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### IDENTIFICATION OF MYCOSPORINE-LIKE AMINO ACIDS AND EXPRESSION OF 3-DEHYDROQUINATE SYNTHASE GENE IN UV RADIATIONS-INDUCED *Deinococcus radiodurans* R1

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#### Abstract

Mycosporine-like amino acids (MAAs) are a group of more than 40 metabolites originated from 4-deoxygadusol featuring antioxidant, growth stimulation, and UV protective properties in many microorganisms. In *D. radiodurans* R1, 3-dehydroquinolate synthase (*DHQS*) gene annotated in chromosome 1 encodes the precursor for all MAAs. In this study, a significant amount of MAAs were identified in *D. radiodurans* R1 after treatment with a different type of UV radiations, namely; the low energy UVA (360 nm) 6W and 100 W, and high energy UVC (254 nm) 6W at a period of 12 to 48 hours. The total RNA and MAAs were isolated from the UV-treated *D. radiodurans* R1. RT-qPCR experiment of the *DHQS* gene resulted in a significant increase of expression. Consequently, specific MAAs were identified using time-of-flight mass spectrometry (TOF-MS). They are mycosporine-taurine, mycosporine-glutamine, mycosporine-glutaminol, mycosporine-glutaminol-glucoside, mycosoprine-glycine, mycosporine-2-glycine, mycosporine-glycine:glutamic acid, shinorine, mycosporine-methylamine:serine, palythine-serine, and palythinol. The results suggested that these compounds play essential roles in *D. radiodurans* R1 radio-tolerance especially mycosporine-methylamine:serine and palythine-serine. This study can help to further understand the mechanism of radiation resistance in *D. radiodurans* R1, and its potential to be utilized as protective compound against radiation risk.

#### INTRODUCTION

*Deinococcus radiodurans* R1 is a well-known extreme radio-resistant and non-pathogenic gram-positive bacterium with a heterotrophic lifestyle. It exhibits radiation survival trait of 15,000 Gy and continuously grow at 60 Gy/h [1] [2]. For comparison, 2 to 5 Gy may kill a human, while *E. coli* can survive up to 200 to 800 Gy [3][4]. The whole genome sequencing of *D. radiodurans* R1 has been performed by White et al. (1999) reporting that the genome comprises two chromosomes and two plasmids resulting in the overall

genome size of 3.2 Mbp [5]. It is known that only 20% of the DNA impairment of *D. radiodurans* R1 directly happens through radiation wave while the other 80% indirectly occurs from the action of reactive oxygen species (ROS) [6].

*D. radiodurans* R1 radiation resistance mechanism is classified into three portions; 1) cellular cleansing, which is when oxidized nucleotide is disintegrated by hydrolases while other harmful constituents are transferred away from the cells. 2) Antioxidant defence by the reactive oxygen species (ROS) scavenging system that comprises superoxide dismutase (SOD), catalase, carotenoids, manganese (Mn<sup>2+</sup>)

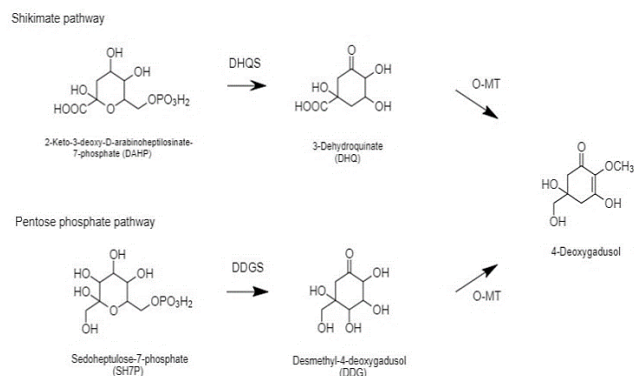
and vitamin A and E. 3) DNA repair through nucleotide excision and strand annealing with energetic homologous rearrangement [7][8].

