1.7 | 11.3 |
| SO2987 | II | | hypothetical protein | - | 1.0 | 1.7 | 12.1 |
| SO2995 | II | | hypothetical protein | - | 1.2 | 2.3 | 18.7 |
| SO2996 | II | | hypothetical protein | - | 1.1 | 1.3 | 2.4 |
| SO2997 | II | | hypothetical protein | - | 0.9 | 2.5 | 17.0 |
| SO2998 | II | | hypothetical protein | - | 0.9 | 2.2 | 14.8 |
| SO2999 | II | | hypothetical protein | - | 0.9 | 1.4 | 8.6 |
| SO3001 | II | | hypothetical protein | - | 1.0 | 2.3 | 20.9 |
| SO3003 | II | | hypothetical protein | - | 1.0 | 2.1 | 22.5 |
| SO3133 | II | | hypothetical protein | - | 1.2 | 1.3 | 3.5 |
| SO0648 | II | | conserved hypothetical protein | - | 1.1 | 1.5 | 8.2 |
| SO0651 | II | | conserved hypothetical protein | - | 1.0 | 1.3 | 3.7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO2660 | II | | conserved hypothetical protein | - | 1.1 | 2.5 | 13.7 |
| SO2663 | II | | conserved hypothetical protein | - | 0.9 | 2.4 | 14.4 |
| SO2984 | II | | conserved hypothetical protein | - | 1.0 | 1.7 | 14.2 |
| SO2988 | II | | conserved hypothetical protein | - | 1.1 | 2.5 | 20.8 |
| SO3000 | II | | conserved hypothetical protein | - | 1.0 | 3.0 | 24.7 |
| SO3002 | II | | conserved hypothetical protein | - | 1.0 | 2.3 | 21.2 |
| SO3005 | II | | conserved hypothetical protein | - | 0.9 | 1.7 | 17.3 |
| SO0643 | II | | transposase, putative | Other categories | 1.0 | 2.1 | 8.2 |
| SO0644 | II | | prophage MuSo1, DNA transposition protein, putative | - | 0.3 | 2.1 | 13.3 |
| SO0652 | II | | prophage MuSo1, positive regulator of late transcription, putative | - | 0.9 | 1.4 | 6.0 |
| SO2654 | II | | transposase, putative | - | 0.5 | 2.2 | 8.5 |
| SO2655 | II | | prophage MuSo2, DNA transposition protein, putative | - | 0.9 | 3.7 | 16.8 |
| SO2985 | II | *O* | prophage LambdaSo, replication protein O | | 1.1 | 1.9 | 15.6 |
| SO2653 | II | | transcriptional regulator, Ner family | Regulatory functions | 1.0 | 2.4 | 10.1 |
| SO3006 | III | | prophage LambdaSo, type II DNA modification methyltransferase, putative | DNA metabolism | 1.0 | 1.3 | 11.4 |
| SO0678 | III | | hypothetical protein | Unknown function | 1.0 | 0.8 | 4.2 |
| SO0836 | III | | hypothetical protein | - | 0.7 | 1.1 | 3.2 |
| SO2343 | III | | hypothetical protein | - | 1.0 | 1.2 | 3.0 |
| SO2665 | III | | hypothetical protein | - | 0.8 | 1.3 | 8.7 |
| SO2666 | III | | hypothetical protein | - | 1.0 | 1.2 | 6.0 |
| SO2945 | III | | hypothetical protein | - | 1.0 | 0.8 | 2.8 |
| SO2946 | III | | hypothetical protein | - | 1.0 | 0.8 | 2.1 |
| SO2959 | III | | hypothetical protein | - | 0.9 | 1.1 | 3.5 |
| SO2962 | III | | hypothetical protein | - | 1.0 | 0.8 | 4.9 |
| SO2970 | III | | hypothetical protein | - | 1.2 | 1.0 | 6.6 |
| SO2972 | III | | hypothetical protein | - | 1.1 | 1.1 | 7.0 |
| SO2974 | III | | hypothetical protein | - | 1.1 | 0.9 | 9.6 |
| SO2977 | III | | hypothetical protein | - | 1.0 | 0.9 | 3.6 |
| SO2979 | III | | hypothetical protein | - | 1.0 | 1.2 | 5.6 |
| SO2980 | III | | hypothetical protein | - | 1.0 | 1.3 | 10.4 |
| SO2981 | III | | hypothetical protein | - | 0.2 | 1.2 | 10.2 |
| SO2982 | III | | hypothetical protein | - | 1.0 | 1.3 | 9.6 |
| SO3008 | III | | hypothetical protein | - | 0.5 | 1.3 | 10.5 |
| SO3009 | III | | hypothetical protein | - | 1.1 | 1.2 | 11.3 |
| SO3010 | III | | hypothetical protein | - | 0.8 | 1.1 | 9.5 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO3011 | III | | hypothetical protein | - | 0.8 | 1.4 | 12.9 |
| SO3012 | III | | hypothetical protein | - | 1.1 | 1.2 | 10.2 |
| SO0650 | III | | hypothetical protein | - | nd | 1.0 | 4.1 |
| SO0676 | III | | conserved hypothetical protein | - | 0.9 | 0.9 | 3.8 |
| SO0677 | III | | conserved hypothetical protein | - | 1.1 | 0.8 | 3.1 |
| SO2667 | III | | conserved hypothetical protein | - | 0.8 | 1.2 | 6.8 |
| SO2858 | III | | conserved hypothetical protein | - | 1.0 | 1.1 | 3.0 |
| SO2955 | III | | conserved hypothetical protein | - | 0.9 | 0.9 | 6.2 |
| SO2957 | III | | conserved hypothetical protein | - | nd | 1.1 | 3.1 |
| SO2958 | III | | conserved hypothetical protein | - | 1.1 | 1.1 | 3.7 |
| SO2960 | III | | conserved hypothetical protein | - | 1.0 | 0.9 | 4.7 |
| SO2961 | III | | conserved hypothetical protein | - | 1.0 | 1.1 | 4.0 |
| SO2967 | III | | conserved hypothetical protein | - | 1.1 | 1.0 | 4.1 |
| SO2968 | III | | conserved hypothetical protein | - | 1.2 | 1.0 | 3.0 |
| SO2971 | III | | conserved hypothetical protein | - | 1.1 | 1.0 | 4.9 |
| SO2976 | III | | conserved hypothetical protein | - | 1.0 | 0.9 | 6.3 |
| SO0674 | III | | prophage MuSo1, protein Gp32, putative | Other categories | 0.8 | 0.8 | 3.8 |
| SO0675 | III | | prophage MuSo1, major head subunit, putative | - | 0.9 | 0.7 | 4.5 |
| SO2668 | III | | prophage MuSo2, positive regulator of late transcription, putative | - | 1.0 | 0.9 | 2.6 |
| SO2941 | III | | prophage LambdaSo, tail assembly protein I | - | 0.9 | 0.9 | 2.2 |
| SO2952 | III | | prophage LambdaSo, minor tail protein M, putative | - | 0.9 | 1.1 | 2.1 |
| SO2956 | III | | prophage LambdaSo, major tail protein V, putative | - | 0.9 | 0.9 | 7.5 |
| SO2963 | III | | prophage LambdaSo, major capsid protein, HK97 family | - | 1.0 | 0.9 | 5.3 |
| SO2965 | III | | prophage LambdaSo, portal protein, HK97 family | - | 1.1 | 1.0 | 6.6 |
| SO2969 | III | | prophage LambdaSo, holin, putative | - | 1.2 | 0.9 | 5.7 |
| SO2973 | III | | prophage LambdaSo, lysozyme, putative | - | 1.2 | 0.9 | 7.7 |
| SO2978 | III | | prophage LambdaSo, site-specific recombinase, phage integrase family | - | 1.0 | 1.1 | 9.9 |
| SO2277 | III | *ibpA* | 16 kDa heat shock protein A | Protein fate | 1.1 | 1.1 | 2.3 |
| SO2964 | III | | ClpP protease family protein | - | 1.0 | 1.0 | 4.5 |
| SO4162 | III | *hslV* | ATP-dependent protease HslV | - | 1.1 | 0.9 | 2.0 |
| SO2857 | III | | sodium/solute symporter family protein | Transport and binding proteins | 1.0 | 1.1 | 3.3 |

[a] the average induction fold at each time point. The data were presented with 95% confidence. nd: no data.

Supplemental Table 3.2. K-means analysis of up-regulated genes in response to UVA radiation

| SO Number | Cluster | Gene | Gene Product | Functional group | [a]Induction fold | | |
|---|---|---|---|---|---|---|---|
| | | | | | T5 | T20 | T60 |
| SO1367 | I | *pheA* | chorismate mutase/prephenate dehydratase | Amino acid biosynthesis | 2.5 | 2.4 | 2.3 |
| SO2067 | I | *hisI* | phosphoribosyl-ATP pyrophosphatase/phosphoribosyl-AMP cyclohydrolase | - | 2.3 | 3.8 | 3.2 |
| SO2068 | I | *hisF* | hisF protein( cyclase) | - | 2.5 | 3.7 | 3.7 |
| SO2069 | I | *hisA* | phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase | - | 2.8 | 4.1 | 3.8 |
| SO2070 | I | *hisH* | amidotransferase HisH | - | 2.9 | 4.4 | 4.1 |
| SO2071 | I | *hisB* | imidazoleglycerol-phosphate dehydratase/histidinol-phosphatase | - | 3.5 | 4.9 | 4.2 |
| SO2072 | I | *hisC* | histidinol-phosphate aminotransferase | - | 3.3 | 4.5 | 3.6 |
| SO2073 | I | *hisD* | histidinol dehydrogenase | - | 3.9 | 5.3 | 4.0 |
| SO2074 | I | *hisG* | ATP phosphoribosyltransferase | - | 4.0 | 4.8 | 3.6 |
| SO3019 | I | *trpE* | anthranilate synthase component I | - | 2.1 | 2.2 | 1.5 |
| SO3020 | I | *trpG* | glutamine amido-transferase | - | 2.0 | 2.2 | 1.5 |
| SO3021 | I | *trpD* | anthranilate phosphoribosyltransferase | - | 1.6 | 2.2 | 1.4 |
| SO3022 | I | *trpC/F* | indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase | - | 1.5 | 2.1 | 1.4 |
| SO3023 | I | *trpB* | tryptophan synthase, beta subunit | | 1.4 | 2.1 | 1.3 |
| SO1820 | I | *polB* | DNA polymerase II | DNA metabolism | 2.4 | 1.8 | 1.5 |
| SO2037 | I | | site-specific recombinase, phage integrase family | - | 1.7 | 2.1 | 1.4 |
| SO2993 | I | | prophage LambdaSo, type II DNA modification methyltransferase, putative, truncation | - | 1.6 | 1.4 | 2.7 |
| SO3061 | I | *topB* | DNA topoisomerase III | - | 3.5 | 3.4 | 2.1 |
| SO3429 | I | *recX* | regulatory protein RecX | - | 3.4 | 3.3 | 2.1 |
| SO3430 | I | *recA* | recA protein | - | 3.5 | 3.5 | 2.2 |
| SO3462 | I | *recN* | DNA repair protein RecN | - | 6.7 | 6.3 | 3.7 |
| SOA0012 | I | *umuC* | umuC protein | - | 2.6 | 3.2 | 2.0 |
| SOA0013 | I | *umuD* | umuD protein | - | 4.6 | 4.1 | 3.9 |
| SO2824 | I | | carbon starvation protein A, authentic point mutation | Energy metabolism | 1.4 | 2.4 | 1.7 |
| SO4480 | I | *aldA* | aldehyde dehydrogenase | - | 6.8 | 5.9 | 3.5 |
| SO1761 | I | | hypothetical protein | Unknown function | 3.7 | 3.6 | 3.9 |
| SO2983 | I | | hypothetical protein | - | 1.3 | 1.1 | 2.1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO4481 | I | | hypothetical protein | - | 7.1 | 3.2 | 2.6 |
| SO4605 | I | | hypothetical protein | - | 2.6 | 3.2 | 2.2 |
| SO2469 | I | | conserved hypothetical protein | - | 2.1 | 1.8 | 1.9 |
| SO0089 | I | | hypothetical protein | - | 2.1 | 2.1 | 1.6 |
| SO0258 | I | | hypothetical protein | - | 1.4 | 1.3 | 2.1 |
| SO1757 | I | | conserved hypothetical protein | - | 22.1 | 28.4 | 13.3 |
| SO1758 | I | | conserved hypothetical protein | - | 11.1 | 11.7 | 7.0 |
| SO1759 | I | | conserved hypothetical protein | - | 10.6 | 7.7 | 3.9 |
| SO2603 | I | | conserved hypothetical protein | - | 2.7 | 2.4 | 1.6 |
| SO2604 | I | | conserved hypothetical protein | - | 3.5 | 3.0 | 2.0 |
| SO2988 | I | | conserved hypothetical protein | - | 1.1 | 1.4 | 2.1 |
| SO3386 | I | | conserved hypothetical protein | - | 28.1 | 15.1 | 5.3 |
| SO4604 | I | | conserved hypothetical protein | - | 6.9 | 6.9 | 3.7 |
| SO2985 | I | *O* | prophage LambdaSo, replication protein O | Other categories | 1.2 | 1.3 | 2.1 |
| SO3422 | I | *yfiA*-2 | ribosomal subunit interface protein | Protein synthesis | 2.6 | 2.0 | 1.6 |
| SO2415 | I | *nrdA* | ribonucleoside-diphosphate reductase, alpha subunit | Purines, pyrimidines, nucleosides, and nucleotides | 1.9 | 2.0 | 1.3 |
| SO3385 | I | | transcriptional regulator, MerR family | Regulatory functions | 20.5 | 12.1 | 3.5 |
| SO4603 | I | *lexA* | LexA repressor | - | 5.3 | 5.9 | 3.3 |
| SO1760 | I | | AzlC family protein | Transport and binding proteins | 7.4 | 4.7 | 2.9 |
| SO1114 | I | *dinP* | DNA-damage-inducible protein P | Unknown function | 4.1 | 4.5 | 2.9 |
| SO1756 | I | | glyoxalase family protein | - | 20.7 | 18.3 | 12.2 |
| SO0613 | II | *pabA* | para-aminobenzoate synthase glutamine amidotransferase, component II | Biosynthesis of cofactors, prosthetic groups, and carriers | 2.2 | 1.0 | 1.1 |
| SO1038 | II | *cobQ* | cobyric acid synthase CobQ | - | 3.8 | 1.3 | 1.0 |
| SO1039 | II | *cobO* | cob(I)alamin adenosyltransferase | - | 3.7 | 1.4 | 1.1 |
| SO1667 | II | | pterin-4-alpha-carbinolamine dehydratase | - | 2.3 | 1.3 | 1.1 |
| SO3559 | II | *gshA* | glutamate--cysteine ligase | - | 2.9 | 1.6 | 1.1 |
| SO0854 | II | | type IV pilin, putative | Cell envelope | 2.0 | 1.0 | 0.9 |
| SO3197 | II | | vacJ lipoprotein, putative | - | 6.0 | 1.7 | 1.1 |
| SO3948 | II | *murA* | UDP-N-acetylglucosamine 1-carboxyvinyltransferase | - | 2.6 | 1.3 | 1.1 |
| SO4636 | II | | lipoprotein, putative | - | 2.5 | 1.3 | 1.1 |
| SO0725 | II | *katG*-1 | catalase/peroxidase HPI | Cellular processes | 3.8 | 1.4 | 1.1 |
| SO0958 | II | *ahpC* | alkyl hydroperoxide reductase, C subunit | - | 4.3 | 1.7 | 1.2 |
| SO0976 | II | *ohr* | organic hydroperoxide resistance protein | - | 8.7 | 1.5 | 0.8 |
| SO1158 | II | | Dps family protein | - | 3.6 | 1.8 | 1.1 |
| SO1354 | II | | hemolysin protein, putative | - | 2.6 | 1.2 | 1.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO1563 | II | | glutathione peroxidase, putative | - | 4.7 | 1.0 | 0.9 |
| SO1989 | II | *cheV*-1 | chemotaxis protein CheV | - | 5.5 | 2.2 | 1.1 |
| SO2178 | II | *ccpA* | cytochrome c551 peroxidase | - | 2.8 | 1.3 | 1.0 |
| SO3247 | II | *flgE* | flagellar hook protein FlgE | - | 2.8 | 1.3 | 1.3 |
| SO3248 | II | *flgD* | basal-body rod modification protein FlgD | - | 6.1 | 1.3 | 1.2 |
| SO3282 | II | | methyl-accepting chemotaxis protein | - | 2.4 | 1.3 | 0.8 |
| SO4149 | II | | RTX toxin, putative | - | 2.0 | 1.3 | 1.0 |
| SO4299 | II | *cat* | chloramphenicol acetyltransferase | - | 2.8 | 0.9 | 0.9 |
| SO4328 | II | | AcrB/AcrD/AcrF family protein, authentic frameshift | - | 10.2 | 3.0 | 0.9 |
| SO4466 | II | | methyl-accepting chemotaxis protein | - | 3.4 | 1.1 | 1.0 |
| SO0485 | II | *nosL* | nosL protein | Central intermediary metabolism | 2.1 | 1.1 | 1.0 |
| SO0560 | II | *fhs* | formate--tetrahydrofolate ligase | - | 3.1 | 1.7 | 1.1 |
| SO2044 | II | *gloA* | lactoylglutathione lyase | - | 4.8 | 1.3 | 1.0 |
| SO3240 | II | | flagellar hook-associated protein FlgK, degenerate | Disrupted reading frame | 2.5 | 1.2 | 1.1 |
| SO0601 | II | *mutL* | DNA mismatch repair protein MutL | DNA metabolism | 2.1 | 1.2 | 1.1 |
| SOA0045 | II | | site-specific recombinase, phage integrase family | - | 3.4 | 1.6 | 1.0 |
| SO0102 | II | *fdnH* | formate dehydrogenase, nitrate-inducible, iron-sulfur subunit | Energy metabolism | 2.2 | 1.2 | 1.3 |
| SO0103 | II | *fdnI* | formate dehydrogenase, nitrate-inducible, cytochrome b556 subunit | - | 2.1 | 1.2 | 1.3 |
| SO0104 | II | *fdhE* | fdhE protein | - | 2.1 | 1.3 | 1.3 |
| SO0397 | II | *fdrC* | fumarate reductase cytochrome B subunit | - | 2.4 | 1.2 | 1.1 |
| SO0398 | II | *frdA* | fumarate reductase flavoprotein subunit | - | 2.6 | 1.5 | 1.2 |
| SO0970 | II | | fumarate reductase flavoprotein subunit precursor | - | 2.9 | 1.2 | 1.2 |
| SO1430 | II | *dmsB*-1 | anaerobic dimethyl sulfoxide reductase, B subunit | - | 2.4 | 1.1 | 1.1 |
| SO4404 | II | | iron-sulfur cluster-binding protein | - | 2.2 | 1.3 | 1.2 |
| SO4515 | II | | formate dehydrogenase, C subunit, putative | - | 2.8 | 1.4 | 1.2 |
| SO0590 | II | *psd* | phosphatidylserine decarboxylase, authentic frameshift | Fatty acid and phospholipid metabolism | 2.3 | 1.2 | 1.0 |
| SO3072 | II | *fabB* | 3-oxoacyl-(acyl-carrier-protein) synthase I | - | 2.4 | 1.2 | 0.9 |
| SO0090 | II | | hypothetical protein | Unknown function | 2.7 | 1.5 | 1.2 |
| SO0338 | II | | hypothetical protein | - | 2.2 | 1.2 | 1.1 |
| SO0975 | II | | hypothetical protein | - | 2.5 | 1.5 | 1.1 |
| SO1432 | II | | hypothetical protein | - | 2.2 | 1.3 | 1.3 |
| SO1702 | II | | hypothetical protein | - | 3.4 | 1.4 | 1.1 |
| SO1837 | II | | hypothetical protein | - | 2.6 | 1.0 | 1.1 |
| SO1983 | II | | hypothetical protein | - | 3.5 | 1.6 | 1.4 |