Oxidative stress is experienced through ROS that could be produced metabolically or when exposed to a physical and chemical substances such as desiccation, ionizing radiation, UV radiation, mitomycin C (MMC), and hydrogen peroxide [9]. Basically, the Earth's surface is exposed to the radiation by sunlight that consists of visible light (700 - 400 nm) and UV light (400 - 10 nm) [10]. UV radiation is divided into three component which are UVA (320 – 400 nm), UVB (290 – 320 nm) and UVC (200 – 290 nm) where Earth is only exposed to UVA and UVB because energy by UVC is used to make up ozone layer in the stratosphere but it can eventually escapes to the Earth upon the depletion of ozone layer [11]. ROS produced upon exposure to UV radiation can destroys lipids, proteins, carbohydrates as well as nucleic acids and induces fatal double-stranded DNA breakdowns (DSBs) in the genome of bacteria, which can upset the entire cellular macromolecules [12]. *D. radiodurans* R1 has the capability to fight against UV radiation as it exhibits significant resistance for the entire ROS-generating agents [9]. The selection of UVA and UVC as the sources of radiation in this study could aid in understanding the effects of the highest and the lowest energy of UV radiation on *D. radiodurans*, R1.

Mycosporine-like amino acids (MAAs) are colourless molecules that can dissolve in water and can take up UVA and UVB radiation and diffuse the energy into a moderate one. MAAs molecules composed of the ring systems of cyclohexenone or cyclohexenimine chromophore with a glycine subunit on the third position of the carbon atom and sulphate ester or glycosidic linkage and have antioxidant properties, growth stimulation activity, and UV protection role in many organisms including in human and micro-organisms like heterotrophic bacteria, cyanobacteria, microalgae, phytoplankton, and protozoan [13][14]. The MAAs display a high variety of molecular arrangement, with molecular weight ranging from 188 to 1050 Daltons which advocate that MAAs are steady and essential molecules permitting the organisms to survive in UV radiation [15]. Therefore, MAAs are believed to be significantly essential at an early stage in life on the globe, which function as a principal sunscreen in reducing the impact of short wavelength light.

In cyanobacteria and other organisms, shikimate pathway and pentose phosphate pathway are believed to be involved in the production of MAAs as sunscreen molecules [16]. Based on Figure 1, the key enzyme in shikimate pathway is *3-dehydroquinase synthase (DHQS)* where it catalyses the conversion of 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) to 3-dehydroquininate (DHQ) while *demethyl-4-deoxygadusol synthase (DDGS)* from pentose phosphate pathway will catalyse sedoheptulose-7-phosphate to demethyl-4-deoxygadusol (DDG). DHQ and DDG will then be

converted to the precursor molecule of MAAs, the 4-deoxygadusol by *O-methyltransferase (OMT)* [17].



**Figure 1.** The possible pathway for the production of 4-deoxygadusol, the precursor for mycosporine-like amino acids (MAAs). *3-dehydroquinase synthase (DHQS)* is the key enzyme in shikimate pathway which catalyses the conversion of 3-deoxy-D-arabinoheptulosonate-7-phosphate (DAHP) to 3-dehydroquininate (DHQ) while *demethyl-4-deoxygadusol synthase (DDGS)* is the key enzyme in pentose phosphate pathway which catalyses sedoheptulose-7-phosphate to demethyl-4-deoxygadusol (DDG). The 4-deoxygadusol will be produced upon conversion of DHQ and DDG by *O-methyltransferase (OMT)*.

*D. radiodurans* R1 has the *DHQS* gene annotated in chromosome 1 with GenBank accession numbers: DR\_0777, while *DDGS* is not reported in the genome. It seems that shikimate pathway is predominated in this bacterium [18]. However, the presence of one or both enzymes can be predicted in *D. radiodurans* R1 through the protein probabilistic studies using profile Hidden Markov Models (HMMs) incorporated in HMMER search tool together with the study on genes expression [19]. Nevertheless, the association of MAAs production concerning the radiation-tolerance in *D. radiodurans* R1 is yet to be revealed. Therefore, the objectives of this study is to identify the MAAs in *D. radiodurans* R1 that accumulated under UV radiation induced stress. Consequently, we observed that the *DHQS* or *DDGS* genes expression correlates with the production of the MAAs.