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|---|---|---|---|---|---|---|
| SO1984 | II | hypothetical protein | - | 3.2 | 1.7 | 1.5 |
| SO1990 | II | hypothetical protein | - | 3.5 | 1.4 | 1.1 |
| SO2010 | II | hypothetical protein | - | 2.8 | 1.4 | nd |
| SO2773 | II | hypothetical protein | - | 4.1 | 1.3 | 1.1 |
| SO2944 | II | hypothetical protein | - | 2.9 | 1.1 | 1.1 |
| SO3198 | II | hypothetical protein | - | 4.0 | 1.6 | 1.0 |
| SO3275 | II | hypothetical protein | - | 8.7 | 2.3 | 1.1 |
| SO3327 | II | hypothetical protein | - | 3.6 | 1.5 | 1.7 |
| SO3558 | II | hypothetical protein | - | 3.0 | 1.5 | 1.1 |
| SO3643 | II | hypothetical protein | - | 3.0 | 1.6 | 1.2 |
| SO3658 | II | hypothetical protein | - | 3.2 | 1.2 | 1.1 |
| SO3777 | II | hypothetical protein | - | 2.7 | 1.3 | 1.0 |
| SO4167 | II | hypothetical protein | - | 2.5 | 1.4 | 1.0 |
| SO4171 | II | hypothetical protein | - | 5.4 | 1.8 | 0.9 |
| SO4464 | II | hypothetical protein | - | 2.2 | 1.1 | 1.0 |
| SO4482 | II | hypothetical protein | - | 2.2 | 1.4 | 1.5 |
| SO4592 | II | hypothetical protein | - | 3.8 | 1.8 | 1.2 |
| SO4593 | II | hypothetical protein | - | 3.3 | 1.7 | 1.2 |
| SO4643 | II | hypothetical protein | - | 2.5 | 1.2 | 0.9 |
| SO4694 | II | hypothetical protein | - | 7.0 | 2.6 | 1.2 |
| SO4695 | II | hypothetical protein | - | 7.3 | 2.0 | 1.3 |
| SO4738 | II | hypothetical protein | - | 2.4 | 1.2 | 1.5 |
| SO0316 | II | conserved hypothetical protein TIGR00481 | - | 3.9 | 1.6 | 1.2 |
| SO0334 | II | conserved hypothetical protein | - | 2.6 | 1.5 | 1.1 |
| SO0335 | II | conserved hypothetical protein | - | 2.6 | 1.4 | 1.1 |
| SO0336 | II | conserved hypothetical protein | - | 2.3 | 1.5 | 1.3 |
| SO0855 | II | conserved hypothetical protein | - | 5.1 | 1.8 | 0.8 |
| SO0856 | II | conserved hypothetical protein | - | 6.5 | 1.7 | 0.9 |
| SO1379 | II | conserved hypothetical protein | - | 2.0 | 1.1 | 1.1 |
| SO1380 | II | conserved hypothetical protein | - | 4.4 | 1.3 | 1.0 |
| SO1381 | II | conserved hypothetical protein | - | 10.0 | 1.6 | 1.4 |
| SO2008 | II | conserved hypothetical protein | - | 8.3 | 2.6 | 1.2 |
| SO2009 | II | conserved hypothetical protein | - | 9.2 | 2.0 | 1.1 |
| SO2043 | II | conserved hypothetical protein | - | 12.3 | 3.1 | 1.2 |
| SO2697 | II | conserved hypothetical protein | - | 2.3 | 1.2 | 1.1 |
| SO2710 | II | conserved hypothetical protein | - | 3.5 | 1.2 | 1.1 |
| SO2922 | II | conserved hypothetical protein | - | 7.3 | 2.3 | 1.1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO3073 | II | | conserved hypothetical protein | - | 6.7 | 1.8 | 1.2 |
| SO3097 | II | | conserved hypothetical protein | - | 7.3 | 2.0 | 1.3 |
| SO3274 | II | | conserved hypothetical protein | - | 4.7 | 1.5 | 1.1 |
| SO3319 | II | | conserved hypothetical protein | - | 4.0 | 1.8 | 1.1 |
| SO3346 | II | | conserved hypothetical protein | - | 2.6 | 1.3 | 1.1 |
| SO3370 | II | | conserved hypothetical protein | - | 3.8 | 1.0 | 0.9 |
| SO3557 | II | | conserved hypothetical protein | - | 2.1 | 1.2 | 1.1 |
| SO3776 | II | | conserved hypothetical protein | - | 2.9 | 1.3 | 1.0 |
| SO3951 | II | | conserved hypothetical protein | - | 2.2 | 1.2 | 1.0 |
| SO3953 | II | | conserved hypothetical protein TIGR00056 | - | 2.8 | 1.3 | 1.0 |
| SO4329 | II | | conserved hypothetical protein | - | 5.8 | 2.0 | 0.9 |
| SO4641 | II | | conserved hypothetical protein | - | 2.5 | 1.2 | 1.1 |
| SO4642 | II | | conserved hypothetical protein | - | 2.4 | 1.2 | 1.0 |
| SO0644 | II | | prophage MuSo1, DNA transposition protein, putative | Other categories | 2.2 | 1.1 | 1.3 |
| SO2699 | II | | prophage MuSo2, 43 kDa tail protein, putative | - | 2.0 | 1.2 | 1.1 |
| SO3854 | II | | ISSo12, transposase | - | 2.3 | 1.5 | 1.1 |
| SO4533 | II | | ISSo10, transposase OrfA | - | 2.1 | 1.1 | 1.1 |
| SO0605 | II | *hflK* | hflK protein | Protein fate | 2.6 | 1.4 | 1.0 |
| SO0606 | II | *hflC* | hflC protein | - | 2.4 | 1.3 | 0.9 |
| SO0696 | II | *dsbD* | thiol:disulfide interchange protein DsbD | - | 4.8 | 1.8 | 1.1 |
| SO0951 | II | *dsbC* | thiol:disulfide interchange protein DsbC | - | 4.7 | 1.6 | 1.2 |
| SO1796 | II | *lon* | ATP-dependent protease La | - | 4.1 | 2.0 | 1.1 |
| SO3302 | II | | serine protease, subtilase family | - | 2.9 | 1.4 | 1.1 |
| SO0602 | II | *miaA* | tRNA delta(2)-isopentenylpyrophosphate transferase | Protein synthesis | 3.9 | 1.6 | 1.1 |
| SO0317 | II | | transcriptional regulator, AraC/XylS family | Regulatory functions | 2.4 | 1.4 | 1.1 |
| SO0502 | II | | transcriptional regulator, ArsR family | - | 2.2 | 1.2 | 1.0 |
| SO0603 | II | *hfq* | host factor-I protein | - | 2.9 | 1.3 | 1.0 |
| SO1578 | II | | transcriptional regulator, TetR family | - | 2.5 | 1.1 | 1.0 |
| SO1762 | II | | transcriptional regulator, AraC/XylS family | - | 4.9 | 2.0 | 1.5 |
| SO1878 | II | | glycine cleavage system transcriptional repressor, putative | - | 2.4 | 1.1 | 1.1 |
| SO3778 | II | | adenylate cyclase CyaB, putative | - | 4.6 | 1.8 | 1.1 |
| SO4326 | II | | transcriptional regulator, TetR family | - | 11.1 | 2.5 | 1.0 |
| SO0860 | II | | response regulator | Signal transduction | 4.2 | 1.3 | 0.9 |
| SO3196 | II | | response regulator | - | 4.8 | 1.8 | 1.2 |
| SO1284 | II | *rpoD* | RNA polymerase sigma-70 factor | Transcription | 2.2 | 1.3 | 1.0 |
| SO3096 | II | | RNA polymerase sigma-70 factor, ECF subfamily | - | 6.3 | 1.8 | 1.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO4599 | II | | ribonuclease, T2 family | - | 3.4 | 1.2 | 1.0 |
| SO0486 | II | *nosD* | copper ABC transporter, periplasmic copper-binding protein | Transport and binding proteins | 2.5 | 1.5 | 1.1 |
| SO0525 | II | | drug resistance transporter, EmrB/QacA family protein | - | 4.5 | 1.6 | 1.4 |
| SO0857 | II | | ABC transporter, ATP-binding protein | - | 10.9 | 3.0 | 1.1 |
| SO1923 | II | | AcrB/AcrD/AcrF family protein | - | 7.8 | 2.7 | 0.9 |
| SO1924 | II | | AcrB/AcrD/AcrF family protein | - | 10.2 | 3.1 | 1.1 |
| SO1925 | II | | HlyD family secretion protein | - | 5.4 | 1.7 | 1.2 |
| SO3098 | II | | sodium/proline symporter, degenerate | - | 4.7 | 1.6 | 1.6 |
| SO3779 | II | *cydC* | ABC transporter, ATP-binding protein CydC | - | 5.4 | 2.0 | 1.1 |
| SO3780 | II | *cydD* | ABC transporter, ATP-binding protein CydD | - | 5.7 | 2.3 | 1.2 |
| SO4148 | II | | HlyD family secretion protein | - | 4.9 | 2.0 | 0.9 |
| SO0589 | II | | integral membrane domain protein, authentic frameshift | Unknown function | 2.2 | 1.1 | 1.0 |
| SO0604 | II | *hflX* | GTP-binding protein HflX | - | 2.6 | 1.4 | 1.0 |
| SO0628 | II | | GGDEF domain protein | - | 3.5 | 1.3 | 1.0 |
| SO0697 | II | *cutA* | periplasmic divalent cation tolerance protein CutA | - | 4.5 | 1.3 | 1.1 |
| SO0841 | II | | GGDEF domain protein | - | 2.2 | 1.1 | 0.9 |
| SO0895 | II | | pirin family protein | - | 2.7 | 1.5 | 1.3 |
| SO1502 | II | | cobalamin synthesis protein/P47K family protein | - | 2.1 | 1.0 | 1.0 |
| SO1876 | II | | NodD transcription activator-related protein | - | 2.2 | 1.1 | 0.9 |
| SO1911 | II | | oxidoreductase, short chain dehydrogenase/reductase family | - | 2.1 | 1.3 | 1.2 |
| SO1975 | II | | Zinc carboxypeptidase-related protein | - | 3.1 | 1.7 | 1.2 |
| SO1976 | II | | hydrolase, alpha/beta fold family | - | 3.2 | 1.7 | 1.3 |
| SO2492 | II | | oxidoreductase, acyl-CoA dehydrogenase family | - | 5.9 | 2.2 | 1.1 |
| SO2607 | II | | spore maturation protein A-related protein | - | 2.3 | 1.2 | 0.9 |
| SO2708 | II | | nitroreductase family protein | - | 2.0 | 1.0 | 1.1 |
| SO3303 | II | | chitinase domain protein | - | 5.4 | 1.8 | 1.2 |
| SO3949 | II | | BolA/YrbA family protein | - | 2.2 | 1.0 | 1.1 |
| SO3950 | II | | SpoIIAA family protein | - | 2.5 | 1.1 | 1.1 |
| SO3952 | II | | mce-related protein | - | 2.7 | 1.3 | 1.1 |
| SO4039 | II | | hydrolase, haloacid dehalogenase-like family | - | 9.8 | 2.6 | 1.2 |
| SO4040 | II | | integral membrane domain protein | - | 8.2 | 1.9 | 1.3 |
| SO4327 | II | | HlyD family secretion domain protein | - | 10.0 | 2.7 | 1.0 |
| SO3348 | III | *hemH-2* | ferrochelatase | Biosynthesis of cofactors, prosthetic groups, and carriers | 10.4 | 5.8 | 1.1 |
| SO0300 | III | | lipoprotein, putative | Cell envelope | 5.1 | 2.4 | 1.7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO0837 | III | | beta-lactamase, putative | Cellular processes | 2.1 | 1.6 | 1.2 |
| SO1773 | III | | catalase-related protein, authentic point mutation | - | 8.0 | 3.1 | 1.0 |
| SO3241 | III | *flgJ* | flagellar protein FlgJ | - | 2.1 | 1.5 | 1.0 |
| SO3349 | III | | glutathione peroxidase, putative | - | 11.1 | 5.6 | 1.4 |
| SO4170 | III | | C-factor, putative | - | 13.3 | 8.5 | 1.2 |
| SO0929 | III | *metK* | S-adenosylmethionine synthetase | Central intermediary metabolism | 2.0 | 1.7 | 0.7 |
| SOA0092 | III | | transposase, IS3 family, interruption | Disrupted reading frame | 2.1 | 1.6 | 1.0 |
| SO3384 | III | *phrB* | deoxyribodipyrimidine photolyase | DNA metabolism | 19.9 | 11.3 | 2.1 |
| SO2098 | III | *hyaB* | quinone-reactive Ni/Fe hydrogenase, large subunit | Energy metabolism | 2.2 | 1.6 | 1.2 |
| SO3379 | III | *cfa* | cyclopropane-fatty-acyl-phospholipid synthase | Fatty acid and phospholipid metabolism | 9.5 | 10.6 | 0.7 |
| SO2873 | III | | hypothetical protein | Unknown function | 2.7 | 2.0 | 1.1 |
| SO3276 | III | | hypothetical protein | - | 15.3 | 4.6 | 1.1 |
| SO4168 | III | | hypothetical protein | - | 8.4 | 4.4 | 1.1 |
| SO4338 | III | | hypothetical protein | - | 2.2 | nd | 1.1 |
| SO4594 | III | | hypothetical protein | - | 2.8 | 2.4 | 1.5 |
| SO0771 | III | | conserved hypothetical protein | - | 13.0 | 3.9 | 1.7 |
| SO1770 | III | | glycerate kinase, putative | - | 2.1 | 1.8 | 1.1 |
| SO1988 | III | | conserved hypothetical protein | - | 15.8 | 7.3 | 1.5 |
| SO2007 | III | | conserved hypothetical protein | - | 10.4 | 3.7 | 1.3 |
| SO2042 | III | | conserved hypothetical protein | - | 13.9 | 3.8 | 1.4 |
| SO3374 | III | | conserved hypothetical protein | - | 4.5 | 2.7 | 1.3 |
| SO3376 | III | | conserved hypothetical protein | - | 3.9 | 7.4 | 0.7 |
| SO3377 | III | | conserved hypothetical protein | - | 5.3 | 8.8 | 0.8 |
| SO3378 | III | | conserved hypothetical protein | - | 6.1 | 10.5 | 0.6 |
| SO3380 | III | | conserved hypothetical protein | - | 9.0 | 9.2 | 0.8 |
| SO3381 | III | | conserved hypothetical protein | - | 15.2 | 10.8 | 1.1 |
| SO3849 | III | | conserved domain protein | - | 7.0 | 4.8 | 1.7 |
| SO4169 | III | | conserved hypothetical protein | - | 17.8 | 8.8 | 1.3 |
| SO4465 | III | | conserved domain protein | - | 12.6 | 4.9 | 1.0 |
| SO4696 | III | | conserved hypothetical protein | - | 7.0 | 3.9 | 1.4 |
| SOA0155 | III | | conserved domain protein | - | 6.0 | 3.2 | 1.7 |
| SO1193 | III | *secD*-1 | protein-export membrane protein SecD | Protein fate | 5.5 | 2.6 | 1.7 |
| SO1194 | III | *secF*-1 | protein-export membrane protein SecF | - | 5.8 | 2.5 | 1.8 |
| SO1987 | III | | ATP-dependent protease La (LON) domain protein | - | 11.2 | 7.0 | 1.3 |
| SO2588 | III | | protein-methionine-S-oxide reductase, PilB family | - | 5.3 | 2.4 | 1.3 |
| SO3403 | III | *yfiA*-1 | ribosomal subunit interface protein | Protein synthesis | 1.7 | 2.1 | 1.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO2834 | III | *nrdD* | anaerobic ribonucleoside-triphosphate reductase | Purines, pyrimidines, nucleosides, and nucleotides | 4.1 | 2.3 | 1.7 |
| SO1985 | III | | transcriptional activator, putative | Regulatory functions | 10.5 | 6.3 | 0.9 |
| SO2366 | III | | response regulator | Signal transduction | 9.9 | 3.4 | 1.2 |
| SO1986 | III | | RNA polymerase sigma-70 factor, ECF subfamily | Transcription | 13.8 | 7.5 | 1.2 |
| SO1689 | III | | cation transport ATPase, E1-E2 family | Transport and binding proteins | 2.1 | 1.7 | 1.2 |
| SO1771 | III | | permease, GntP family | - | 3.2 | 1.8 | 1.2 |
| SO4597 | III | | heavy metal efflux system protein, putative | - | 6.4 | 3.3 | 1.3 |
| SO4598 | III | | heavy metal efflux pump, CzcA family | - | 7.2 | 2.8 | 1.2 |
| SOA0153 | III | | heavy metal efflux pump, CzcA family | - | 6.3 | 2.7 | 1.3 |
| SOA0154 | III | | heavy metal efflux protein, putative | - | 6.5 | 3.2 | 1.6 |
| SO1722 | III | | ACT domain protein | Unknown function | 2.8 | 1.7 | 1.2 |
| SO1877 | III | *bcp* | bacterioferritin comigratory protein | - | 7.0 | 2.8 | 1.2 |
| SO3382 | III | | oxidoreductase, short-chain dehydrogenase/reductase family | - | 11.0 | 9.8 | 1.2 |
| SO3383 | III | | transcriptional regulator-related protein | - | 10.5 | 8.3 | 1.6 |
| SO4596 | III | | copper-transporting ATPase domain protein | - | 3.8 | 3.2 | 1.5 |
| SO0818 | IV | *metE* | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase | Amino acid biosynthesis | 0.8 | 5.0 | 0.6 |
| SO1030 | IV | *metH* | 5-methyltetrahydrofolate--homocysteine methyltransferase | - | 0.8 | 2.9 | 0.6 |
| SO1676 | IV | *metA* | homoserine O-succinyltransferase | - | 0.8 | 2.9 | 0.7 |
| SO4054 | IV | *metF* | 5,10-methylenetetrahydrofolate reductase | - | 1.1 | 5.9 | 0.7 |
| SO4055 | IV | *metL* | aspartokinase II/homoserine dehydrogenase, methionine-sensitive | - | 0.9 | 5.1 | 0.6 |
| SO4056 | IV | *metB* | cystathionine gamma-synthase | - | 1.2 | 5.7 | 0.6 |
| SO1031 | IV | | alpha-ribazole-5-phosphate phosphatase, putative | Biosynthesis of cofactors, prosthetic groups, and carriers | 0.8 | 2.1 | 0.8 |
| SO4053 | IV | | methyl-accepting chemotaxis protein | Cellular processes | 1.1 | 2.0 | 0.9 |
| SO4163 | IV | *hslU* | heat shock protein HslVU, ATPase subunit HslU | - | 1.0 | 2.1 | 1.0 |
| SO1087 | IV | | Na+/H+ antiporter family protein | Unknown function | 1.1 | 4.2 | 0.7 |
| SO1126 | IV | *dnaK* | chaperone protein DnaK | Protein fate | 1.0 | 2.2 | 0.9 |
| SO2016 | IV | *htpG* | heat shock protein HtpG | - | 1.0 | 2.1 | 0.9 |
| SO3577 | IV | *clpB* | clpB protein | - | 1.2 | 2.1 | 0.9 |
| SO0817 | IV | *metR* | transcriptional activator protein MetR | Regulatory functions | 1.1 | 3.2 | 0.7 |
| SO2997 | V | | hypothetical protein | Unknown function | 1.0 | 1.1 | 2.5 |
| SO2998 | V | | hypothetical protein | - | 0.8 | 1.4 | 2.2 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| SO3001 | V | | hypothetical protein | - | 0.8 | 1.3 | 2.1 |
| SO3002 | V | | conserved hypothetical protein | - | 0.9 | 1.4 | 2.0 |
| SO3344 | V | | hypothetical protein | - | 0.7 | 2.3 | 2.0 |
| SO2984 | V | | conserved hypothetical protein | - | 1.0 | 1.0 | 2.1 |
| SO3000 | V | | conserved hypothetical protein | - | 1.0 | 1.5 | 3.0 |
| SO3667 | V | | conserved hypothetical protein | - | 0.6 | 1.7 | 2.4 |
| SO3668 | V | | conserved hypothetical protein | - | 0.6 | 1.9 | 2.4 |
| SO1482 | V | | TonB-dependent receptor, putative | Transport and binding proteins | 1.0 | 1.3 | 2.2 |
| SO3669 | V | *hugA* | heme transport protein | - | 0.3 | 2.3 | 2.1 |
| SO3670 | V | *tonB*1 | TonB1 protein | - | 0.4 | 2.2 | 1.9 |
| SO3671 | V | *exbB*1 | TonB system transport protein ExbB1 | - | 0.3 | 2.3 | 1.8 |
| SO3673 | V | *hmuT* | hemin ABC transporter, periplasmic hemin-binding protein | - | 0.4 | 1.6 | 2.0 |
| SO3674 | V | *hmuU* | hemin ABC transporter, permease protein | - | 0.5 | 1.5 | 2.2 |
| SO3675 | V | *hmuV* | hemin ABC transporter, ATP-binding protein | - | 0.3 | 1.4 | 2.1 |

[a]the average induction fold at each time point. The data were presented with 95% confidence. nd: no data.