## MATERIALS AND METHODS

### *D. radiodurans* R1 Collection and UV Radiation Treatment

The *D. radiodurans* R1 was grown in Tryptone Glucose Yeast Extract (TGY) agar media containing 0.5% tryptone, 0.1% glucose, 0.3% yeast extract, 0.1% potassium phosphate dibasic, and 1.5% agar at pH 7.0, 30 °C and irradiated under three different UV-radiation lamps; UVA (360 nm) 100 watts (Black-Ray long wave ultraviolet lamp model B 100AP USA), UVA (360 nm) 6 watts (Handheld UV Lamps UV 365nm 6 watts USA), and UVC (254 nm) 6 watts

(Handheld UV Lamp UVGL-58 UV: 254nm 6 watts USA). The irradiation time periods used were 12 h, 24 h, 36 h, 48 h. The *D. radiodurans* R1 was grown in TGY agar at 30 °C without UV irradiation was used as a control in this experiment.

### Primer Design

Based on the nucleotide sequence of the 3-dehydroquinate synthase (*DHQS*) gene (GenBank accession number DR\_0777, Gene ID: 1800287) and 16S ribosomal RNA gene (GenBank accession number DR\_r06, Gene ID: 1798264), a

pair of primers for each gene was designed and synthesized by Eurofins Genomics (Japan) (Table 1). The designing of primers for *DDGS* was based on the multiple sequence alignment of *DDGS* in related organisms which were *demethyl 4-deoxygadusol synthase MysA* in *Rhodococcus* sp. (GenBank accession number: NY08\_2748), *demethyl 4-deoxygadusol synthase MysA* in *Microcystis panniformis* (GenBank accession number: VL20\_4964), *demethyl-4-deoxygadusol synthase* in *Nostoc punctiforme* (GenBank accession number: Npun\_R5600) and *demethyl-4-deoxygadusol synthase* in *Trichormus variabilis* (GenBank accession number: Ava\_3858).

**Table 1.** Primers used in qRT-PCR for amplification of *DHQS*, *DDGS* and 16S ribosomal RNA genes.

Gene	Primer's name	Sequence (5'-3')	Position* (5'-3')	Product length
<i>DHQS</i>	DHQS-RTF	GTGCAAGACGCTGGAAGTTT	192-211	135 bp
	DHQS-RTR	AGGTAACCTGCCGCAACAAA	326-307	
16S	16S-RTF	GTAGTCCACACCCTAAACGAT	768-788	294 bp
	16S-RTR	CTTAACCCAACATCTCAGACA	1061-1040	
<i>DDGS</i>	DDGS-RTF	GCAAGCAGGACAATATGCGA	NA	156 bp
	DDGS-RTR	AAACATCTCAGCGACGCTCT	NA	

GenBank accession numbers: DR\_0777 (DHQ) and DR\_r06 (16S rRNA).

### *D. radiodurans* R1 Sequence Similarity Scanning Using HMMER

The nucleotide sequences of chromosome 1 in *D. radiodurans* R1 was translated to protein sequences using BLASTP in NCBI to predict the presence of the homologous protein of *DHQS* or *DDGS*. The multiple sequence alignment was applied to find the conserved motifs on the protein sequences of *DHQS* and *DDGS*, then the conserved motifs was used to search on the protein sequence of *D. radiodurans* R1 using HMMER software tools [19].

### RNA Isolation and Quantitative Real-Time PCR (qRT-PCR)

The total RNA was extracted from *D. radiodurans* R1 after being irradiated. The sample was harvested using 5 mL bacteria and incubated for 30 min at 25 °C after adding lysozyme (lysozyme egg pH 8.0). The extraction of RNA was done using RNeasy® kit 50. (QIAGEN, Germany). The RNA was suspended in RNase-free water and RNA purity was analyzed by NanoDrop (Thermo Fisher Scientific, USA) to check the absorbance ratio at 260/280 nm, and 260/230 nm for the determination of contaminants exist in the sample and 1.2% agarose gel electrophoresis was run to check the bands and smear representing the total RNA in the sample. The qRT-PCR was performed using Power SYBR® Green RNA-to-C<sub>T</sub><sup>™</sup> 1- step kit (Applied Biosystems, USA) in 10