Supplemental Table 3.3. K-means analysis of up-regulated genes in response to UVB radiation

| SO Number | Cluster | Gene | Gene Product | Functional group | [a]Induction fold | | |
|---|---|---|---|---|---|---|---|
| | | | | | T5 | T20 | T60 |
| SO1751 | I | | membrane protein, putative | Cell envelope | 2.1 | 1.2 | 2.9 |
| SO4568 | I | *nrfD*-2 | formate-dependent nitrite reductase, nrfD protein | Central intermediary metabolism | 2.1 | 1.6 | 2.4 |
| SO2037 | I | | site-specific recombinase, phage integrase family | DNA metabolism | 1.1 | 1.6 | 2.1 |
| SO3061 | I | *topB* | DNA topoisomerase III | - | 2.9 | 6.9 | 9.7 |
| SO3429 | I | *recX* | regulatory protein RecX | - | 4.1 | 6.2 | 9.6 |
| SO3430 | I | *recA* | recA protein | - | 3.9 | 6.8 | 10.0 |
| SO3462 | I | *recN* | DNA repair protein RecN | - | 7.3 | 11.4 | 20.3 |
| SO4364 | I | *recG* | ATP-dependent DNA helicase RecG | - | 1.7 | 2.3 | 2.5 |
| SOA0012 | I | *umuC* | umuC protein | - | 2.5 | 6.0 | 7.7 |
| SOA0013 | I | *umuD* | umuD protein | - | 4.0 | 10.7 | 19.6 |
| SO1812 | I | *mdeA* | methionine gamma-lyase | Energy metabolism | 7.4 | 1.8 | 3.9 |
| SO0089 | I | | hypothetical protein | Unknown function | 2.3 | 4.6 | 6.7 |
| SO1116 | I | | hypothetical protein | - | 1.2 | 2.3 | 2.8 |
| SO1753 | I | | hypothetical protein | - | 1.8 | 1.4 | 2.3 |
| SO1761 | I | | hypothetical protein | - | 4.2 | 3.3 | 2.5 |
| SO3327 | I | | hypothetical protein | - | 2.4 | 1.7 | 4.7 |
| SO4363 | I | | hypothetical protein | - | 1.5 | 2.1 | 2.6 |
| SO4605 | I | | hypothetical protein | - | 4.7 | 8.6 | 8.0 |
| SO0179 | I | | conserved hypothetical protein | - | 2.5 | 2.4 | 2.1 |
| SO1757 | I | | conserved hypothetical protein | - | 19.2 | 15.9 | 32.5 |
| SO1758 | I | | conserved hypothetical protein | - | 0.8 | 3.9 | 0.9 |
| SO1759 | I | | conserved hypothetical protein | - | 12.9 | 10.7 | 11.0 |
| SO1816 | I | | conserved hypothetical protein | - | 23.0 | 3.6 | 59.2 |
| SO2602 | I | | conserved hypothetical protein | - | 1.3 | 1.8 | 2.0 |
| SO2603 | I | | conserved hypothetical protein | - | 2.6 | 4.8 | 5.7 |
| SO2604 | I | | conserved hypothetical protein | - | 2.9 | 5.3 | 7.2 |
| SO3849 | I | | conserved domain protein | - | 10.7 | 10.1 | 9.2 |
| SO4604 | I | | conserved hypothetical protein | - | 7.5 | 9.1 | 20.1 |
| SO3854 | I | | ISSo12, transposase | Other categories | 2.8 | 5.3 | 4.0 |
| SO1115 | I | *pepD* | aminoacyl-histidine dipeptidase | Protein fate | 1.5 | 2.1 | 3.0 |
| SO4603 | I | *lexA* | LexA repressor | Regulatory functions | 7.9 | 9.7 | 17.5 |
| SO1750 | I | | ABC transporter, ATP-binding protein | Transport and binding proteins | 2.1 | 1.3 | 3.7 |

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|---|---|---|---|---|---|---|---|
| SO1760 | I | | AzlC family protein | - | 0.9 | 3.0 | 0.8 |
| SO3060 | I | | outer membrane porin, putative | - | 1.6 | 3.2 | 2.8 |
| SO1114 | I | *dinP* | DNA-damage-inducible protein P | Unknown function | 3.6 | 5.6 | 12.2 |
| SO1756 | I | | glyoxalase family protein | - | 0.8 | 5.6 | 1.1 |
| SO1813 | I | | DNA-binding protein, putative | - | 10.2 | 2.6 | 14.5 |
| SO1815 | I | | histone deacetylase/AcuC/AphA family protein | - | 11.6 | 2.8 | 13.4 |
| SO3348 | II | *hemH-2* | ferrochelatase | Biosynthesis of cofactors, prosthetic groups, and carriers | 10.7 | 1.4 | 0.9 |
| SO3349 | II | | glutathione peroxidase, putative | Cellular processes | 9.3 | 1.5 | 0.9 |
| SO4170 | II | | C-factor, putative | - | 6.9 | 1.7 | 1.1 |
| SO4328 | II | | AcrB/AcrD/AcrF family protein, authentic frameshift | - | 4.9 | 1.0 | 0.8 |
| SO1276 | II | *gabT* | 4-aminobutyrate aminotransferase | Central intermediary metabolism | 2.3 | 0.8 | 0.8 |
| SO3384 | II | *phrB* | deoxyribodipyrimidine photolyase | DNA metabolism | 6.6 | 1.5 | 0.9 |
| SO3379 | II | *cfa* | cyclopropane-fatty-acyl-phospholipid synthase | Fatty acid and phospholipid metabolism | 15.0 | 2.0 | 0.9 |
| SO1246 | II | | hypothetical protein | Unknown function | 2.3 | 0.9 | 0.9 |
| SO1588 | II | | hypothetical protein | - | 10.3 | 1.0 | 0.9 |
| SO1589 | II | | hypothetical protein | - | 12.9 | 1.0 | 0.9 |
| SO1590 | II | | hypothetical protein | - | 13.6 | 1.1 | 0.9 |
| SO1592 | II | | hypothetical protein | - | 12.7 | 1.1 | 0.8 |
| SO3275 | II | | hypothetical protein | - | 2.4 | 1.1 | 1.0 |
| SO3276 | II | | hypothetical protein | - | 5.5 | 1.1 | 0.8 |
| SO4168 | II | | hypothetical protein | - | 3.1 | 1.1 | 1.0 |
| SO4694 | II | | hypothetical protein | - | 2.7 | 1.1 | 1.0 |
| SO4695 | II | | hypothetical protein | - | 2.0 | 1.3 | 1.1 |
| SO0771 | II | | conserved hypothetical protein | - | 7.1 | 1.2 | 1.1 |
| SO0855 | II | | conserved hypothetical protein | - | 2.3 | 1.0 | 1.0 |
| SO1274 | II | | conserved hypothetical protein | - | 2.1 | 0.8 | 0.9 |
| SO1587 | II | | conserved hypothetical protein | - | 2.1 | 0.9 | 0.8 |
| SO1649 | II | | conserved hypothetical protein | - | 3.4 | 1.0 | 0.9 |
| SO1650 | II | | conserved hypothetical protein | - | 2.8 | 0.9 | 0.9 |
| SO1810 | II | | conserved hypothetical protein | - | 4.8 | 1.5 | 2.0 |
| SO3073 | II | | conserved hypothetical protein | - | 2.0 | 1.1 | 0.9 |
| SO3376 | II | | conserved hypothetical protein | - | 6.4 | 1.6 | 0.9 |
| SO3377 | II | | conserved hypothetical protein | - | 8.1 | 1.9 | 0.9 |
| SO3378 | II | | conserved hypothetical protein | - | 10.2 | 1.9 | 0.9 |
| SO3380 | II | | conserved hypothetical protein | - | 11.5 | 2.1 | 0.9 |

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|---|---|---|---|---|---|---|---|
| SO3381 | II | | conserved hypothetical protein | - | 21.2 | 2.3 | 0.9 |
| SO3386 | II | | conserved hypothetical protein | - | 7.0 | 1.5 | 1.0 |
| SO4169 | II | | conserved hypothetical protein | - | 7.5 | 0.4 | 1.0 |
| SO4329 | II | | conserved hypothetical protein | - | 3.0 | 1.1 | 0.8 |
| SO4465 | II | | conserved domain protein | - | 8.3 | 1.2 | 0.9 |
| SO4696 | II | | conserved hypothetical protein | - | 4.6 | 1.2 | 1.0 |
| SO3385 | II | | transcriptional regulator, MerR family | Regulatory functions | 6.1 | 1.6 | 0.9 |
| SO4326 | II | | transcriptional regulator, TetR family | - | 3.9 | 1.0 | 0.8 |
| SO1559 | II | *phoR* | phosphate regulon sensor protein PhoR | Signal transduction | 3.0 | 1.0 | 0.9 |
| SO2366 | II | | response regulator | - | 2.3 | 1.1 | 0.9 |
| SO0157 | II | | proton/glutamate symporter | Transport and binding proteins | 2.2 | 1.4 | 1.0 |
| SO0857 | II | | ABC transporter, ATP-binding protein | - | 2.8 | 1.0 | 1.0 |
| SO1647 | II | | glutathione-regulated potassium-efflux system protein KefB, putative | - | 2.0 | 1.0 | 1.0 |
| SO2260 | II | *suhB* | extragenic suppressor protein SuhB | Unknown function | 3.5 | 1.7 | 1.4 |
| SO3382 | II | | oxidoreductase, short-chain dehydrogenase/reductase family | - | 14.6 | 2.4 | 1.0 |
| SO3383 | II | | transcriptional regulator-related protein | - | 11.7 | 2.4 | 0.9 |
| SO4039 | II | | hydrolase, haloacid dehalogenase-like family | - | 2.3 | 1.1 | 0.9 |
| SO4327 | II | | HlyD family secretion domain protein | - | 4.3 | 1.0 | 0.9 |
| SO2277 | III | *ibpA* | 16 kDa heat shock protein A | Protein fate | 1.1 | 1.1 | 2.0 |
| SO2964 | III | | ClpP protease family protein | - | 1.1 | 1.0 | 3.7 |
| SO0648 | III | | conserved hypothetical protein | Unknown function | 1.1 | 1.2 | 6.1 |
| SO0651 | III | | conserved hypothetical protein | - | 1.0 | 1.1 | 3.3 |
| SO2660 | III | | conserved hypothetical protein | - | 0.8 | 1.6 | 14.8 |
| SO2663 | III | | conserved hypothetical protein | - | 0.7 | 1.4 | 13.3 |
| SO2667 | III | | conserved hypothetical protein | - | 0.9 | 1.0 | 6.0 |
| SO2955 | III | | conserved hypothetical protein | - | 1.0 | 0.9 | 4.5 |
| SO2957 | III | | conserved hypothetical protein | - | 0.7 | 0.9 | 2.0 |
| SO2958 | III | | conserved hypothetical protein | - | 0.9 | 0.9 | 2.7 |
| SO2960 | III | | conserved hypothetical protein | - | 0.9 | 0.9 | 2.6 |
| SO2961 | III | | conserved hypothetical protein | - | 1.0 | 1.0 | 2.4 |
| SO2967 | III | | conserved hypothetical protein | - | 1.1 | 0.9 | 3.2 |
| SO2968 | III | | conserved hypothetical protein | - | 1.2 | 0.9 | 2.4 |
| SO2971 | III | | conserved hypothetical protein | - | 1.1 | 0.9 | 3.5 |
| SO2976 | III | | conserved hypothetical protein | - | 0.7 | 0.9 | 6.4 |
| SO2984 | III | | conserved hypothetical protein | - | 1.0 | 1.3 | 14.7 |

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|---|---|---|---|---|---|---|---|
| SO2988 | III | | conserved hypothetical protein | - | 0.9 | 1.3 | 21.4 |
| SO3000 | III | | conserved hypothetical protein | - | 0.9 | 1.7 | 37.2 |
| SO3002 | III | | conserved hypothetical protein | - | 0.9 | 1.4 | 23.5 |
| SO3005 | III | | conserved hypothetical protein | - | 0.8 | 0.6 | 23.1 |
| SO3326 | III | | conserved hypothetical protein | - | 1.4 | 1.0 | 2.5 |
| SO4163 | III | hslU | heat shock protein HslVU, ATPase subunit HslU | Cellular processes | 1.0 | 0.9 | 2.1 |
| SO0646 | III | | hypothetical protein | Unknown function | 0.9 | 1.2 | 8.5 |
| SO0836 | III | | hypothetical protein | - | 0.9 | 0.9 | 2.3 |
| SO2343 | III | | hypothetical protein | - | 1.0 | 1.1 | 3.7 |
| SO2656 | III | | hypothetical protein | - | 0.8 | 1.4 | 18.0 |
| SO2657 | III | | hypothetical protein | - | 1.0 | 1.5 | 11.8 |
| SO2658 | III | | hypothetical protein | - | 0.7 | 1.3 | 13.7 |
| SO2659 | III | | hypothetical protein | - | 0.9 | 1.4 | 11.5 |
| SO2661 | III | | hypothetical protein | - | 1.0 | 1.4 | 11.1 |
| SO2662 | III | | hypothetical protein | - | 1.1 | 1.9 | 6.5 |
| SO2664 | III | | hypothetical protein | - | 0.7 | 1.0 | 6.8 |
| SO2665 | III | | hypothetical protein | - | 0.7 | 1.0 | 5.6 |
| SO2666 | III | | hypothetical protein | - | 1.0 | 1.0 | 4.0 |
| SO2954 | III | | hypothetical protein | - | 1.3 | 1.1 | 2.2 |
| SO2962 | III | | hypothetical protein | - | 1.0 | 0.9 | 3.5 |
| SO2970 | III | | hypothetical protein | - | 1.1 | 0.9 | 4.7 |
| SO2972 | III | | hypothetical protein | - | 1.2 | 1.0 | 5.6 |
| SO2974 | III | | hypothetical protein | - | 0.9 | 0.8 | 7.2 |
| SO2979 | III | | hypothetical protein | - | 1.0 | 1.2 | 3.7 |
| SO2980 | III | | hypothetical protein | - | 0.9 | 1.2 | 12.8 |
| SO2981 | III | | hypothetical protein | - | ND | 0.6 | 11.9 |
| SO2982 | III | | hypothetical protein | - | 1.0 | 1.1 | 8.5 |
| SO2983 | III | | hypothetical protein | - | 0.8 | 1.3 | 17.2 |
| SO2986 | III | | hypothetical protein | - | 1.2 | 1.3 | 11.5 |
| SO2987 | III | | hypothetical protein | - | 1.0 | 1.2 | 12.3 |
| SO2995 | III | | hypothetical protein | - | 0.8 | 1.2 | 23.3 |
| SO2997 | III | | hypothetical protein | - | 0.9 | 1.4 | 18.8 |
| SO2998 | III | | hypothetical protein | - | 0.8 | 1.3 | 14.1 |
| SO2999 | III | | hypothetical protein | - | 0.9 | 1.3 | 7.2 |
| SO3001 | III | | hypothetical protein | - | 0.8 | 1.3 | 17.2 |
| SO3003 | III | | hypothetical protein | - | 0.5 | 1.2 | 26.6 |
| SO3008 | III | | hypothetical protein | - | 0.8 | 1.2 | 10.3 |

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|---|---|---|---|---|---|---|---|
| SO3009 | III | | hypothetical protein | - | 0.9 | 0.8 | 11.0 |
| SO3010 | III | | hypothetical protein | - | 1.0 | 1.0 | 7.4 |
| SO3011 | III | | hypothetical protein | - | 1.0 | 1.2 | 15.8 |
| SO3012 | III | | hypothetical protein | - | 1.1 | 1.2 | 8.8 |
| SO3004 | III | | prophage LambdaSo, DNA modification methyltransferase, putative | DNA metabolism | 0.9 | 1.3 | 15.6 |
| SO2969 | III | | prophage LambdaSo, holin, putative | Other categories | 1.2 | 1.0 | 4.0 |
| SO2963 | III | | prophage LambdaSo, major capsid protein, HK97 family | - | 1.0 | 0.9 | 3.8 |
| SO2956 | III | | prophage LambdaSo, major tail protein V, putative | - | 0.9 | 0.9 | 4.9 |
| SO2965 | III | | prophage LambdaSo, portal protein, HK97 family | - | 1.2 | 1.0 | 4.7 |
| SO2985 | III | *O* | prophage LambdaSo, replication protein O | - | 1.0 | 1.3 | 15.7 |
| SO2978 | III | | prophage LambdaSo, site-specific recombinase, phage integrase family | - | 0.9 | 1.0 | 9.7 |
| SO3006 | III | | prophage LambdaSo, type II DNA modification methyltransferase, putative | DNA metabolism | 0.9 | 1.1 | 11.0 |
| SO2993 | III | | prophage LambdaSo, type II DNA modification methyltransferase, putative, truncation | - | 1.0 | 1.7 | 35.3 |
| SO0644 | III | | prophage MuSo1, DNA transposition protein, putative | Other categories | 1.0 | 1.4 | 18.9 |
| SO0652 | III | | prophage MuSo1, positive regulator of late transcription, putative | - | 0.9 | 1.1 | 5.8 |
| SO0674 | III | | prophage MuSo1, protein Gp32, putative | - | 1.0 | 0.8 | 2.8 |
| SO2655 | III | | prophage MuSo2, DNA transposition protein, putative | - | 0.9 | 2.1 | 19.7 |
| SO2653 | III | | transcriptional regulator, Ner family | Regulatory functions | 0.6 | 1.3 | 9.8 |
| SO0643 | III | | transposase, putative | Other categories | nd | 1.2 | 8.2 |
| SO2654 | III | | transposase, putative | - | 1.0 | 1.8 | 7.6 |
| SO0645 | IV | | hypothetical protein | Unknown function | nd | 0.0 | 7.6 |
| SO0649 | IV | | hypothetical protein | - | nd | 0.5 | 10.9 |
| SO0678 | IV | | hypothetical protein | - | 1.0 | 0.7 | 3.3 |
| SO2959 | IV | | hypothetical protein | - | 0.7 | 0.7 | 2.2 |
| SO2975 | IV | | hypothetical protein | - | 0.8 | 0.5 | 2.0 |
| SO0676 | IV | | conserved hypothetical protein | - | 0.9 | 0.6 | 3.1 |
| SO0677 | IV | | conserved hypothetical protein | - | 1.0 | 0.4 | 2.6 |
| SO0675 | IV | | prophage MuSo1, major head subunit, putative | Other categories | 1.0 | 0.7 | 3.5 |
| SO2973 | IV | | prophage LambdaSo, lysozyme, putative | - | 1.1 | 0.6 | 6.7 |

[a] the average induction fold at each time point. The data were presented with 95% confidence. nd: no data.