μL reactions containing 5 μL Power SYBR® Green RT-PCR Mix (2X), primers 100 nm, 0.08 μL RT Enzyme Mix (125X), RNA template (up to 100 ng), RNase free H<sub>2</sub>O to 10 μL. The assays were performed using an Applied Biosystems StepOne Real Time PCR (Thermo Fisher Scientific USA). The reaction was run with *DHQS* primers, *DDGS* primers and 16S rRNA primers [21]. The individual sample was run in triplicate to insure the C<sub>T</sub> value. The change in gene expression of *DHQS* and *DDGS* was determined by the normalization of C<sub>T</sub> value for *DHQS* and *DDGS* to the C<sub>T</sub> value of housekeeping gene, 16S rRNA by using the 2<sup>-ΔΔCT</sup> method as described for technical triplicate samples in at least two independent experiments [22].

### MAAs Extraction and TOF-MS Identifications

The MAAs were extracted from *D. radiodurans* R1 after UV irradiation treatment. The extraction and purification of MAAs were done according to Agostino et al., 2016 [23]. The *D. radiodurans* R1 was washed with 20 mM NaH<sub>2</sub>PO<sub>4</sub> and centrifuged at 5.000 xg for 15 min at 4°C. Then, the *D. radiodurans* R1 cells were resuspended in HPLC grade methanol and lysed on ice by sonication with five 20-S pulses at 40% amplitude with 1 min of recovery time between pulses using QSonica LLC (QSonica, USA). The lysed cellular debris was removed via centrifugation (5.000 xg for 15 min at 4°C). *D. radiodurans* R1 was dissolved in 1 mL of Milli-Q water and mixed vigorously with 500 mL of

chloroform to remove pigments. Thereafter, the aqueous phase was carefully aspirated and filtered through a filtration of PVDF membrane filter (0.22 µm) to obtain partially purified MAA.

ESI-TOF-MS technique was used to determine the MAAs types. 200 µL of the sudation was injected to the ESI-TOF-MS AccuTOF LC-PLUS (JEOL Ltd. Tokyo, Japan). The method for ESI operation was in positive mode. The capillary voltage was set at 2000 V and nitrogen was used as a nebulizing and drying gas. The ion guide radio frequency (RF) was 1500 V.

## RESULTS AND DISCUSSION

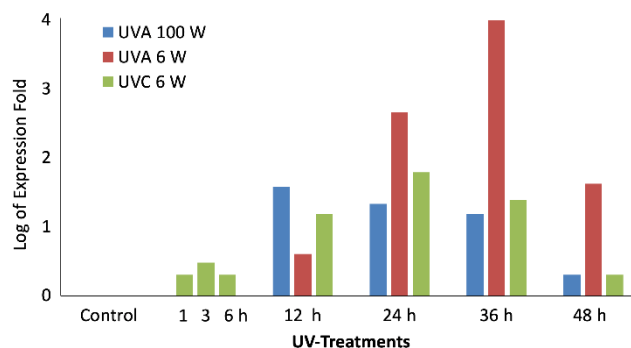
### Gene expressed in *D. radiodurans* R1

*DHQS* gene is expressed after exposure to the UV radiation at most of the times meanwhile *DDGS* gene is not expressed in any times of exposure. Based on the results in qPCR, the  $C_T$  value is undetermined for *DDGS* which conveys that the expression of DNA in the cycle of *DDGS* is unable to exceed the threshold level during the reaction. On the other hand, the produced mRNA of *DDGS* was meagre during the exposure to UV radiation in *D. radiodurans* R1 and this causes the  $C_T$  value to be high and exceed the limit for detection [24]. Based on the sequence searches of *D. radiodurans* R1 by HMMER, the E-value score for *DHQS* (E-value =  $2.2e^{-222}$ ) was much closer to 0 or smaller as compared to *DDGS* (E-value =  $2.9e^{-21}$ ). This shows that the *DHQS* alignment has better quality than *DDGS* because the probability to find expected sequence in the database by chance is lower, and it has a high or equal alignment bit score that reflects high sequence similarity of the alignment [25]. *DHQS* and *DDGS* genes fall into the same superfamily sugar phosphate cyclases but they utilize different substrates and involved in different pathways to produce the same precursor for MAAs, the 4-deoxygadusol [26]. However, in *D. radiodurans* R1, shikimate pathway is dominating over pentose phosphate pathway because of the increased expression of *DHQS* gene over time [18].

### UV-induced *DHQS* Gene Expression in *D. radiodurans* R1

*DHQS* gene was expressed in *D. radiodurans* R1 under all UV treatments; UVA 100W, UVA 6W and UVC 6W for 12 h, 24 h, 36 h, and 48 h. The qPCR results exhibited high expressions of *DHQS* in *D. radiodurans* R1 especially upon exposure to UVA 6W lamp after 36 h of irradiation with the expression fold of 9273. On the treatment of UVA 100W the *DHQS* showed highest expression at 12 h while the expression was decreased in further time of treatment. The expression was 37-fold for 12 h, 21-fold for 24 h, 15-fold for 36 h, and 2-fold for 48 h. These findings suggest a slight inhibition of *DHQS* expression occurs under an extremely

strong UV (UVA 100W) with a prolonged time treatment (Figure 2).



**Figure 2.** *DHQS* gene expressions fold under three types of UV-lamp namely UVA 100W, UVA 6W and UVC 6W at 12 h to 48 h. UVC 6W was also taken at shorter period 1 h - 6 h.

On the treatment of UVA 6W, which is not as harsh as the UVA 100W but sufficient to cause the cell damage. Under this lamp, for 12 h treatment the expression was 4-fold. As the treatment time increased to 24 h the expression increased to 438-fold, and for 36 h the *DHQS* expression was estimated at 9273-fold. The finding suggests that the *D. radiodurans* R1 bacteria starts to use the defence mechanism of *DHQS* gene expression when treated continuously with such UVR for a long time. However, for 48 h the expression was decreased to 41-fold. Finally, in UVC 6W lamp, which is shorter wavelength than UVA, the *DHQS* expression fold was 15, 60, 24, and 2 fold for 12 h, 24 h, 36 h, 48 h treatment, respectively. Besides, short exposure times (1 h, 3 hr and 6 hr) to UVC also leads to the expression of *DHQS* which shows that ROS start to increase after the irradiation. The expression fold of *DHQS* was the highest after 24 h irradiation under UVC 6W lamp and start to decrease upon time. This finding shows that a prolonged exposure to UVC lamp might be harsh to *D. radiodurans* R1 and was not able to express more *DHQS* to compensate with the damage.

The *DHQS* showed relatively strong response to UV induction in *D. radiodurans* R1 compared to the control condition. Particularly it shows high stimulation and in UVA 6W. The gene expression induction or stimulation increases, or decreases is depending to the types of UV and exposure time. The same gene can be highly expressed under specific UV-wavelength and can have lower expression under another wavelength [27][28]. The absorption maxima of MAAs (310–360 nm) is one of the reason contributes to high expression of *DHQS* under UVA (360 nm) radiation as more energy is absorbed by the cells and increases the synthesis of MAAs [29][30]. This shows that wavelength has an important role in managing the damage from UV radiation because the difference in wavelength also exerts different effect on genetic material like DNA [31]. The exposure to UVC or high energy UVA might cause impairment on the DNA and subsequently causing the death of the cells while low energy UVA has a low possibility to cause damage to

the DNA [32]. Therefore, DNA damage might be the cause for a lower expression of *DHQS* under UVC 6W and UVA 100W radiation as compared to the high expression of *DHQS* under UVA 6W radiation.

Other observations for the *DHQS* displayed strong response towards UVA, UVB and UVC [33][34]. The same gene was also reported in respond to oxidative stress [35][36][37]. In plants, the bifunctional *DHQS* catalyses the conversion of dehydroquininate into shikimate [38]. The *DHQS* was also regarded as multifunctional that response to salt, heat, and drought stress [39][40]. The *DHQS* converts of 3-dehydroquininate to 4-deoxygadusol (4-DG), the direct precursor of the MAAs [41]. *DHQS* is the precursor for the fungal mycosporines and all MAAs [42]. It was proposed that 3-dehydroquininate acts as the MAAs core structure [43][44][45][14].