Supplemental Table 3.4. Up-regulated hypothetical and conserved hypothetical genes in response to UVR

| SO number | Induction | Description | UVC | | | UVB | | | UVA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | T5 | T20 | T60 | T5 | T20 | T60 | T5 | T20 | T60 |
| SO0086 | UVC only | hypothetical protein | 1.0 | 2.6 | 1.1 | [a]- | - | - | - | - | - |
| SO2038 | UVC only | hypothetical protein | 1.2 | 2.0 | 2.0 | - | - | - | - | - | - |
| SO2858 | UVC only | conserved hypothetical protein | 1.0 | 1.1 | 3.0 | - | - | - | - | - | - |
| SO2945 | UVC only | hypothetical protein | 1.0 | 0.8 | 2.8 | - | - | - | - | - | - |
| SO2946 | UVC only | hypothetical protein | 1.0 | 0.8 | 2.1 | - | - | - | - | - | - |
| SO2977 | UVC only | hypothetical protein | 1.0 | 0.9 | 3.6 | - | - | - | - | - | - |
| SO2996 | UVC only | hypothetical protein | 1.1 | 1.3 | 2.4 | - | - | - | - | - | - |
| SO3133 | UVC only | hypothetical protein | 1.2 | 1.3 | 3.5 | - | - | - | - | - | - |
| SO0179 | Both UVC and UVB | conserved hypothetical protein | 3.3 | 2.6 | 1.9 | 2.5 | 2.4 | 2.1 | - | - | - |
| SO0645 | Both UVC and UVB | hypothetical protein | [b]nd | 2.0 | 10.5 | nd | 0.0 | 7.6 | - | - | - |
| SO0646 | Both UVC and UVB | hypothetical protein | 1.0 | 1.6 | 8.4 | 0.9 | 1.2 | 8.5 | - | - | - |
| SO0647 | Both UVC and UVB | hypothetical protein | nd | 1.5 | 8.8 | nd | nd | 8.5 | - | - | - |
| SO0648 | Both UVC and UVB | conserved hypothetical protein | 1.1 | 1.5 | 8.2 | 1.1 | 1.2 | 6.1 | - | - | - |
| SO0649 | Both UVC and UVB | hypothetical protein | 0.6 | 1.6 | 8.3 | nd | 0.5 | 10.9 | - | - | - |
| SO0650 | Both UVC and UVB | hypothetical protein | nd | 1.0 | 4.1 | nd | nd | 3.7 | - | - | - |
| SO0651 | Both UVC and UVB | conserved hypothetical protein | 1.0 | 1.3 | 3.7 | 1.0 | 1.1 | 3.3 | - | - | - |
| SO0676 | Both UVC and UVB | conserved hypothetical protein | 0.9 | 0.9 | 3.8 | 0.9 | 0.6 | 3.1 | - | - | - |
| SO0677 | Both UVC and UVB | conserved hypothetical protein | 1.1 | 0.8 | 3.1 | 1.0 | 0.4 | 2.6 | - | - | - |
| SO0678 | Both UVC and UVB | hypothetical protein | 1.0 | 0.8 | 4.2 | 1.0 | 0.7 | 3.3 | - | - | - |
| SO0836 | Both UVC and UVB | hypothetical protein | 0.7 | 1.1 | 3.2 | 0.9 | 0.9 | 2.3 | - | - | - |
| SO1116 | Both UVC and UVB | hypothetical protein | 1.2 | 2.3 | 2.2 | 1.2 | 2.3 | 2.8 | - | - | - |
| SO1753 | Both UVC and UVB | hypothetical protein | 1.4 | 2.0 | 1.9 | 1.8 | 1.4 | 2.3 | - | - | - |
| SO1810 | Both UVC and UVB | conserved hypothetical protein | 1.9 | 2.2 | 1.5 | 4.8 | 1.5 | 2.0 | - | - | - |
| SO1816 | Both UVC and UVB | conserved hypothetical protein | 6.4 | 27.7 | 14.8 | 23.0 | 3.6 | 59.2 | - | - | - |
| SO2343 | Both UVC and UVB | hypothetical protein | 1.0 | 1.2 | 3.0 | 1.0 | 1.1 | 3.7 | - | - | - |
| SO2656 | Both UVC and UVB | hypothetical protein | 0.9 | 3.4 | 16.1 | 0.8 | 1.4 | 18.0 | - | - | - |
| SO2657 | Both UVC and UVB | hypothetical protein | 1.1 | 2.5 | 11.5 | 1.0 | 1.5 | 11.8 | - | - | - |
| SO2658 | Both UVC and UVB | hypothetical protein | 0.7 | 2.6 | 13.0 | 0.7 | 1.3 | 13.7 | - | - | - |
| SO2659 | Both UVC and UVB | hypothetical protein | 0.7 | 2.4 | 10.3 | 0.9 | 1.4 | 11.5 | - | - | - |
| SO2660 | Both UVC and UVB | conserved hypothetical protein | 1.1 | 2.5 | 13.7 | 0.8 | 1.6 | 14.8 | - | - | - |
| SO2661 | Both UVC and UVB | hypothetical protein | 0.7 | 2.0 | 12.3 | 1.0 | 1.4 | 11.1 | - | - | - |
| SO2662 | Both UVC and UVB | hypothetical protein | 1.4 | 2.5 | 7.7 | 1.1 | 1.9 | 6.5 | - | - | - |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SO2663 | Both UVC and UVB | conserved hypothetical protein | 0.9 | 2.4 | 14.4 | 0.7 | 1.4 | 13.3 | - | - | - |
| SO2664 | Both UVC and UVB | hypothetical protein | 0.8 | 1.4 | 9.5 | 0.7 | 1.0 | 6.8 | - | - | - |
| SO2665 | Both UVC and UVB | hypothetical protein | 0.8 | 1.3 | 8.7 | 0.7 | 1.0 | 5.6 | - | - | - |
| SO2666 | Both UVC and UVB | hypothetical protein | 1.0 | 1.2 | 6.0 | 1.0 | 1.0 | 4.0 | - | - | - |
| SO2667 | Both UVC and UVB | conserved hypothetical protein | 0.8 | 1.2 | 6.8 | 0.9 | 1.0 | 6.0 | - | - | - |
| SO2954 | Both UVC and UVB | hypothetical protein | 1.3 | 1.4 | 3.1 | 1.3 | 1.1 | 2.2 | - | - | - |
| SO2955 | Both UVC and UVB | conserved hypothetical protein | 0.9 | 0.9 | 6.2 | 1.0 | 0.9 | 4.5 | - | - | - |
| SO2957 | Both UVC and UVB | conserved hypothetical protein | nd | 1.1 | 3.1 | 0.7 | 0.9 | 2.0 | - | - | - |
| SO2958 | Both UVC and UVB | conserved hypothetical protein | 1.1 | 1.1 | 3.7 | 0.9 | 0.9 | 2.7 | - | - | - |
| SO2959 | Both UVC and UVB | hypothetical protein | 0.9 | 1.1 | 3.5 | 0.7 | 0.7 | 2.2 | - | - | - |
| SO2960 | Both UVC and UVB | conserved hypothetical protein | 1.0 | 0.9 | 4.7 | 0.9 | 0.9 | 2.6 | - | - | - |
| SO2961 | Both UVC and UVB | conserved hypothetical protein | 1.0 | 1.1 | 4.0 | 1.0 | 1.0 | 2.4 | - | - | - |
| SO2962 | Both UVC and UVB | hypothetical protein | 1.0 | 0.8 | 4.9 | 1.0 | 0.9 | 3.5 | - | - | - |
| SO2967 | Both UVC and UVB | conserved hypothetical protein | 1.1 | 1.0 | 4.1 | 1.1 | 0.9 | 3.2 | - | - | - |
| SO2968 | Both UVC and UVB | conserved hypothetical protein | 1.2 | 1.0 | 3.0 | 1.2 | 0.9 | 2.4 | - | - | - |
| SO2970 | Both UVC and UVB | hypothetical protein | 1.2 | 1.0 | 6.6 | 1.1 | 0.9 | 4.7 | - | - | - |
| SO2971 | Both UVC and UVB | conserved hypothetical protein | 1.1 | 1.0 | 4.9 | 1.1 | 0.9 | 3.5 | - | - | - |
| SO2972 | Both UVC and UVB | hypothetical protein | 1.1 | 1.1 | 7.0 | 1.2 | 1.0 | 5.6 | - | - | - |
| SO2974 | Both UVC and UVB | hypothetical protein | 1.1 | 0.9 | 9.6 | 0.9 | 0.8 | 7.2 | - | - | - |
| SO2976 | Both UVC and UVB | conserved hypothetical protein | 1.0 | 0.9 | 6.3 | 0.7 | 0.9 | 6.4 | - | - | - |
| SO2979 | Both UVC and UVB | hypothetical protein | 1.0 | 1.2 | 5.6 | 1.0 | 1.2 | 3.7 | - | - | - |
| SO2980 | Both UVC and UVB | hypothetical protein | 1.0 | 1.3 | 10.4 | 0.9 | 1.2 | 12.8 | - | - | - |
| SO2981 | Both UVC and UVB | hypothetical protein | 0.2 | 1.2 | 10.2 | nd | 0.6 | 11.9 | - | - | - |
| SO2982 | Both UVC and UVB | hypothetical protein | 1.0 | 1.3 | 9.6 | 1.0 | 1.1 | 8.5 | - | - | - |
| SO2986 | Both UVC and UVB | hypothetical protein | 1.1 | 1.7 | 11.3 | 1.2 | 1.3 | 11.5 | - | - | - |
| SO2987 | Both UVC and UVB | hypothetical protein | 1.0 | 1.7 | 12.1 | 1.0 | 1.2 | 12.3 | - | - | - |
| SO2995 | Both UVC and UVB | hypothetical protein | 1.2 | 2.3 | 18.7 | 0.8 | 1.2 | 23.3 | - | - | - |
| SO2999 | Both UVC and UVB | hypothetical protein | 0.9 | 1.4 | 8.6 | 0.9 | 1.3 | 7.2 | - | - | - |
| SO3003 | Both UVC and UVB | hypothetical protein | 1.0 | 2.1 | 22.5 | 0.5 | 1.2 | 26.6 | - | - | - |
| SO3005 | Both UVC and UVB | conserved hypothetical protein | 0.9 | 1.7 | 17.3 | 0.8 | 0.6 | 23.1 | - | - | - |
| SO3007 | Both UVC and UVB | hypothetical protein | nd | nd | 2.4 | nd | | 3.5 | - | - | - |
| SO3008 | Both UVC and UVB | hypothetical protein | 0.5 | 1.3 | 10.5 | 0.8 | 1.2 | 10.3 | - | - | - |
| SO3009 | Both UVC and UVB | hypothetical protein | 1.1 | 1.2 | 11.3 | 0.9 | 0.8 | 11.0 | - | - | - |
| SO3010 | Both UVC and UVB | hypothetical protein | 0.8 | 1.1 | 9.5 | 1.0 | 1.0 | 7.4 | - | - | - |
| SO3011 | Both UVC and UVB | hypothetical protein | 0.8 | 1.4 | 12.9 | 1.0 | 1.2 | 15.8 | - | - | - |
| SO3012 | Both UVC and UVB | hypothetical protein | 1.1 | 1.2 | 10.2 | 1.1 | 1.2 | 8.8 | - | - | - |
| SO4363 | Both UVC and UVB | hypothetical protein | 1.8 | 2.5 | 2.4 | 1.5 | 2.1 | 2.6 | - | - | - |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SO1246 | UVB only | hypothetical protein | - | - | - | 2.285 | 0.9 | 0.86 | - | - | - |
| SO1274 | UVB only | conserved hypothetical protein | - | - | - | 2.097 | 0.8 | 0.92 | - | - | - |
| SO1587 | UVB only | conserved hypothetical protein | - | - | - | 2.092 | 0.9 | 0.79 | - | - | - |
| SO1588 | UVB only | hypothetical protein | - | - | - | 10.28 | 1 | 0.9 | - | - | - |
| SO1589 | UVB only | hypothetical protein | - | - | - | 12.9 | 1 | 0.91 | - | - | - |
| SO1590 | UVB only | hypothetical protein | - | - | - | 13.58 | 1.1 | 0.9 | - | - | - |
| SO1592 | UVB only | hypothetical protein | - | - | - | 12.65 | 1.1 | 0.81 | - | - | - |
| SO1649 | UVB only | conserved hypothetical protein | - | - | - | 3.39 | 1 | 0.94 | - | - | - |
| SO1650 | UVB only | conserved hypothetical protein | - | - | - | 2.826 | 0.9 | 0.92 | - | - | - |
| SO2602 | UVB only | conserved hypothetical protein | - | - | - | 1.255 | 1.8 | 2.01 | - | - | - |
| SO2975 | UVB only | hypothetical protein | - | - | - | 0.825 | 0.5 | 2.02 | - | - | - |
| SO3326 | UVB only | conserved hypothetical protein | - | - | - | 1.442 | 1 | 2.55 | - | - | - |
| SO0771 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 7.1 | 1.2 | 1.1 | 13.0 | 3.9 | 1.7 |
| SO0855 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 2.3 | 1.0 | 1.0 | 5.1 | 1.8 | 0.8 |
| SO1758 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 0.8 | 3.9 | 0.9 | 11.1 | 11.7 | 7.0 |
| SO3073 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 2.0 | 1.1 | 0.9 | 6.7 | 1.8 | 1.2 |
| SO3275 | Both UVB and UVA | hypothetical protein | - | - | - | 2.4 | 1.1 | 1.0 | 8.7 | 2.3 | 1.1 |
| SO3276 | Both UVB and UVA | hypothetical protein | - | - | - | 5.5 | 1.1 | 0.8 | 15.3 | 4.6 | 1.1 |
| SO3376 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 6.4 | 1.6 | 0.9 | 3.9 | 7.4 | 0.7 |
| SO3377 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 8.1 | 1.9 | 0.9 | 5.3 | 8.8 | 0.8 |
| SO3378 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 10.2 | 1.9 | 0.9 | 6.1 | 10.5 | 0.6 |
| SO3380 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 11.5 | 2.1 | 0.9 | 9.0 | 9.2 | 0.8 |
| SO3381 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 21.2 | 2.3 | 0.9 | 15.2 | 10.8 | 1.1 |
| SO3386 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 7.0 | 1.5 | 1.0 | 28.1 | 15.1 | 5.3 |
| SO4168 | Both UVB and UVA | hypothetical protein | - | - | - | 3.1 | 1.1 | 1.0 | 8.4 | 4.4 | 1.1 |
| SO4169 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 7.5 | 0.4 | 1.0 | 17.8 | 8.8 | 1.3 |
| SO4329 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 3.0 | 1.1 | 0.8 | 5.8 | 2.0 | 0.9 |
| SO4465 | Both UVB and UVA | conserved domain protein | - | - | - | 8.3 | 1.2 | 0.9 | 12.6 | 4.9 | 1.0 |
| SO4694 | Both UVB and UVA | hypothetical protein | - | - | - | 2.7 | 1.1 | 1.0 | 7.0 | 2.6 | 1.2 |
| SO4695 | Both UVB and UVA | hypothetical protein | - | - | - | 2.0 | 1.3 | 1.1 | 7.3 | 2.0 | 1.3 |
| SO4696 | Both UVB and UVA | conserved hypothetical protein | - | - | - | 4.6 | 1.2 | 1.0 | 7.0 | 3.9 | 1.4 |
| SO0090 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.7 | 1.5 | 1.2 |
| SO0258 | UVA only | hypothetical protein | - | - | - | - | - | - | 1.4 | 1.3 | 2.1 |
| SO0316 | UVA only | conserved hypothetical protein TIGR00481 | - | - | - | - | - | - | 3.9 | 1.6 | 1.2 |
| SO0334 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.6 | 1.5 | 1.1 |
| SO0335 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.6 | 1.4 | 1.1 |
| SO0336 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.3 | 1.5 | 1.3 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SO0338 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.2 | 1.2 | 1.1 |
| SO0856 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 6.5 | 1.7 | 0.9 |
| SO0975 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.5 | 1.5 | 1.1 |
| SO1379 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.0 | 1.1 | 1.1 |
| SO1380 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 4.4 | 1.3 | 1.0 |
| SO1381 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 10.0 | 1.6 | 1.4 |
| SO1432 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.2 | 1.3 | 1.3 |
| SO1702 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.4 | 1.4 | 1.1 |
| SO1837 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.6 | 1.0 | 1.1 |
| SO1983 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.5 | 1.6 | 1.4 |
| SO1984 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.2 | 1.7 | 1.5 |
| SO1988 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 15.8 | 7.3 | 1.5 |
| SO1990 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.5 | 1.4 | 1.1 |
| SO2007 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 10.4 | 3.7 | 1.3 |
| SO2008 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 8.3 | 2.6 | 1.2 |
| SO2009 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 9.2 | 2.0 | 1.1 |
| SO2010 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.8 | 1.4 | nd |
| SO2042 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 13.9 | 3.8 | 1.4 |
| SO2043 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 12.3 | 3.1 | 1.2 |
| SO2469 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.1 | 1.8 | 1.9 |
| SO2697 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.3 | 1.2 | 1.1 |
| SO2710 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 3.5 | 1.2 | 1.1 |
| SO2773 | UVA only | hypothetical protein | - | - | - | - | - | - | 4.1 | 1.3 | 1.1 |
| SO2873 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.7 | 2.0 | 1.1 |
| SO2922 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 7.3 | 2.3 | 1.1 |
| SO2944 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.9 | 1.1 | 1.1 |
| SO3097 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 7.3 | 2.0 | 1.3 |
| SO3198 | UVA only | hypothetical protein | - | - | - | - | - | - | 4.0 | 1.6 | 1.0 |
| SO3274 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 4.7 | 1.5 | 1.1 |
| SO3319 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 4.0 | 1.8 | 1.1 |
| SO3344 | UVA only | hypothetical protein | - | - | - | - | - | - | 0.7 | 2.3 | 2.0 |
| SO3346 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.6 | 1.3 | 1.1 |
| SO3370 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 3.8 | 1.0 | 0.9 |
| SO3374 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 4.5 | 2.7 | 1.3 |
| SO3557 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.1 | 1.2 | 1.1 |
| SO3558 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.0 | 1.5 | 1.1 |
| SO3643 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.0 | 1.6 | 1.2 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SO3658 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.2 | 1.2 | 1.1 |
| SO3667 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 0.6 | 1.7 | 2.4 |
| SO3668 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 0.6 | 1.9 | 2.4 |
| SO3776 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.9 | 1.3 | 1.0 |
| SO3777 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.7 | 1.3 | 1.0 |
| SO3951 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.2 | 1.2 | 1.0 |
| SO3953 | UVA only | conserved hypothetical protein TIGR00056 | - | - | - | - | - | - | 2.8 | 1.3 | 1.0 |
| SO4167 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.5 | 1.4 | 1.0 |
| SO4171 | UVA only | hypothetical protein | - | - | - | - | - | - | 5.4 | 1.8 | 0.9 |
| SO4338 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.2 | nd | 1.1 |
| SO4464 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.2 | 1.1 | 1.0 |
| SO4481 | UVA only | hypothetical protein | - | - | - | - | - | - | 7.1 | 3.2 | 2.6 |
| SO4482 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.2 | 1.4 | 1.5 |
| SO4592 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.8 | 1.8 | 1.2 |
| SO4593 | UVA only | hypothetical protein | - | - | - | - | - | - | 3.3 | 1.7 | 1.2 |
| SO4594 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.8 | 2.4 | 1.5 |
| SO4641 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.5 | 1.2 | 1.1 |
| SO4642 | UVA only | conserved hypothetical protein | - | - | - | - | - | - | 2.4 | 1.2 | 1.0 |
| SO4643 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.5 | 1.2 | 0.9 |
| SO4738 | UVA only | hypothetical protein | - | - | - | - | - | - | 2.4 | 1.2 | 1.5 |
| SOA0155 | UVA only | conserved domain protein | - | - | - | - | - | - | 6.0 | 3.2 | 1.7 |

[a]no change in gene expression. [b]nd stands for "no data".