**MAAs Types Identified in *D. radiodurans* R1 UV-induced**

Various types of MAAs were biosynthesized in *D. radiodurans* R1 after the treatment of UVR as shown in Table 2. It was demonstrated that the most accumulated MAAs under the three types of UV-lamps (UVA 100W, UVA 6W, UVC 6W) were mycosporine-methylene:serine and palythine-serine, which were present in 90% of the treatment. This followed by shinorine and mycosporine-aurine that were present in 50% of the treatment. Mycosporine-2-glycine and mycosporine-glycine:glutamic acid were identified in the long time of treatment (at 48 h) in UVA 100W and 6W. Meanwhile, mycosporine-glutamine was identified at only two times, which were at 12 h under UVA 100W and at 36 h under UVA 6W. The lowest accumulated MAAs were mycosporine-glutaminol, mycosporine-glutaminol glucoside, mycosporine-glycine, and palythanol that were identified only once under UVA 100W and 6W, and none in UVC treatment. In contrast, there was no synthesized MAAs observed in untreated sample.

**Table 2.** Outcome of ESI-TOF-MS 11 types of Mycosporine-like amino acid (MAAs) in *D. radiodurans* R1 produced under 3 different lamps UVA 100W, UVA 6W and UVC 6W at different exposure time from 12 h to 48 h, the marker '+' mean MAAs was present and the marker '-' means MAAs was not present.

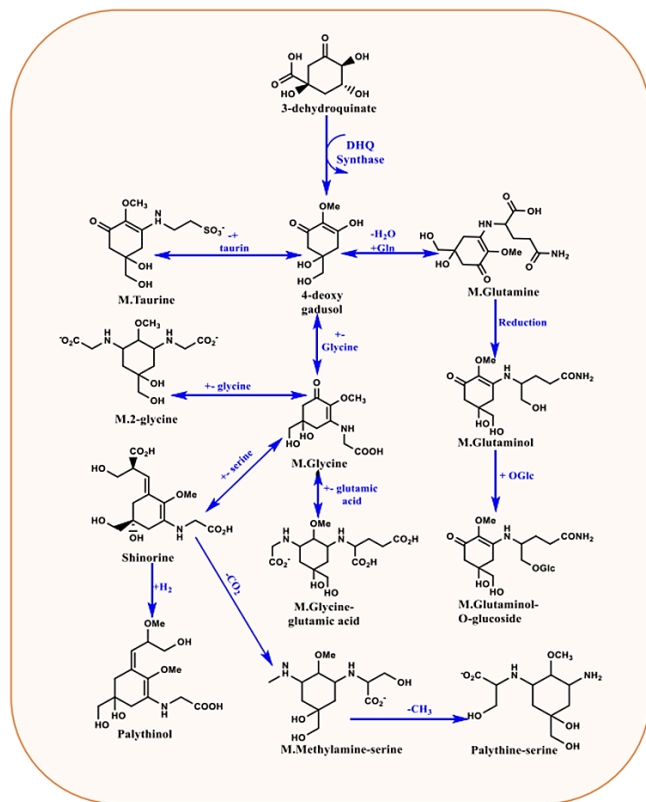
MAAs Types	UVA 360 nm 100W				UVA 360 nm 6W				UVC 254 nm 6W			
	12h	24h	36h	48h	12h	24h	36h	48h	12h	24h	36h	48h
1- Mycosporine-Taurine	-	+	+	-	-	+	+	-	-	-	+	+
2- Mycosporine-Glutamine	+	-	-	-	-	-	+	-	-	-	-	-
3- Mycosporine-Glutaminol	-	-	+	-	-	-	-	-	-	-	-	-
4- Mycosporine-Glutaminol Glucoside	-	-	-	+	-	-	-	-	-	-	-	-
5- Mycosporine-Glycine	-	-	-	-	+	-	-	-	-	-	-	-
6- Mycosporine -2-Glycine	+	-	-	+	-	-	-	+	-	-	-	-
7- Mycosporine-Glycine: Glutamic Acid	-	-	-	+	-	-	-	+	-	-	-	-
8- Shinorine	+	-	-	-	+	-	+	-	+	-	+	+
9- Mycosporine-Methylamine:Serine	+	+	+	-	-	+	+	+	-	+	+	+
10- Palythine-Serine	+	+	+	-	+	-	+	-	+	+	+	+
11- Palythanol	-	-	-	+	-	-	-	-	-	-	-	-