Supplemental Table 4.1 The relative expression of other stress-related genes following solar radiation exposure

| ID | Gene | Gene product | Function | [a]T5 | [a]T20 | [a]T60 |
|---|---|---|---|---|---|---|
| SO3248 | *flgD* | basal-body rod modification protein FlgD | Cellular processes | 0.85 | 2.10 | 2.60 |
| SO1989 | *cheV*-1 | chemotaxis protein CheV | - | 2.09 | 2.56 | 1.76 |
| SO3235 | *fliD* | flagellar hook-associated protein FliD | - | 0.97 | 1.58 | 2.03 |
| SO3242 | *flgI* | flagellar P-ring protein FlgI | - | 1.26 | 2.49 | 2.07 |
| SO3241 | *flgJ* | flagellar protein FlgJ | - | 1.23 | 2.91 | 2.54 |
| SO3236 | *flaG* | flagellin FlaG | - | 1.01 | 1.58 | 2.12 |
| SO0584 | | methyl-accepting chemotaxis protein | - | 0.51 | 2.48 | 1.31 |
| SO1278 | | methyl-accepting chemotaxis protein | - | 1.75 | 2.63 | 1.39 |
| SO3052 | | methyl-accepting chemotaxis protein | - | 1.71 | 3.20 | 1.98 |
| SO3351 | *pilT* | twitching motility protein PilT | - | 2.75 | 1.73 | 1.71 |
| SO3350 | *pilU* | twitching motility protein PilU | - | 2.05 | 1.51 | 1.55 |
| SO2787 | | cold shock domain family protein | - | 2.00 | 0.74 | 1.17 |
| SO1807 | *pspA* | phage shock protein A | - | 0.72 | 1.87 | 3.48 |
| SO1808 | *pspB* | phage shock protein B | - | 0.86 | 1.60 | 2.92 |
| SO1809 | *pspC* | phage shock protein C | - | 0.77 | 1.73 | 2.97 |
| SO4394 | *pspE*-2 | phage shock protein E | - | 5.78 | 3.99 | 1.92 |
| SO3681 | | universal stress protein family | - | 1.07 | 3.82 | 1.88 |
| SO3585 | | azoreductase, putative | - | 4.25 | 3.31 | 1.90 |
| SO4170 | | C-factor, putative | - | 4.30 | 6.20 | 8.10 |
| SO2824 | | carbon starvation protein A, authentic point mutation | General stress | 0.96 | 3.15 | 4.28 |
| SO2588 | | protein-methionine-S-oxide reductase, PilB family | Protein modification and repair | 3.41 | 5.95 | 6.10 |
| SO1193 | *secD*-1 | protein-export membrane protein SecD | Protein fate | 3.04 | 8.07 | 2.76 |
| SO1194 | *secF*-1 | protein-export membrane protein SecF | - | 3.28 | 8.52 | 2.65 |
| SO0696 | *dsbD* | thiol:disulfide interchange protein DsbD | protein folding and stabilization | 2.01 | 3.66 | 2.20 |
| SO0951 | *dsbC* | thiol:disulfide interchange protein DsbC | - | 5.22 | 5.96 | 3.67 |
| SO1126 | *dnaK* | chaperone protein DnaK | - | 0.71 | 4.32 | 3.12 |
| SO1127 | *dnaJ* | chaperone protein DnaJ | - | 0.50 | 2.15 | 2.40 |
| SO2277 | *ibpA* | 16 kDa heat shock protein A | - | 0.85 | 3.54 | 4.37 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SO2267 | *hscB* | co-chaperone Hsc20 | - | 0.69 | 0.54 | 2.63 |
| SO2268 | *hscA* | chaperone protein HscA | - | 0.51 | 0.38 | 2.31 |

[a]the average induction fold at 5 (T5), 20 (T20) and 60 (T60) min. The data were presented with 99% confidence.

Supplemental Table 4.2 Cluster analysis and the relative expression of metabolic genes in MR-1 following solar radiation exposure

| ID | Gene | Gene product | [a]Cluster | [b]T5 | [b]T20 | [b]T60 |
|---|---|---|---|---|---|---|
| SO1666 | *phhA* | phenylalanine-4-hydroxylase | I | 3.29 | 4.39 | 4.09 |
| SO1962 | | 4-hydroxyphenylpyruvate dioxygenase | I | 1.65 | 4.34 | 3.80 |
| SO4480 | *aldA* | aldehyde dehydrogenase | I | 2.16 | 5.11 | 4.42 |
| SO2248 | *sdaA* | L-serine dehydratase 1 | I | 3.07 | 2.98 | 1.41 |
| SO1678 | *mmsA* | methylmalonate-semialdehyde dehydrogenase | I | 1.99 | 6.51 | 2.96 |
| SO1682 | *mmsB* | 3-hydroxyisobutyrate dehydrogenase | I | 1.50 | 2.35 | 1.78 |
| SO0095 | *hutI* | imidazolonepropionase | I | 2.66 | 3.67 | 2.04 |
| SO1812 | *mdeA* | methionine gamma-lyase | I | 2.09 | 2.26 | 1.73 |
| SO1897 | *ivd* | isovaleryl-CoA dehydrogenase | I | 1.35 | 2.21 | 1.39 |
| SO0847 | *napG* | iron-sulfur cluster-binding protein NapG | I | 1.48 | 3.94 | 1.60 |
| SO0848 | *napA* | periplasmic nitrate reductase | I | 3.10 | 8.46 | 0.88 |
| SO0849 | *napD* | napD protein | I | 2.81 | 5.20 | 0.94 |
| SO1430 | *dmsB*-1 | anaerobic dimethyl sulfoxide reductase, B subunit | I | 1.27 | 3.09 | 1.36 |
| SO3980 | | cytochrome c552 nitrite reductase | I | 1.96 | 3.93 | 0.88 |
| SO4510 | *fdhB*-1 | formate dehydrogenase, iron-sulfur subunit | I | 1.09 | 2.19 | 0.88 |
| SO4511 | | formate dehydrogenase, C subunit, putative | I | 1.07 | 2.19 | 1.05 |
| SO1232 | *torA* | trimethylamine-N-oxide reductase | I | 1.29 | 2.38 | 1.64 |
| SO1538 | | isocitrate dehydrogenase, NAD-dependent | I | 1.36 | 1.23 | 2.12 |
| SO1519 | | iron-sulfur cluster-binding protein | I | 2.93 | 3.52 | 2.38 |
| SO1782 | *mtrD* | decaheme cytochrome c MtrD | I | 1.75 | 3.20 | 1.57 |
| SO2097 | *hydC* | quinone-reactive Ni/Fe hydrogenase, cytochrome b subunit | I | 0.84 | 4.35 | 0.91 |
| SO4047 | | cytochrome c family protein | I | 2.58 | 3.03 | 1.54 |
| SO4048 | | cytochrome c family protein | I | 2.92 | 2.96 | 1.71 |
| SO4142 | | cytochrome c family protein | I | 1.74 | 4.30 | 1.74 |
| SO4453 | | electron transfer flavoprotein-ubiquinone oxidoreductase, putative | I | 3.00 | 2.28 | 1.30 |
| SO4483 | | cytochrome b, putative | I | 2.14 | 5.25 | 2.81 |
| SO4484 | | cytochrome c-type protein Shp | I | 2.28 | 5.49 | 3.40 |
| SO4485 | | diheme cytochrome c | I | 1.90 | 5.60 | 3.46 |
| SO4606 | | cytochrome c oxidase, subunit II | I | 1.71 | 3.12 | 3.57 |
| SO4607 | | cytochrome c oxidase, subunit I | I | 0.76 | 1.73 | 2.58 |

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|---|---|---|---|---|---|---|
| SO2727 | | cytochrome c3 (4 heme) | I | 1.62 | 2.20 | 0.89 |
| SO0747 | *fpr* | ferredoxin--NADP reductase | I | 2.50 | 1.69 | 1.79 |
| SO1483 | *aceB* | malate synthase A | I | 4.67 | 10.65 | 9.03 |
| SO1484 | *aceA* | isocitrate lyase | I | 1.38 | 7.05 | 7.30 |
| SO0694 | *galK* | galactokinase | I | 3.11 | 1.35 | 1.17 |
| SO0049 | *gpmA* | phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent | I | 2.36 | 1.85 | 1.12 |
| SO0967 | | dienelactone hydrolase family protein | I | 1.97 | 1.61 | 2.05 |
| SO1237 | | acetoin utilization protein AcuB, putative | I | 2.06 | 1.76 | 1.41 |
| SO1902 | *gnd* | 6-phosphogluconate dehydrogenase, decarboxylating | I | 2.16 | 1.59 | 1.05 |
| SO2213 | | oligo-1,6-glucosidase | I | 2.53 | 1.55 | 1.38 |
| SO4702 | *gor* | glutathione reductase | I | 2.02 | 1.49 | 1.13 |
| SO3496 | | aldehyde dehydrogenase | I | 2.90 | 3.49 | 4.22 |
| SO3683 | | coniferyl aldehyde dehydrogenase | I | 1.58 | 1.76 | 2.13 |
| SO0397 | *fdrC* | fumarate reductase cytochrome B subunit | II | 0.55 | 2.02 | 0.33 |
| SO0970 | | fumarate reductase flavoprotein subunit precursor | II | 1.21 | 2.11 | 0.32 |
| SO0398 | *frdA* | fumarate reductase flavoprotein subunit | II | 0.46 | 1.83 | 0.47 |
| SO0399 | *frdB* | fumarate reductase iron-sulfur protein | II | 0.47 | 1.14 | 0.56 |
| SO0902 | *nqrA*-1 | NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit | II | 2.02 | 2.06 | 0.27 |
| SO0903 | *nqrB*-1 | NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB | II | 1.17 | 2.04 | 0.31 |
| SO0904 | *nqrC*-1 | NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit | II | 0.83 | 2.12 | 0.44 |
| SO0905 | *nqrD*-1 | NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrD | II | 0.68 | 2.20 | 0.54 |
| SO0906 | *nqrE*-1 | NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE | II | 0.74 | 2.50 | 0.58 |
| SO1490 | *adhB* | alcohol dehydrogenase II | II | 1.89 | 3.28 | 0.33 |
| SO2912 | *pflB* | formate acetyltransferase | II | 1.03 | 1.63 | 0.23 |
| SO2913 | *pflA* | pyruvate formate-lyase 1 activating enzyme | II | 0.65 | 1.27 | 0.33 |
| SO2915 | *ackA* | acetate kinase | II | 0.48 | 1.21 | 0.37 |
| SO2916 | *pta* | phosphate acetyltransferase | II | 0.77 | 1.17 | 0.47 |
| SO3285 | *cydB* | cytochrome d ubiquinol oxidase, subunit II | II | 1.14 | 1.44 | 0.42 |
| SO3286 | *cydA* | cytochrome d ubiquinol oxidase, subunit I | II | 1.26 | 1.60 | 0.47 |
| SO4404 | | iron-sulfur cluster-binding protein | II | 1.12 | 2.08 | 0.34 |
| SO4513 | | formate dehydrogenase, alpha subunit | II | 0.73 | 2.22 | 0.09 |
| SO4515 | | formate dehydrogenase, C subunit, putative | II | 0.94 | 3.04 | 0.39 |
| SO4591 | *cymA* | tetraheme cytochrome c | II | 2.40 | 1.77 | 0.39 |

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|---|---|---|---|---|---|---|
| SO1427 | | decaheme cytochrome c | II | 0.76 | 1.70 | 0.43 |
| SO1777 | *mtrA* | decaheme cytochrome c MtrA | II | 0.81 | 1.46 | 0.41 |
| SO1778 | *omcB* | decaheme cytochrome c | II | 1.11 | 1.75 | 0.45 |
| SO1779 | *omcA* | decaheme cytochrome c | II | 1.14 | 1.83 | 0.46 |
| SO2098 | *hyaB* | quinone-reactive Ni/Fe hydrogenase, large subunit | II | 0.94 | 5.65 | 0.34 |
| SO2099 | *hoxK* | quinone-reactive Ni/Fe hydrogenase, small subunit precursor | II | 1.98 | 4.82 | 0.41 |
| SO0162 | *pckA* | phosphoenolpyruvate carboxykinase(ATP) | III | 0.82 | 0.40 | 0.47 |
| SO0274 | *ppc* | phosphoenolpyruvate carboxylase | III | 0.66 | 0.63 | 0.22 |
| SO0343 | *acnA* | aconitate hydratase 1 | III | 1.01 | 0.50 | 0.49 |
| SO0344 | *prpC* | methylcitrate synthase | III | 0.84 | 0.39 | 0.46 |
| SO0345 | *prpB* | methylisocitrate lyase | III | 0.68 | 0.47 | 0.61 |
| SO0424 | *aceE* | pyruvate dehydrogenase complex, E1 component, pyruvate dehydrogenase | III | 1.02 | 0.34 | 0.46 |
| SO0425 | *aceF* | pyruvate dehydrogenase complex, E2 component, dihydrolipoamide acetyltransferase | III | 0.95 | 0.37 | 0.44 |
| SO0426 | *lpdA* | pyruvate dehydrogenase complex, E3 component, lipoamide dehydrogenase | III | 0.59 | 0.44 | 0.52 |
| SO1103 | *nqrA*-2 | NADH:ubiquinone oxidoreductase, Na translocating, alpha subunit | III | 0.47 | 0.40 | 0.78 |
| SO1104 | *nqrB*-2 | NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrB | III | 0.60 | 0.32 | 0.66 |
| SO1105 | *nqrC*-2 | NADH:ubiquinone oxidoreductase, Na translocating, gamma subunit | III | 0.96 | 0.37 | 0.63 |
| SO1106 | *nqrD*-2 | NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrD | III | 1.07 | 0.36 | 0.61 |
| SO1107 | *nqrE*-2 | NADH:ubiquinone oxidoreductase, Na translocating, hydrophobic membrane protein NqrE | III | 1.08 | 0.34 | 0.64 |
| SO1108 | *nqrF*-2 | NADH:ubiquinone oxidoreductase, Na translocating, beta subunit | III | 0.98 | 0.37 | 0.64 |
| SO1200 | *tpiA* | triosephosphate isomerase | III | 0.32 | 0.36 | 0.45 |
| SO2347 | *gapA*-3 | glyceraldehyde 3-phosphate dehydrogenase | III | 0.57 | 0.28 | 0.54 |
| SO2644 | *ppsA* | phosphoenolpyruvate synthase | III | 0.51 | 0.24 | 0.71 |
| SO1927 | *sdhC* | succinate dehydrogenase, cytochrome b556 subunit | III | 0.45 | 0.20 | 0.57 |
| SO1928 | *sdhA* | succinate dehydrogenase, flavoprotein subunit | III | 0.72 | 0.28 | 0.62 |
| SO1929 | *sdhB* | succinate dehydrogenase, iron-sulfur protein | III | 1.08 | 0.45 | 0.78 |
| SO1930 | *sucA* | 2-oxoglutarate dehydrogenase, E1 component | III | 0.79 | 0.33 | 0.61 |
| SO1931 | *sucB* | 2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase | III | 0.60 | 0.37 | 0.57 |
| SO1932 | *sucC* | succinyl-CoA synthase, beta subunit | III | 0.62 | 0.36 | 0.42 |
| SO1933 | *sucD* | succinyl-CoA synthase, alpha subunit | III | 0.77 | 0.55 | 0.44 |
| SO0608 | *petA* | ubiquinol-cytochrome c reductase, iron-sulfur subunit | III | 0.51 | 0.42 | 1.00 |
| SO0609 | *petB* | ubiquinol-cytochrome c reductase, cytochrome b | III | 0.91 | 0.48 | 0.82 |
| SO0610 | *petC* | ubiquinol-cytochrome c reductase, cytochrome c1 | III | 1.03 | 0.44 | 0.72 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SO2303 | *trxB* | thioredoxin reductase | III | 0.84 | 0.39 | 0.43 |
| SO1251 | | ferredoxin, 4Fe-4S | III | 0.40 | 0.28 | 0.52 |
| SO2361 | *ccoP* | cytochrome c oxidase, cbb3-type, subunit III | III | 1.22 | 0.59 | 0.39 |
| SO2362 | *ccoQ* | cytochrome c oxidase, cbb3-type, CcoQ subunit | III | 1.32 | 0.51 | 0.32 |
| SO2363 | *ccoO* | cytochrome c oxidase, cbb3-type, subunit II | III | 1.28 | 0.52 | 0.41 |
| SO2364 | *ccoN* | cytochrome c oxidase, cbb3-type, subunit I | III | 1.27 | 0.44 | 0.41 |
| SO4295 | | NAD(P) dehydrogenase (quinone) | III | 0.56 | 0.41 | 0.29 |
| SO1012 | *nuoK* | NADH dehydrogenase I, K subunit | III | 0.94 | 0.87 | 0.44 |
| SO0779 | *gcvT* | glycine cleavage system T protein | III | 0.53 | 0.50 | 0.39 |
| SO0780 | *gcvH* | glycine cleavage system H protein | III | 0.82 | 0.60 | 0.43 |
| SO0781 | *gcvP* | glycine cleavage system P protein | III | 0.60 | 0.47 | 0.76 |
| SO2338 | *astE* | succinylglutamate desuccinylase | III | 0.42 | 0.29 | 0.50 |
| SO3365 | *glsA* | glutaminase A | III | 0.60 | 0.33 | 0.64 |
| SO2304 | *ald* | alanine dehydrogenase, authentic point mutation | III | 1.47 | 0.45 | 0.94 |
| SO4374 | | histidine ammonia-lyase, putative | III | 0.70 | 0.36 | 0.57 |
| SO4746 | *atpC* | ATP synthase F1, epsilon subunit | III | 1.14 | 0.76 | 0.20 |
| SO4747 | *atpD* | ATP synthase F1, beta subunit | III | 1.07 | 0.69 | 0.25 |
| SO4748 | *atpG* | ATP synthase F1, gamma subunit | III | 1.08 | 0.48 | 0.22 |
| SO4749 | *atpA* | ATP synthase F1, alpha subunit | III | 0.97 | 0.48 | 0.28 |
| SO4750 | *atpH* | ATP synthase F1, delta subunit | III | 1.03 | 0.47 | 0.32 |
| SO4751 | *atpF* | ATP synthase F0, B subunit | III | 1.02 | 0.40 | 0.24 |
| SO4752 | *atpE* | ATP synthase F0, C subunit | III | 1.05 | 0.36 | 0.23 |
| SO4753 | *atpB* | ATP synthase F0, A subunit | III | 1.01 | 0.36 | 0.24 |
| SO4754 | *atpI* | ATP synthase protein I | III | 1.03 | 0.45 | 0.32 |

[a] cluster analysis was performed using GeneSpring. The distance was calculated using the standard correlation. [b] the average induction fold at 5 (T5), 20 (T20) and 60 (T60) min. The data were presented with 99% confidence.

Supplemental Table 4.3 The relative expression of biosynthesis genes in MR-1 following solar radiation exposure

| ID | Gene | Gene product | Possible Function | [a]T5 | [a]T20 | [a]T60 |
|---|---|---|---|---|---|---|
| SO0275 | *argC* | N-acetyl-gamma-glutamyl-phosphate reductase | Amino acid biosynthesis | 0.41 | 0.33 | 0.09 |
| SO0276 | *argB* | acetylglutamate kinase | - | 0.40 | 0.43 | 0.10 |
| SO0277 | *argF* | ornithine carbamoyltransferase | - | 0.31 | 0.41 | 0.08 |
| SO0278 | *argG* | argininosuccinate synthase | - | 0.41 | 0.42 | 0.05 |
| SO0279 | *argH* | argininosuccinate lyase | - | 0.45 | 0.91 | 0.09 |
| SO0287 | *aroB* | 3-dehydroquinate synthase | - | 0.57 | 0.40 | 0.83 |
| SO0340 | *ilvE* | branched-chain amino acid aminotransferase | - | 0.98 | 0.33 | 0.83 |
| SO0617 | *argD* | acetylornithine aminotransferase | - | 1.82 | 1.32 | 2.18 |
| SO0818 | *metE* | 5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase | - | 0.54 | 0.18 | 0.08 |
| SO0862 | *serA* | D-3-phosphoglycerate dehydrogenase | - | 0.84 | 0.23 | 0.25 |
| SO1030 | *metH* | 5-methyltetrahydrofolate--homocysteine methyltransferase | - | 1.24 | 0.41 | 0.39 |
| SO1095 | | O-acetylhomoserine (thiol)-lyase, putative | - | 0.82 | 0.39 | 0.22 |
| SO1122 | *proA* | gamma-glutamyl phosphate reductase | - | 0.74 | 0.46 | 0.72 |
| SO1223 | *serB* | phosphoserine phosphatase | - | 0.89 | 0.44 | 0.77 |
| SO1268 | | glutamine synthetase | - | 3.94 | 2.60 | 4.14 |
| SO1324 | *gltD* | glutamate synthase, small subunit | - | 0.74 | 1.19 | 0.21 |
| SO1325 | *gltB* | glutamate synthase, large subunit | - | 0.72 | 0.59 | 0.21 |
| SO1361 | *aroF* | phospho-2-dehydro-3-deoxyheptonate aldolase, tyr-sensitive | - | 1.33 | 0.38 | 0.09 |
| SO1362 | *tyrA* | chorismate mutase/prephenate dehydrogenase | - | 0.77 | 0.86 | 0.24 |
| SO1367 | *pheA* | chorismate mutase/prephenate dehydratase | - | 3.66 | 2.92 | 3.41 |
| SO1625 | *dapD* | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase | - | 0.80 | 0.40 | 0.56 |
| SO1676 | *metA* | homoserine O-succinyltransferase | - | 0.93 | 0.52 | 0.38 |
| SO1879 | *dapA* | dihydrodipicolinate synthase | - | 0.74 | 0.47 | 0.66 |
| SO2070 | *hisH* | amidotransferase HisH | - | 0.47 | 1.16 | 1.61 |
| SO2073 | *hisD* | histidinol dehydrogenase | - | 1.32 | 2.41 | 1.53 |
| SO2074 | *hisG* | ATP phosphoribosyltransferase | - | 3.73 | 2.88 | 1.25 |
| SO2278 | *ilvH* | acetolactate synthase III, small subunit | - | 0.82 | 0.30 | 0.49 |
| SO2279 | *ilvI* | acetolactate synthase III, large subunit | - | 1.43 | 0.48 | 0.72 |
| SO2350 | asp*C*-1 | aspartate aminotransferase | - | 0.57 | 0.29 | 0.15 |
| SO2406 | asp*C*-2 | aspartate aminotransferase | - | 2.68 | 1.05 | 1.06 |

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|---|---|---|---|---|---|---|
| SO2903 | *cysK* | cysteine synthase A | - | 2.11 | 1.15 | 0.42 |
| SO3019 | *trpE* | anthranilate synthase component I | - | 7.25 | 2.92 | 1.70 |
| SO3020 | *trpG* | glutamine amido-transferase | - | 8.39 | 3.63 | 1.86 |
| SO3021 | *trpD* | anthranilate phosphoribosyltransferase | - | 5.56 | 2.74 | 1.72 |
| SO3022 | *trpC/F* | indole-3-glycerol phosphate synthase/phosphoribosylanthranilate isomerase | - | 2.18 | 2.48 | 1.54 |
| SO3023 | *trpB* | tryptophan synthase, beta subunit | - | 1.26 | 3.47 | 1.65 |
| SO3024 | *trpA* | tryptophan synthase, alpha subunit | - | 1.07 | 3.05 | 1.17 |
| SO3175 | asn*B*-2 | asparagine synthetase, glutamine-hydrolyzing | - | 0.75 | 0.71 | 0.43 |
| SO3986 | *lysC* | aspartokinase III, lysine-sensitive | - | 0.42 | 0.19 | 0.17 |
| SO4054 | *metF* | 5,10-methylenetetrahydrofolate reductase | - | 0.79 | 0.11 | 0.10 |
| SO4055 | *metL* | aspartokinase II/homoserine dehydrogenase, methionine-sensitive | - | 0.42 | 0.22 | 0.11 |
| SO4056 | *metB* | cystathionine gamma-synthase | - | 0.88 | 0.14 | 0.10 |
| SO4245 | *argA* | amino-acid acetyltransferase | - | 0.49 | 0.30 | 0.24 |
| SO4309 | *lysA* | diaminopimelate decarboxylase | - | 2.15 | 1.03 | 1.22 |
| SO4346 | *ilvM* | acetolactate synthase II, small subunit | - | 2.36 | 1.43 | 1.31 |
| SO4347 | *ilvG* | acetolactate synthase II, large subunit | - | 4.56 | 1.14 | 1.87 |
| SO4349 | *ilvC* | ketol-acid reductoisomerase | - | 1.05 | 0.21 | 0.18 |
| SO4410 | *glnA* | glutamine synthetase, type I | - | 0.43 | 0.56 | 0.22 |
| SO0027 | | protoporphyrinogen oxidase, putative | Biosynthesis of cofactors, prosthetic groups, and carriers | 1.12 | 3.52 | 0.30 |
| SO0142 | *ribB* | 3,4-dihydroxy-2-butanone 4-phosphate synthase | - | 1.43 | 0.40 | 0.36 |
| SO0435 | *hemE* | uroporphyrinogen decarboxylase | - | 1.07 | 0.60 | 0.33 |
| SO0741 | *ggt-1* | gamma-glutamyltranspeptidase | - | 2.14 | 1.73 | 2.72 |
| SO0869 | *panC* | pantoate--beta-alanine ligase | - | 0.76 | 0.41 | 0.58 |
| SO0870 | *panB* | 3-methyl-2-oxobutanoate hydroxymethyltransferase | - | 0.54 | 0.36 | 0.47 |
| SO0931 | *epd* | D-erythrose-4-phosphate dehydrogenase | - | 0.29 | 0.34 | 0.57 |
| SO1109 | *apbE* | thiamin biosynthesis lipoprotein ApbE | - | 0.53 | 0.40 | 0.61 |
| SO1161 | *lipA* | lipoic acid synthetase | - | 0.39 | 0.52 | 1.00 |
| SO1198 | *folP* | dihydropteroate synthase | - | 0.46 | 0.36 | 0.62 |
| SO1300 | *hemL* | glutamate-1-semialdehyde-2,1-aminomutase | - | 0.70 | 0.55 | 0.36 |
| SO1351 | *pdxJ* | pyridoxal phosphate biosynthetic protein PdxJ | - | 0.48 | 0.52 | 0.71 |
| SO1526 | *ispA* | geranyltranstransferase | - | 0.64 | 0.42 | 0.45 |
| SO1667 | | pterin-4-alpha-carbinolamine dehydratase | - | 1.66 | 2.89 | 2.33 |

| | | | | | | |
|---|---|---|---|---|---|---|
| SO1910 | | 1,4-dihydroxy-2-naphthoate octaprenyltransferase, putative | - | 0.74 | 0.40 | 0.39 |
| SO1952 | *ggt*-2 | gamma-glutamyltranspeptidase | - | 2.40 | 1.14 | 1.52 |
| SO2019 | *hemH*-1 | ferrochelatase | - | 0.77 | 0.72 | 0.32 |
| SO2221 | *pabB* | para-aminobenzoate synthase, component I | - | 0.51 | 0.43 | 1.24 |
| SO2264 | *iscS* | cysteine desulfurase | - | 0.88 | 0.87 | 5.51 |
| SO2410 | *serC* | phosphoserine aminotransferase | - | 0.62 | 0.41 | 0.71 |
| SO2443 | *thiF* | thiF protein, putative | - | 0.50 | 0.36 | 0.51 |
| SO2444 | *thiDE* | phosphomethylpyrimidine kinase/thiamin-phosphate pyrophosphorylase, putative | - | 0.64 | 0.47 | 0.86 |
| SO2567 | *menG*-1 | S-adenosylmethionine:2-demethylmenaquinone methyltransferase | - | 2.98 | 1.82 | 1.72 |
| SO2615 | *pabC* | 4-amino-4-deoxychorismate lyase | - | 0.59 | 0.39 | 0.76 |
| SO2737 | *bioD* | dethiobiotin synthase | - | 0.51 | 0.42 | 0.89 |
| SO2738 | *bioC* | biotin synthesis protein BioC | - | 0.55 | 0.37 | 0.96 |
| SO2739 | *bioF* | 8-amino-7-oxononanoate synthase | - | 0.71 | 0.38 | 0.95 |
| SO2740 | *bioB* | biotin synthase | - | 0.95 | 0.38 | 0.87 |
| SO2831 | *ribA* | GTP cyclohydrolase II | - | 0.49 | 0.68 | 1.37 |
| SO2921 | *folX* | D-erythro-7,8-dihydroneopterin triphosphate epimerase | - | 2.37 | 1.23 | 1.29 |
| SO3348 | *hemH*-2 | ferrochelatase | - | 5.92 | 4.98 | 11.60 |
| SO3464 | *thiL* | thiamin-monophosphate kinase | - | 0.30 | 0.25 | 0.31 |
| SO3466 | *ribH* | riboflavin synthase, beta subunit | - | 0.37 | 0.45 | 0.60 |
| SO3471 | *glyA* | serine hydroxymethyltransferase | - | 0.48 | 0.29 | 0.20 |
| SO3533 | *ribF* | riboflavin biosynthesis protein RibF | - | 0.73 | 0.49 | 1.05 |
| SO3559 | *gshA* | glutamate--cysteine ligase | - | 2.18 | 5.54 | 3.65 |
| SO3728 | *cobA* | uroporphyrin-III C-methyltransferase | - | 2.37 | 0.71 | 0.22 |
| SO3836 | *ispE* | 4-diphosphocytidyl-2C-methyl-D-erythritol kinase | - | 0.45 | 0.37 | 0.72 |
| SO4197 | *menG*-2 | S-adenosylmethionine:2-demethylmenaquinone methyltransferase | - | 1.67 | 2.12 | 1.01 |
| SO4199 | *ubiE* | ubiquinone/menaquinone biosynthesis methlytransferase UbiE | - | 1.01 | 0.87 | 0.43 |
| SO4201 | *aarF* | ubiquinone biosynthesis protein AarF | - | 0.65 | 0.45 | 0.40 |
| SO4208 | *hemB*-2 | delta-aminolevulinic acid dehydratase | - | 0.97 | 0.71 | 0.31 |
| SO4313 | *hemC* | porphobilinogen deaminase | - | 2.35 | 1.31 | 0.40 |
| SO4314 | *hemD* | uroporphyrinogen-III synthase | - | 1.24 | 0.99 | 0.44 |
| SO4449 | *moaE* | molybdenum cofactor biosynthesis protein E | - | 0.62 | 2.67 | 1.32 |
| SO4450 | *moaD* | molybdenum cofactor biosynthesis protein D | - | 0.78 | 2.95 | 1.25 |
| SO4451 | *moaC* | molybdenum cofactor biosynthesis protein C | - | 0.74 | 3.03 | 1.53 |

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|---|---|---|---|---|---|---|
| SO4452 | *moaA* | molybdenum cofactor biosynthesis protein A | - | 1.45 | 2.89 | 0.86 |
| SO4520 | | oxygen-independent coproporphyrinogen III oxidase, putative | - | 2.16 | 1.13 | 0.29 |
| SO4576 | | O-succinylbenzoic acid--CoA ligase, putative | - | 0.48 | 0.55 | 0.74 |
| SO4614 | *ctaB* | protoheme IX farnesyltransferase | - | 1.09 | 2.48 | 1.71 |
| SO4626 | *bioH* | bioH protein | - | 0.41 | 0.55 | 0.96 |
| SO4722 | *mobA* | molybdopterin-guanine dinucleotide biosynthesis protein | - | 0.63 | 0.43 | 0.40 |
| SO4723 | | molybdopterin biosynthesis MoeA protein, putative | - | 0.78 | 0.65 | 0.48 |
| SO4724 | | molybdenum cofactor biosynthesis protein A, putative | - | 0.49 | 0.50 | 0.47 |
| SO4730 | *hemN* | oxygen-independent coproporphyrinogen III oxidase | - | 1.48 | 1.28 | 0.37 |
| SO0007 | *rpmH* | ribosomal protein L34 | Protein synthesis | 0.36 | 0.37 | 0.59 |
| SO0196 | *selD* | selenide, water dikinase | - | 0.50 | 0.52 | 0.64 |
| SO0206 | *trmA* | tRNA (uracil-5-)-methyltransferase | - | 0.46 | 0.50 | 0.77 |
| SO0220 | *rplK* | ribosomal protein L11 | - | 0.58 | 0.36 | 0.54 |
| SO0221 | *rplA* | ribosomal protein L1 | - | 0.58 | 0.38 | 0.54 |
| SO0222 | *rplJ* | ribosomal protein L10 | - | 0.80 | 0.35 | 0.39 |
| SO0223 | *rplL* | ribosomal protein L7/L12 | - | 0.85 | 0.42 | 0.46 |
| SO0226 | *rpsL* | ribosomal protein S12 | - | 0.66 | 0.31 | 0.51 |
| SO0227 | *rpsG* | ribosomal protein S7 | - | 0.68 | 0.27 | 0.50 |
| SO0228 | *fusA*-1 | translation elongation factor G | - | 0.87 | 0.29 | 0.58 |
| SO0229 | *tufA* | translation elongation factor Tu | - | 0.94 | 1.68 | 2.34 |
| SO0230 | *rpsJ* | ribosomal protein S10 | - | 0.57 | 0.19 | 0.40 |
| SO0231 | *rplC* | ribosomal protein L3 | - | 0.61 | 0.26 | 0.52 |
| SO0232 | *rplD* | ribosomal protein L4 | - | 0.82 | 0.25 | 0.36 |
| SO0233 | *rplW* | ribosomal protein L23 | - | 1.00 | 0.24 | 0.40 |
| SO0234 | *rplB* | ribosomal protein L2 | - | 1.08 | 0.29 | 0.44 |
| SO0235 | *rpsS* | ribosomal protein S19 | - | 1.36 | 0.30 | 0.36 |
| SO0236 | *rplV* | ribosomal protein L22 | - | 1.41 | 0.32 | 0.39 |
| SO0237 | *rpsC* | ribosomal protein S3 | - | 1.37 | 0.37 | 0.36 |
| SO0238 | *rplP* | ribosomal protein L16 | - | 1.32 | 0.38 | 0.36 |
| SO0239 | *rpmC* | ribosomal protein L29 | - | 1.37 | 0.41 | 0.26 |
| SO0240 | *rpsQ* | ribosomal protein S17 | - | 1.28 | 0.45 | 0.32 |
| SO0241 | *rplN* | ribosomal protein L14 | - | 0.66 | 0.30 | 0.61 |
| SO0242 | *rplX* | ribosomal protein L24 | - | 0.65 | 0.30 | 0.62 |

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|---|---|---|---|---|---|---|
| SO0243 | *rplE* | ribosomal protein L5 | - | 0.72 | 0.32 | 0.59 |
| SO0244 | *rpsN* | ribosomal protein S14 | - | 0.70 | 0.31 | 0.59 |
| SO0245 | *rpsH* | ribosomal protein S8 | - | 0.89 | 0.35 | 0.60 |
| SO0246 | *rplF* | ribosomal protein L6 | - | 0.95 | 0.34 | 0.57 |
| SO0247 | *rplR* | ribosomal protein L18 | - | 1.07 | 0.36 | 0.55 |
| SO0248 | *rpsE* | ribosomal protein S5 | - | 1.15 | 0.42 | 0.55 |
| SO0249 | *rpmD* | ribosomal protein L30 | - | 1.23 | 0.43 | 0.50 |
| SO0250 | *rplO* | ribosomal protein L15 | - | 1.26 | 0.44 | 0.52 |
| SO0252 | *rpmJ* | ribosomal protein L36 | - | 1.32 | 0.46 | 0.44 |
| SO0255 | *rpsD* | ribosomal protein S4 | - | 0.94 | 0.46 | 0.51 |
| SO0257 | *rplQ* | ribosomal protein L17 | - | 0.90 | 0.38 | 0.41 |
| SO0842 | *fusA*-2 | translation elongation factor G | - | 0.34 | 0.27 | 0.80 |
| SO0991 | *prfB* | peptide chain release factor 2, programmed frameshift | - | 0.46 | 0.34 | 0.75 |
| SO0992 | *lysS* | lysyl-tRNA synthetase | - | 0.99 | 0.50 | 0.77 |
| SO1184 | *pth* | peptidyl-tRNA hydrolase | - | 0.39 | 0.48 | 0.72 |
| SO1211 | *prfC* | peptide chain release factor 3, authentic frameshift | - | 0.35 | 0.27 | 0.65 |
| SO1288 | *rpsU* | ribosomal protein S21 | - | 0.40 | 0.21 | 0.44 |
| SO1315 | *tyrS* | tyrosyl-tRNA synthetase | - | 1.13 | 0.47 | 0.82 |
| SO1357 | *rpsP* | ribosomal protein S16 | - | 0.54 | 0.22 | 0.39 |
| SO1359 | *trmD* | tRNA (guanine-N1)-methyltransferase | - | 0.65 | 0.25 | 0.42 |
| SO1360 | *rplS* | ribosomal protein L19 | - | 0.80 | 0.23 | 0.31 |
| SO1575 | | NOL1/NOP2/sun family putative RNA methylase | - | 0.39 | 0.42 | 0.56 |
| SO1629 | *rpsB* | ribosomal protein S2 | - | 0.81 | 0.22 | 0.40 |
| SO1630 | *tsf* | translation elongation factor Ts | - | 0.83 | 0.23 | 0.35 |
| SO1632 | *frr* | ribosome recycling factor | - | 0.64 | 0.46 | 0.50 |
| SO1855 | *rmf* | ribosome modulation factor | - | 1.21 | 3.34 | 2.34 |
| SO2085 | *pheS* | phenylalanyl-tRNA synthetase, alpha subunit | - | 0.65 | 0.45 | 1.02 |
| SO2112 | *rplY* | ribosomal protein L25 | - | 0.40 | 0.40 | 0.44 |
| SO2215 | | sun protein, putative | - | 0.32 | 0.40 | 0.70 |
| SO2218 | *asnS* | asparaginyl-tRNA synthetase | - | 0.84 | 0.35 | 0.93 |
| SO2261 | | RNA methyltransferase, TrmH family, group 1 | - | 0.39 | 0.29 | 0.60 |
| SO2270 | *rimK*-2 | ribosomal protein S6 modification protein | - | 0.28 | 0.52 | 0.77 |
| SO2300 | *infC* | translation initiation factor IF-3 | - | 0.56 | 0.45 | 0.61 |

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|---|---|---|---|---|---|---|
| SO2328 | *efp* | translation elongation factor P | - | 0.63 | 0.28 | 0.43 |
| SO2402 | *rpsA* | ribosomal protein S1 | - | 0.93 | 0.48 | 0.75 |
| SO2619 | *metG* | methionyl-tRNA synthetase | - | 0.52 | 0.35 | 0.77 |
| SO2625 | *infA* | translation initiation factor IF-1 | - | 0.34 | 0.33 | 0.63 |
| SO2633 | *trmU* | tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase | - | 0.35 | 0.47 | 0.82 |
| SO2780 | *rpmF* | ribosomal protein L32 | - | 0.71 | 0.44 | 0.54 |
| SO2784 | *rluC* | ribosomal large subunit pseudouridine synthase C | - | 0.48 | 0.60 | 1.02 |
| SO3130 | *gltX* | glutamyl-tRNA synthetase, authentic point mutation | - | 0.78 | 0.41 | 0.66 |
| SO3154 | *proS* | prolyl-tRNA synthetase | - | 0.85 | 0.44 | 0.72 |
| SO3403 | *yfiA*-1 | ribosomal subunit interface protein | - | 3.93 | 8.18 | 1.36 |
| SO3422 | *yfiA*-2 | ribosomal subunit interface protein | - | 1.72 | 20.29 | 2.29 |
| SO3532 | *ileS* | isoleucyl-tRNA synthetase | - | 0.85 | 0.38 | 0.77 |
| SO3651 | *rpmA* | ribosomal protein L27 | - | 0.37 | 0.45 | 0.50 |
| SO3652 | *rplU* | ribosomal protein L21 | - | 0.26 | 0.36 | 0.53 |
| SO3843 | *rsuA*-2 | ribosomal small subunit pseudouridine synthase A | - | 0.43 | 0.42 | 0.77 |
| SO3927 | *rplI* | ribosomal protein L9 | - | 0.74 | 0.36 | 0.43 |
| SO3928 | *rpsR* | ribosomal protein S18 | - | 0.75 | 0.35 | 0.35 |
| SO3930 | *rpsF* | ribosomal protein S6 | - | 0.60 | 0.33 | 0.44 |
| SO3939 | *rpsI* | ribosomal protein S9 | - | 0.68 | 0.38 | 0.51 |
| SO3940 | *rplM* | ribosomal protein L13 | - | 0.57 | 0.37 | 0.57 |
| SO4123 | *argS* | arginyl-tRNA synthetase | - | 0.73 | 0.41 | 0.72 |
| SO4246 | *rpmG* | ribosomal protein L33 | - | 1.11 | 0.41 | 0.51 |
| SO4247 | *rpmB* | ribosomal protein L28 | - | 1.01 | 0.45 | 0.56 |
| SO4426 | | RNA pseudouridylate synthase family protein | - | 0.48 | 0.53 | 1.01 |
| SO4529 | | RNA methyltransferase, TrmH family, group 2 | - | 0.34 | 0.36 | 0.64 |

[a] the average induction fold at 5 (T5), 20 (T20) and 60 min (T60). The data were presented with 99% confidence.