Mycosporine-glutamine (M-Gln) was detected in *D. radiodurans* R1 and presented only in UVA 100W 12 h and UVA 6W 36 h. The M-Gln is a well-known UV protective compound in *Glomerella Cingulata*, it was reported that the M-Gln is the first MAA molecule that has been shown to have a definite biological role [46]. In microalgae, M-Gln is present to protect the cell from UVR [47][48]. It is also known that glutamine and MAAs promote recovery, improve protein nutritional conditions and extend antioxidant effects in rats that were exposed to X-ray irradiation [49][50]. *D. radiodurans* R1 system produces oxidative stress that involves flavins [6]. A UV-light can convert the M-Gln to aminocyclohexenone and hence scavenges the flavins [51][52]. Thus, it is hypothesized that the M-Gln involved in the scavenging system to reduce the flavins in *D. radiodurans* R1.

In *D. radiodurans* R1, mycosporine-glutaminol (M-Gln(OH)) was presented only at UVA 100W 36h. Its presence was alternated with mycosporine-glutaminol-glucoside (MGG), which appears at 48 h of the same treatment. This may be due to the conversion of M-Gln (OH) to MGG (Figure 3). On other hand, its precursor, M-Gln was not found after 12 h of the same UVA 100 W lamp treatment. It was reported that the M-Gln(OH) offers the defense to some terrestrial cyanobacteria from UVR [53][54]. At 48 h of UVA 100 W treatment, only peripheral MAAs were found, namely; MGG, M2G, M-Gly:Glu, and palythanol. We also observed same alternation function between MMS and PS that appeared from 12 h until 36 h and later was replaced by the MGG that has different biochemical routes at 48 h. This 'contingency' mechanism was not observed for the other two weaker lamps. The MGG is suggested to prevent the *D. radiodurans* R1 from cyclobutane pyrimidine dimers



(CPDs), an example of CPDs is thymine dimers, which is caused by UVR at two thymine neighbors of DNA. Hence, blocking important functions as transcription and translation [55].



**Figure 3.** The 11 MAAs types synthesized from 3-dehydroquininate synthase (DHQS) and their pathway in *D. radiodurans* R1 bacteria. 3-dehydroquininate (DHQ) is converted to the precursor molecule, 4-deoxygadusol by the bi-functional role of DHQS. Then amino acid glycine is added to the core structure and producing primary MAAs, the M.Glycine. Different kinds of amino acid attached either to 4-deoxygadusol or M.Glycine and producing other secondary or peripheral MAAs [14].

Mycosporine-glycine (M-Gly) was synthesized in *D. radiodurans* R1 under 12 h of UVA 6W treatment. It is worth to note that this is the weakest UVR and the shortest treatment period in this study. It is well-known that M-Gly is the driver of all MAAs in many organisms [56][57]. Interestingly, most of MAAs in *D. radiodurans* R1 in this study was synthesized through M-Gly (Figure 3). M-Gly is often described as the major MAAs which can be converted to various other MAAs [58]. It was the first MAAs discovered in the zoanthid *Palythoa tuberculosa* in 1977 [14].

Mycosporine-glycine:glutamic acid (M-Gly:Glu) and mycosporine-2-glycine (M2G) were identified in the long treatment (48 h) of UVA both in 100W and 6W, while it was not stimulated under UVC, (Table 2). The expression of M-

Gly:Glu and M2G under long period of treatment demonstrated their importance whilst hypothesized to be degraded in the shorter wavelength of UVC.

Meanwhile, M2G was for first time identified in 1994 from the sea anemone as a protection compound from the deleterious effects of solar UVR [59]. It was also reported in cyanobacterium as a major compound of MAAs [15], that has photoprotection mechanism against UVR. Its anti-oxidation mechanism against ROS was studied in other organisms such as, coral [60][61], *Heterocapsa sp* [62], microalgae *Chlorella vulgaris* [63][64], and *Aplysia dactylomela* [65]. Moreover, M2G was also reported to have other protective mechanisms, which can present under salt stress [66][67].