Supplemental Table 5.1 The relative gene expression of up-regulated gene following ionizing radiation exposure in MR-1

| ID | Gene | Gene product | Possible Function | [a]T5 | [a]T20 | [a]T60 |
|---|---|---|---|---|---|---|
| SO3646 | *folA* | dihydrofolate reductase | Biosynthesis of cofactors, prosthetic groups, and carriers | 1.3 | 1.3 | 2.0 |
| SO0150 | | lipoprotein, putative | Cell envelope | 1.9 | 1.5 | 2.1 |
| SO0659 | | lysozyme, putative | - | 1.0 | 1.0 | 4.5 |
| SO1215 | | outer membrane protein OmpK, putative | - | 1.6 | 2.2 | 1.1 |
| SO3916 | *alr* | alanine racemase, biosynthetic | - | 1.0 | 1.1 | 2.2 |
| SO0725 | *katG*-1 | catalase/peroxidase HPI | Cellular processes | 5.5 | 1.6 | 1.1 |
| SO0956 | *ahpF* | alkyl hydroperoxide reductase, F subunit | - | 3.9 | 1.1 | 1.0 |
| SO0958 | *ahpC* | alkyl hydroperoxide reductase, C subunit | - | 11.0 | 1.9 | 1.0 |
| SO1070 | *katB* | catalase | - | 12.6 | 1.1 | 1.2 |
| SO1158 | | Dps family protein | - | 3.0 | 1.2 | 1.0 |
| SO2178 | *ccpA* | cytochrome c551 peroxidase | - | 3.8 | 1.3 | 0.9 |
| SO4163 | *hslU* | heat shock protein HslVU, ATPase subunit HslU | - | 0.9 | 1.1 | 3.3 |
| SO4697 | *gst* | glutathione S-transferase | Central intermediary metabolism | 0.7 | 1.3 | 3.6 |
| SO4422 | | siderophore receptor, putative, degenerate | Disrupted reading frame | 1.3 | 2.6 | 2.4 |
| SO0690 | | type II DNA modification methyltransferase, putative | DNA metabolism | 0.9 | 0.9 | 4.4 |
| SO1286 | *dnaG* | DNA primase | - | 1.3 | 1.5 | 2.1 |
| SO1330 | *mutH* | DNA mismatch repair protein MutH | - | 1.4 | 1.3 | 2.4 |
| SO1817 | | primosomal replication protein n, putative | - | 2.2 | 2.0 | 1.2 |
| SO1819 | *dinG* | ATP-dependent helicase DinG | - | 3.2 | 3.3 | 2.7 |
| SO1820 | *polB* | DNA polymerase II | - | 3.3 | 4.6 | 2.8 |
| SO2993 | | prophage LambdaSo, type II DNA modification methyltransferase, putative, truncation | - | 1.1 | 23.3 | 45.1 |
| SO3004 | | prophage LambdaSo, DNA modification methyltransferase, putative | - | 0.9 | 6.2 | 20.5 |
| SO3006 | | prophage LambdaSo, type II DNA modification methyltransferase, putative | - | 1.0 | 4.8 | 18.9 |
| SO3013 | | site-specific recombinase, phage integrase family | - | 1.2 | 1.4 | 2.6 |
| SO3061 | *topB* | DNA topoisomerase III | - | 3.4 | 7.7 | 7.1 |
| SO3429 | *recX* | regulatory protein RecX | - | 6.7 | 8.0 | 6.6 |
| SO3430 | *recA* | recA protein | - | 6.9 | 8.8 | 6.5 |

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|---|---|---|---|---|---|---|
| SO3462 | *recN* | DNA repair protein RecN | - | 11.6 | 13.7 | 12.4 |
| SO3917 | *dnaB* | replicative DNA helicase | - | 0.9 | 1.1 | 2.6 |
| SO4364 | *recG* | ATP-dependent DNA helicase RecG | - | 1.8 | 2.4 | 2.2 |
| SO4669 | *polA* | DNA polymerase I | - | 1.1 | 1.2 | 3.3 |
| SOA0012 | *umuC* | umuC protein | - | 3.5 | 7.9 | 8.0 |
| SOA0013 | *umuD* | umuD protein | - | 3.2 | 14.3 | 15.6 |
| SO0406 | *trxA* | thioredoxin 1 | Energy metabolism | 1.1 | 1.2 | 2.1 |
| SO0452 | *trxC* | thioredoxin 2 | - | 2.0 | 1.7 | 2.4 |
| SO1217 | *deoC* | deoxyribose-phosphate aldolase | - | 2.3 | 2.0 | 1.0 |
| SO1493 | *malQ* | 4-alpha-glucanotransferase | - | 1.5 | 2.3 | 1.1 |
| SO3057 | | Pal/histidase family protein | - | 0.8 | 1.0 | 3.0 |
| SO3861 | | iron-sulfur cluster-binding protein | - | 1.1 | 1.1 | 3.8 |
| SO4480 | *aldA* | aldehyde dehydrogenase | - | 1.1 | 2.1 | 1.5 |
| SO4606 | | cytochrome c oxidase, subunit II | - | 3.1 | 1.9 | 1.5 |
| SO0054 | | conserved hypothetical protein TIGR00275 | Unknown function | 1.2 | 1.4 | 2.1 |
| SO0394 | | conserved hypothetical protein | - | 1.4 | 1.9 | 2.6 |
| SO0648 | | conserved hypothetical protein | - | 0.8 | 2.7 | 15.4 |
| SO0651 | | conserved hypothetical protein | - | 1.2 | 2.1 | 5.8 |
| SO0676 | | conserved hypothetical protein | - | 1.0 | 1.2 | 12.5 |
| SO0677 | | conserved hypothetical protein | - | 1.1 | 1.0 | 12.3 |
| SO1556 | | conserved hypothetical protein | - | 1.1 | 1.2 | 3.2 |
| SO1757 | | conserved hypothetical protein | - | 10.1 | 14.8 | 8.7 |
| SO1758 | | conserved hypothetical protein | - | 6.4 | 7.4 | 5.0 |
| SO1759 | | conserved hypothetical protein | - | 5.5 | 6.4 | 4.4 |
| SO1818 | | conserved hypothetical protein | - | 2.8 | 2.9 | 2.2 |
| SO1977 | | conserved hypothetical protein | - | 2.5 | 1.3 | 0.8 |
| SO2603 | | conserved hypothetical protein | - | 4.5 | 5.3 | 3.4 |
| SO2604 | | conserved hypothetical protein | - | 5.2 | 5.8 | 3.9 |
| SO2660 | | conserved hypothetical protein | - | 0.7 | 5.3 | 14.7 |
| SO2663 | | conserved hypothetical protein | - | 0.6 | 3.4 | 14.5 |
| SO2667 | | conserved hypothetical protein | - | 0.9 | 3.4 | 11.1 |
| SO2689 | | conserved hypothetical protein | - | 1.1 | 1.0 | 2.3 |
| SO2702 | | conserved hypothetical protein | - | 1.1 | 1.0 | 3.8 |

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|---|---|---|---|---|---|
| SO2863 | conserved hypothetical protein | - | 1.0 | 0.7 | 2.6 |
| SO2955 | conserved hypothetical protein | - | 1.0 | 1.1 | 23.6 |
| SO2957 | conserved hypothetical protein | - | 1.2 | 1.3 | 18.4 |
| SO2958 | conserved hypothetical protein | - | 1.1 | 1.8 | 19.3 |
| SO2960 | conserved hypothetical protein | - | 1.1 | 1.4 | 24.6 |
| SO2961 | conserved hypothetical protein | - | 1.1 | 1.0 | 24.3 |
| SO2966 | conserved hypothetical protein | - | 1.0 | 1.0 | 7.6 |
| SO2967 | conserved hypothetical protein | - | 1.2 | 1.6 | 26.5 |
| SO2968 | conserved hypothetical protein | - | 1.1 | 0.9 | 18.8 |
| SO2971 | conserved hypothetical protein | - | 1.1 | 0.9 | 27.2 |
| SO2976 | conserved hypothetical protein | - | 1.0 | 3.0 | 16.9 |
| SO2984 | conserved hypothetical protein | - | 1.0 | 10.9 | 22.4 |
| SO2988 | conserved hypothetical protein | - | 1.1 | 15.2 | 38.4 |
| SO3000 | conserved hypothetical protein | - | 1.0 | 22.7 | 40.9 |
| SO3002 | conserved hypothetical protein | - | 1.2 | 11.6 | 29.7 |
| SO3005 | conserved hypothetical protein | - | ND | 6.0 | 26.2 |
| SO3014 | conserved hypothetical protein | - | 1.1 | 1.2 | 2.1 |
| SO3085 | conserved domain protein | - | 1.2 | 0.8 | 2.3 |
| SO3326 | conserved hypothetical protein | - | 1.4 | 2.6 | 2.3 |
| SO3364 | conserved hypothetical protein | - | 2.1 | 2.2 | 1.8 |
| SO3647 | conserved hypothetical protein | - | 1.3 | 1.4 | 2.9 |
| SO3648 | conserved hypothetical protein | - | 1.3 | 1.5 | 3.3 |
| SO3941 | conserved hypothetical protein | - | 0.9 | 1.0 | 2.4 |
| SO4164 | conserved hypothetical protein | - | 0.9 | 1.0 | 2.8 |
| SO4604 | conserved hypothetical protein | - | 10.9 | 13.2 | 11.2 |
| SO4698 | conserved hypothetical protein | - | 1.0 | 1.1 | 3.0 |
| SO4715 | conserved hypothetical protein | - | 1.5 | 1.5 | 2.6 |
| SOA0059 | conserved hypothetical protein | - | 1.1 | 1.2 | 2.2 |
| SOA0069 | conserved hypothetical protein | - | 1.1 | 1.2 | 3.7 |
| SOA0072 | conserved hypothetical protein | - | 1.2 | 1.1 | 3.8 |
| SO0643 | transposase, putative | Other categories | 1.2 | 4.0 | 8.5 |
| SO0644 | prophage MuSo1, DNA transposition protein, putative | - | 1.3 | 4.9 | 21.4 |
| SO0652 | prophage MuSo1, positive regulator of late transcription, putative | - | 1.4 | 2.7 | 8.5 |

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|---|---|---|---|---|---|---|
| SO0666 | | prophage MuSo1, portal protein, putative | - | 1.0 | 1.1 | 2.2 |
| SO0674 | | prophage MuSo1, protein Gp32, putative | - | 1.2 | 1.1 | 19.2 |
| SO0675 | | prophage MuSo1, major head subunit, putative | - | 1.1 | 1.0 | 21.7 |
| SO2654 | | transposase, putative | - | 1.0 | 12.8 | 15.9 |
| SO2655 | | prophage MuSo2, DNA transposition protein, putative | - | 1.1 | 16.5 | 22.3 |
| SO2668 | | prophage MuSo2, positive regulator of late transcription, putative | - | 0.8 | 1.0 | 5.6 |
| SO2684 | | prophage MuSo2, protein Gp32, putative | - | 1.0 | 1.2 | 4.1 |
| SO2685 | | prophage MuSo2, major head subunit, putative | - | 1.2 | 1.0 | 4.8 |
| SO2693 | | prophage MuSo2, tail sheath protein, putative | - | 1.1 | 0.9 | 2.5 |
| SO2940 | | prophage LambdaSo, host specificity protein J, putative | - | 1.1 | 1.0 | 7.7 |
| SO2941 | | prophage LambdaSo, tail assembly protein I | - | 1.0 | 0.8 | 10.2 |
| SO2948 | | prophage LambdaSo, tail assembly protein K, putative | - | 1.3 | 1.0 | 12.0 |
| SO2949 | | prophage LambdaSo, minor tail protein L | - | 1.1 | 0.9 | 9.3 |
| SO2952 | | prophage LambdaSo, minor tail protein M, putative | - | 1.1 | 0.9 | 10.1 |
| SO2953 | *H* | prophage LambdaSo, tail length tape meausure protein | - | 1.0 | 1.0 | 9.7 |
| SO2956 | | prophage LambdaSo, major tail protein V, putative | - | 1.0 | 3.1 | 34.1 |
| SO2963 | | prophage LambdaSo, major capsid protein, HK97 family | - | 1.1 | 1.3 | 25.9 |
| SO2965 | | prophage LambdaSo, portal protein, HK97 family | - | 1.1 | 1.1 | 33.8 |
| SO2969 | | prophage LambdaSo, holin, putative | - | 1.1 | 1.1 | 23.6 |
| SO2973 | | prophage LambdaSo, lysozyme, putative | - | 0.9 | 1.3 | 34.7 |
| SO2978 | | prophage LambdaSo, site-specific recombinase, phage integrase family | - | 0.9 | 3.0 | 21.6 |
| SO2985 | *O* | prophage LambdaSo, replication protein O | - | 1.0 | 15.5 | 24.3 |
| SOA0023 | *higA* | proteic killer suppressor protein | - | 1.1 | 1.1 | 2.5 |
| SO0704 | *groEL* | chaperonin GroEL | Protein fate | 1.0 | 1.0 | 2.1 |
| SO1115 | *pepD* | aminoacyl-histidine dipeptidase | - | 2.1 | 4.6 | 4.0 |
| SO2964 | | ClpP protease family protein | - | 1.1 | 1.0 | 25.0 |
| SO3577 | *clpB* | clpB protein | - | 1.2 | 0.9 | 2.9 |
| SO4162 | *hslV* | ATP-dependent protease HslV | - | 1.0 | 1.0 | 3.8 |
| SO4699 | *prlC* | oligopeptidase A | - | 0.9 | 1.1 | 2.9 |
| SO0442 | *purH* | phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase | Purines, pyrimidines, nucleosides, and nucleotides | 1.0 | 1.5 | 2.1 |
| SO2001 | *ushA* | 5-nucleotidase | - | 2.5 | 3.0 | 0.9 |

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|---|---|---|---|---|---|---|
| SO0393 | *fis* | DNA-binding protein Fis | Regulatory functions | 1.3 | 2.1 | 2.5 |
| SO1762 | | transcriptional regulator, AraC/XylS family | - | 3.6 | 3.1 | 2.9 |
| SO2653 | | transcriptional regulator, Ner family | - | ND | 4.5 | 15.9 |
| SO4603 | *lexA* | LexA repressor | - | 11.0 | 12.7 | 11.3 |
| SO1066 | | extracellular nuclease | Transcription | 1.7 | 2.1 | 1.1 |
| SO0760 | *amt* | ammonium transporter | Transport and binding proteins | 1.2 | 2.0 | 1.0 |
| SO1072 | | chitin-binding protein, putative | - | 2.3 | 0.9 | 1.0 |
| SO1145 | *mgtE*-1 | magnesium transporter | - | 0.9 | 1.5 | 5.1 |
| SO1156 | | TonB-dependent receptor | - | 1.0 | 1.2 | 2.7 |
| SO1214 | | NupC family protein | - | 1.8 | 2.1 | 0.9 |
| SO1760 | | AzlC family protein | - | 2.8 | 3.7 | 2.1 |
| SO3060 | | outer membrane porin, putative | - | 2.5 | 5.6 | 6.4 |
| SO3134 | *dctP* | C4-dicarboxylate-binding periplasmic protein | - | 2.5 | 2.4 | 1.6 |
| SO3801 | | ABC transporter, permease protein | - | 1.0 | 1.0 | 2.8 |
| SO3802 | | ABC transporter, ATP-binding protein | - | 1.0 | 1.0 | 2.7 |
| SO0037 | | Sua5/YciO/YrdC/YwlC family protein | Unknown function | 1.2 | 1.2 | 2.1 |
| SO0661 | | zinc finger-related protein | - | 0.9 | 0.7 | 2.1 |
| SO0698 | *fsxA* | fxsA protein | - | 1.0 | 0.9 | 2.6 |
| SO1114 | *dinP* | DNA-damage-inducible protein P | - | 5.1 | 9.8 | 7.3 |
| SO1756 | | glyoxalase family protein | - | 6.5 | 12.9 | 6.8 |
| SO1976 | | hydrolase, alpha/beta fold family | - | 2.0 | 1.2 | 1.1 |
| SO2228 | | CBS domain protein | - | 2.2 | 0.8 | 0.5 |
| SO3649 | | GTP-binding protein, GTP1/Obg family | - | 1.2 | 1.3 | 3.3 |
| SO3926 | | GTP-binding protein | - | 1.4 | 1.5 | 2.1 |
| SO0089 | | hypothetical protein | Unknown function | 2.3 | 5.0 | 4.6 |
| SO0158 | | hypothetical protein | - | 1.3 | 1.6 | 2.5 |
| SO0645 | | hypothetical protein | - | 1.0 | 3.2 | 12.2 |
| SO0646 | | hypothetical protein | - | 1.3 | 3.0 | 11.6 |
| SO0647 | | hypothetical protein | - | ND | 2.8 | 7.5 |
| SO0649 | | hypothetical protein | - | 0.7 | 2.4 | 9.8 |
| SO0650 | | hypothetical protein | - | ND | 1.4 | 5.0 |
| SO0653 | | hypothetical protein | - | 0.9 | 1.1 | 4.0 |
| SO0658 | | hypothetical protein | - | 1.1 | 1.1 | 3.5 |

| | | | | | |
|---|---|---|---|---|---|
| SO0660 | hypothetical protein | - | 1.1 | 1.2 | 3.1 |
| SO0678 | hypothetical protein | - | 1.1 | 0.8 | 22.2 |
| SO0679 | hypothetical protein | - | 1.0 | 1.2 | 11.9 |
| SO0680 | hypothetical protein | - | 1.1 | 1.3 | 5.5 |
| SO0681 | hypothetical protein | - | ND | ND | 2.5 |
| SO0682 | hypothetical protein | - | 1.0 | 1.0 | 5.1 |
| SO0683 | hypothetical protein | - | 1.0 | 1.0 | 3.1 |
| SO0689 | hypothetical protein | - | 0.9 | 0.9 | 2.1 |
| SO0724 | hypothetical protein | - | 2.2 | 1.1 | ND |
| SO0836 | hypothetical protein | - | ND | 2.5 | 2.0 |
| SO0957 | hypothetical protein | - | 2.2 | 1.1 | 1.1 |
| SO1116 | hypothetical protein | - | 2.2 | 4.3 | 3.6 |
| SO1146 | hypothetical protein | - | 1.6 | 1.5 | 4.6 |
| SO1761 | hypothetical protein | - | 4.2 | 2.4 | 4.0 |
| SO2343 | hypothetical protein | - | 1.2 | 3.1 | 8.1 |
| SO2656 | hypothetical protein | - | 0.8 | 8.4 | 18.0 |
| SO2657 | hypothetical protein | - | 0.7 | 4.9 | 11.1 |
| SO2658 | hypothetical protein | - | 0.8 | 3.9 | 11.5 |
| SO2659 | hypothetical protein | - | 0.9 | 5.6 | 13.6 |
| SO2661 | hypothetical protein | - | 1.1 | 3.9 | 13.0 |
| SO2662 | hypothetical protein | - | 1.3 | 3.5 | 8.3 |
| SO2664 | hypothetical protein | - | ND | 2.6 | 7.8 |
| SO2665 | hypothetical protein | - | 0.7 | 3.6 | 6.2 |
| SO2666 | hypothetical protein | - | 0.9 | 2.1 | 7.2 |
| SO2669 | hypothetical protein | - | 0.7 | 1.0 | 2.7 |
| SO2670 | hypothetical protein | - | 0.9 | 0.8 | 2.4 |
| SO2673 | hypothetical protein | - | 1.1 | 1.0 | 2.1 |
| SO2687 | hypothetical protein | - | 1.0 | 0.9 | 3.1 |
| SO2688 | hypothetical protein | - | 1.1 | 1.0 | 2.5 |
| SO2691 | hypothetical protein | - | 0.9 | 0.9 | 2.2 |
| SO2696 | hypothetical protein | - | 0.9 | 1.3 | 2.8 |
| SO2936 | hypothetical protein | - | 1.0 | 1.0 | 2.2 |
| SO2938 | hypothetical protein | - | 0.8 | 0.7 | 2.7 |

| | | | | | |
|---|---|---|---|---|---|
| SO2939 | hypothetical protein | - | 0.9 | 0.8 | 4.7 |
| SO2942 | hypothetical protein | - | 0.9 | 0.8 | 4.6 |
| SO2944 | hypothetical protein | - | 1.0 | 0.9 | 7.5 |
| SO2945 | hypothetical protein | - | 1.0 | 1.0 | 13.9 |
| SO2946 | hypothetical protein | - | 1.2 | 0.9 | 11.4 |
| SO2947 | hypothetical protein | - | 0.7 | 0.8 | 12.9 |
| SO2950 | hypothetical protein | - | 1.1 | 1.1 | 8.0 |
| SO2951 | hypothetical protein | - | 1.1 | 1.0 | 13.4 |
| SO2954 | hypothetical protein | - | 0.9 | 1.4 | 16.5 |
| SO2959 | hypothetical protein | - | 1.3 | 0.8 | 19.7 |
| SO2962 | hypothetical protein | - | 1.2 | 1.0 | 27.1 |
| SO2970 | hypothetical protein | - | 1.1 | 1.8 | 25.3 |
| SO2972 | hypothetical protein | - | 1.1 | 1.1 | 29.4 |
| SO2974 | hypothetical protein | - | 1.1 | 0.4 | 39.5 |
| SO2975 | hypothetical protein | - | 0.8 | 1.4 | 7.8 |
| SO2977 | hypothetical protein | - | ND | 2.8 | 15.1 |
| SO2979 | hypothetical protein | - | 1.0 | 2.5 | 11.8 |
| SO2980 | hypothetical protein | - | 1.0 | 4.6 | 23.2 |
| SO2981 | hypothetical protein | - | ND | 2.9 | 19.6 |
| SO2982 | hypothetical protein | - | 1.1 | 4.1 | 16.6 |
| SO2983 | hypothetical protein | - | 1.0 | 7.8 | 25.3 |
| SO2986 | hypothetical protein | - | 1.1 | 8.1 | 20.4 |
| SO2987 | hypothetical protein | - | ND | 6.9 | 26.5 |
| SO2995 | hypothetical protein | - | 0.8 | 11.4 | 23.1 |
| SO2996 | hypothetical protein | - | 1.0 | 1.9 | 3.2 |
| SO2997 | hypothetical protein | - | 0.8 | 16.5 | 23.6 |
| SO2998 | hypothetical protein | - | 0.8 | 14.8 | 22.7 |
| SO2999 | hypothetical protein | - | 1.1 | 7.5 | 14.5 |
| SO3001 | hypothetical protein | - | 1.0 | 12.9 | 36.5 |
| SO3003 | hypothetical protein | - | ND | 7.7 | 36.1 |
| SO3007 | hypothetical protein | - | ND | ND | 3.4 |
| SO3008 | hypothetical protein | - | 1.0 | 3.0 | 18.9 |
| SO3009 | hypothetical protein | - | 1.1 | 3.2 | 22.3 |

| | | | | | |
|---|---|---|---|---|---|
| SO3010 | hypothetical protein | - | 1.1 | 2.4 | 12.0 |
| SO3011 | hypothetical protein | - | 1.1 | 3.0 | 18.0 |
| SO3012 | hypothetical protein | - | 1.2 | 2.9 | 13.5 |
| SO3132 | hypothetical protein | - | 1.9 | 2.2 | 1.6 |
| SO3133 | hypothetical protein | - | 1.2 | 1.8 | 2.8 |
| SO3327 | hypothetical protein | - | 2.5 | 2.8 | 2.2 |
| SO3910 | hypothetical protein | - | 1.9 | 2.1 | 2.0 |
| SO4137 | hypothetical protein | - | 1.2 | 1.5 | 2.3 |
| SO4161 | hypothetical protein | - | 1.0 | 1.0 | 2.4 |
| SO4210 | hypothetical protein | - | 1.5 | 2.1 | 3.3 |
| SO4605 | hypothetical protein | - | 3.8 | 8.4 | 4.3 |
| SO4644 | hypothetical protein | - | 1.1 | 1.2 | 3.1 |
| SO4736 | hypothetical protein | - | 1.3 | 1.5 | 2.3 |
| SOA0067 | hypothetical protein | - | 1.1 | 1.1 | 2.6 |

[a]the average induction fold at 5 (T5), 20 (T20) and 60 min (T60). The data were presented with 95% confidence. ND: no data.

Supplemental table 5.2 The relative expression of genes involved in defending against oxidative stress in MR-1 following ionizing radiation exposure

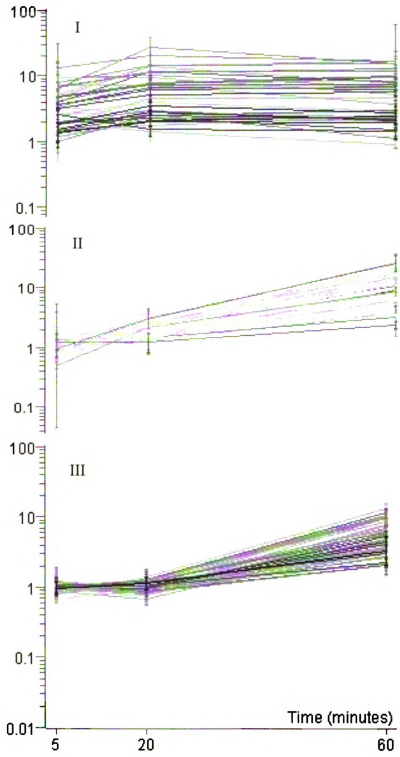
| ID | Gene | Gene product | [a]T5 | [a]T20 | [a]T60 |
|---|---|---|---|---|---|
| SO0139 | *ftn* | ferritin | 1.0 | 1.3 | 1.1 |
| SO1111 | *bfr*2 | bacterioferritin subunit 2 | 0.8 | 0.9 | 0.6 |
| SO1112 | *bfr*1 | bacterioferritin subunit 1 | 0.8 | 0.8 | 0.6 |
| SO0583 | *bfd* | bacterioferritin-associated ferredoxin | 1.1 | 1.0 | 1.1 |
| SO1877 | *bcp* | bacterioferritin comigratory protein | 0.9 | 1.0 | 0.8 |
| SO2881 | *sodB* | superoxide dismutase, Fe | 1.5 | 0.8 | 0.6 |
| SO0725 | *katG*-1 | catalase/peroxidase HPI | 5.5 | 1.6 | 1.1 |
| SO4405 | *katG*-2 | catalase/peroxidase HPI | 0.7 | 0.8 | 0.7 |
| SO1070 | *katB* | catalase | 12.6 | 1.1 | 1.2 |
| SO1773 | | catalase-related protein, authentic point mutation | 0.9 | 1.0 | 0.6 |
| SO0956 | *ahpF* | alkyl hydroperoxide reductase, F subunit | 3.9 | 1.1 | 1.1 |
| SO0958 | *ahpC* | alkyl hydroperoxide reductase, C subunit | 11.0 | 1.9 | 1.0 |
| SO2756 | | antioxidant, AhpC/Tsa family | 1.0 | 0.9 | 0.8 |
| SO3341 | | antioxidant, AhpC/Tsa family | 1.0 | 0.7 | 0.6 |
| SO4640 | | antioxidant, AhpC/Tsa family | 1.0 | 1.1 | 0.9 |
| SO0976 | *ohr* | organic hydroperoxide resistance protein | 0.9 | 0.8 | 0.9 |
| SO1158 | | Dps family protein | 3.0 | 1.2 | 1.0 |
| SO2178 | *ccpA* | cytochrome c551 peroxidase | 3.8 | 1.3 | 0.9 |
| SO3349 | | glutathione peroxidase | 0.9 | 0.9 | 0.6 |
| SO1563 | | glutathione peroxidase | 1.2 | 1.2 | 0.9 |
| SO4697 | *gst* | glutathione S-transferase | 0.8 | 1.3 | 3.6 |
| SO1576 | | glutathione S-transferase family protein | 0.8 | 1.0 | 0.6 |
| SO1577 | | glutathione S-transferase family protein | 1.0 | 0.9 | 0.7 |
| SO1671 | | glutathione S-transferase family protein | 1.1 | 0.9 | 0.7 |
| SO0746 | | glutathione S-transferase family protein | 0.7 | 0.8 | 0.5 |
| SO2745 | | glutaredoxin | 0.9 | 1.0 | 0.9 |
| SO2880 | | glutaredoxin domain protein | 1.2 | 1.2 | 1.2 |
| SO4702 | *gor* | glutathione reductase | 0.9 | 1.1 | 1.1 |
| SO0831 | *gshB* | glutathione synthetase | 0.9 | 1.0 | 0.9 |
| SO2415 | *nrdA* | ribonucleoside-diphosphate reductase, alpha subunit | 1.0 | 1.1 | 0.8 |
| SO3117 | | thioredoxin | 0.9 | 0.8 | 0.8 |
| SO0269 | | thioredoxin | 0.7 | 0.8 | 0.8 |

| | | | | | |
|---|---|---|---|---|---|
| SO0406 | *trxA* | thioredoxin 1 | 1.1 | 1.2 | 2.1 |
| SO0452 | *trxC* | thioredoxin 2 | 2.0 | 1.7 | 2.4 |
| SO0476 | | thioredoxin (cytochrome c biogenesis protein) | 0.7 | 1.1 | 0.7 |
| SO2017 | | conserved hypothetical thioredoxin-like protein | 0.9 | 0.8 | 1.6 |
| SO2100 | | thioredoxin family protein | 0.8 | 0.8 | 0.8 |
| SO1762 | | transcriptional regulator, AraC/XylS family | 3.6 | 3.1 | 2.9 |

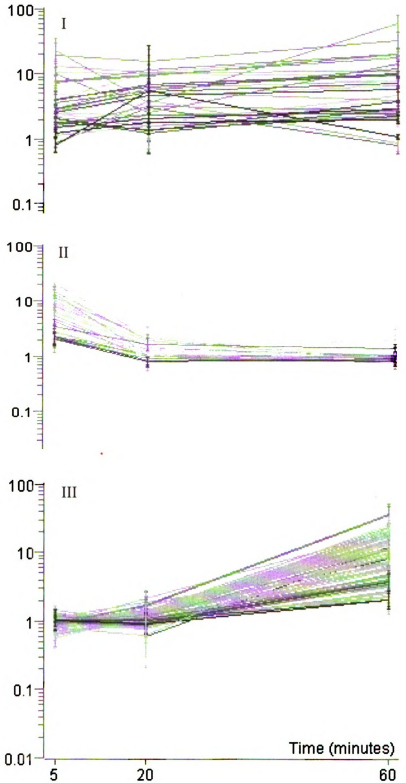
[a]the average induction fold at 5 (T5), 20 (T20) and 60 min (T60). The data were presented with 95% confidence.

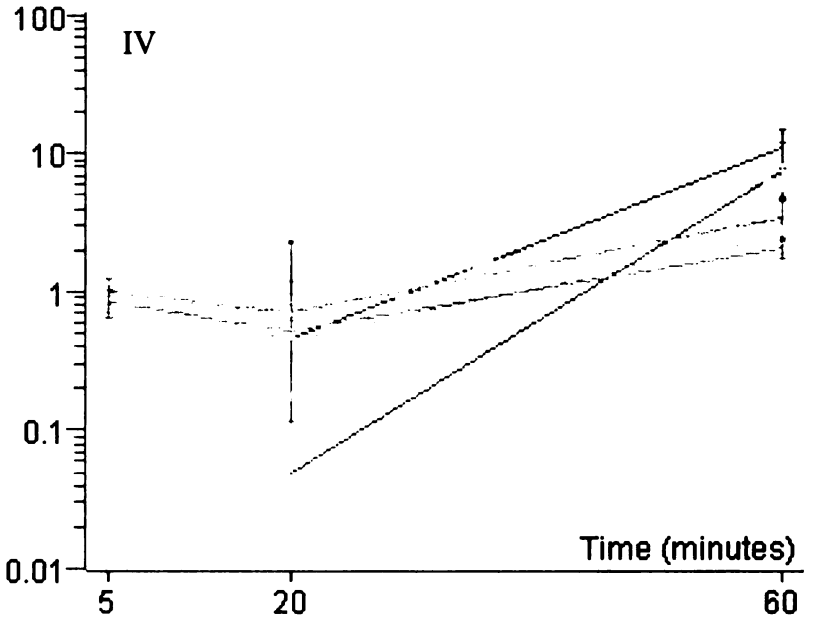
# APPENDIX B

# SUPPLEMENTAL FIGURES

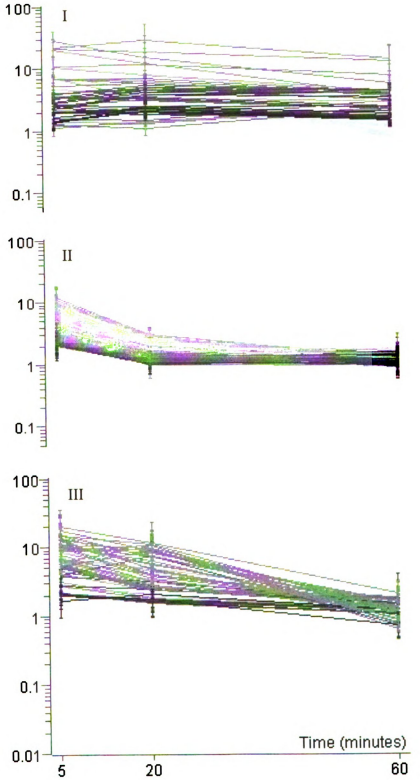


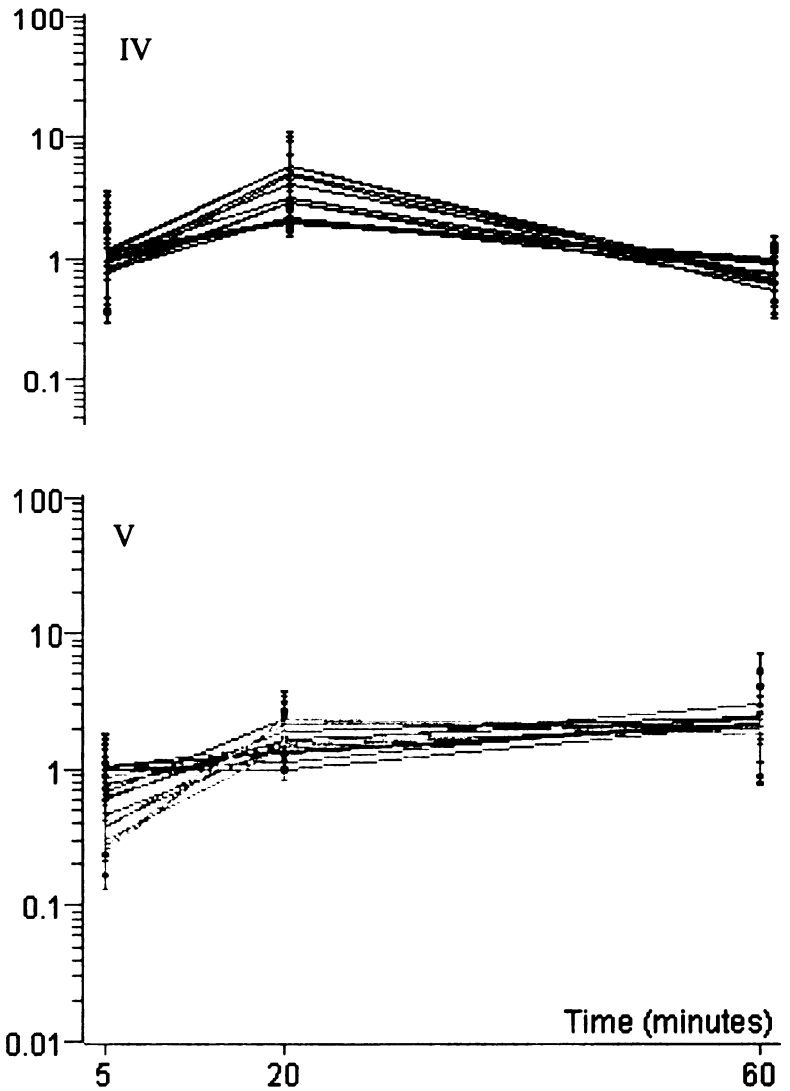

Supplemental Figure 3.1A: K-means analysis of up-regulated genes in response to UVC.


100
10
1
0.1
I
100
10
1
0.1
II
100
10
1
0.1
0.01
III
Time (minutes)
5
20
60

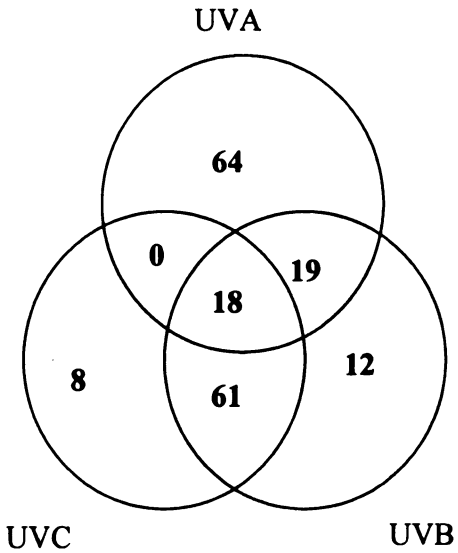



Supplemental Figure 3.1B: K-means analysis of up-regulated genes in response to UVB.


I
100
10
1
0.1
II
100
10
1
0.1
III
100
10
1
0.1
0.01
Time (minutes)
5
20
60




Supplemental Figure 3.1C: K-means analysis of up-regulated genes in response to UVA.




Supplemental Figure 3.2. Venn diagram of up-regulated hypothetical genes in response to UVC, UVB and UVA irradiation.