The M-Gly:Glu has been isolated and characterized for the first time from the extract of the sponge *Dysidea herbacea* which live in shallow water of the Great Barrier Reef [68]. Such places are normally exposed to intense UVR, which has significant biological effects on the organism. However, these invertebrates have evolved a variety of photo-adaptive mechanisms and strategies for successful growth. The organism produces UV-absorbing metabolites, which act as an effective UV shield. This is considered to be one of such strategies.

Shinorine (SH) and mycosporine-aurine (M-Tau) were synthesized under all three lamps of UVR. Interestingly while many MAAs could be degraded in the shorter wavelength of UV, ie UVC, both SH and M-Tau were found stable. This suggests a defense mechanism for *D. radiodurans* against UVC. SH was first found in the edible mussel *Mytilus galloprovincialis* [69]. It is known as a stable oxidative [70] and UV-photoprotective molecules [71]. Besides, it is among the most dominant expression MAAs [72][73][74]. It was reported as UVR protective agent in many organisms, such as fish species, algal species [75], green sea urchin [76], subtidal marine organisms [77], stony corals [78], terrestrial microalgae [79], cyanobacterium [45].

The mycosporine-aurine (M-Tau) is involved in UVR radio-resistance mechanism in *D. radiodurans* R1. The compound is capable to scavenge the ROS generated by the UVR. M-Tau has antioxidant and it can protect organism against cellular damages induced by high level of ROS under different stresses [58][48]. The *D. radiodurans* R1 is highly resistant to lipid peroxidation and hydrogen peroxide induced by the UV-radiation. M-Tau, therefore, plays important role in inhibiting lipid peroxidation as what has been shown in marine organisms [71][80].

Mycosporine-methylamine:serine (MMS) and palythine-serine (PS) are among the most important MAAs that were identified in *D. radiodurans* R1 which constitute about 75% of the whole UV-treatments in this study. This shows their importance roles in combating the UVR. MMS was reported to protect many organisms from UVR and also from oxidative forms of oxygen (HO<sub>2</sub>, O<sub>2</sub><sup>-</sup>, HO.) [77]. The MMS was one of the most common MAAs that was found in the tissues of the hermatypic coral *Pocillopora capitata* in

Mexico. The *P. capitata* contains a high diversity of primary and secondary MAAs [81]. Meanwhile, three studies on different organisms indicated that PS is the highest MAAs expressed among other MAAs [82][83].

Mycosporine-palythanol (Palythanol) was identified in *D. radiodurans* R1 only in UVA 100W at 48 h. It was expressed under long exposure towards UV-treatment (48 h) and under the high energy of UVA 100W radiation. While in UVA 6W and UVC 6W the Palythanol was not observed in *D. radiodurans* R1. Palythanol was also identified in four marine species extracted under the pressure of ROS [65]. It was shown that exposure to photon fluxes of UVB wavelengths, UVA and PAR for 24 h caused double palythanol accumulation per volume culture [84].

## CONCLUSION

*D. radiodurans* R1 withstands reactive oxygen species (ROS), and doses of radiation that would be lethal to most organisms by the effective defence mechanism played by *DHQS* gene. The expression of *DHQS* in *D. radiodurans* R1 is stimulated upon exposure to lower energy UVA and high energy UVC radiation which is responsible for the production of MAAs. From the TOF-MS results, 11 MAAs were successfully identified namely; mycosporine-aurine, mycosporine-glutamine, mycosporine-glutaminol, mycosporine-glutaminol-glucoside, mycosporine-glycine, mycosporine-2-glycine, mycosporine-glycine:glutamic acid, shinorine, mycosporine-methylamine:serine, palythine-serine, and palythanol. These MAAs were thought to be the important MAAs in *D. radiodurans* R1 defense mechanism against UVR and UV-initiated ROS. Therefore, the increased accumulation of MAAs after exposure to UVR correlates with the increased of the expression fold of *DHQS* over time of treatment.

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