UNIVERSITI PUTRA MALAYSIA

IDENTIFICATION OF MYCOSPORINELIKE AMINO ACIDS AND 3-DEHYDROQUINATE SYNTHASE GENE EXPRESSION IN UV RADIATION-INDUCED Deinococcus radiodurans R1

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By

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July 2018

Chairman : Amir Syahir bin Amir Hamzah, PhD
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Deinococcus radiodurans R1 is a well-known heterotrofic bacterium with extreme radio-resistant capability. It exhibits radiation survival up to 15,000 Gy while still be able to grow normally at 60 Gy/h. The radiation-tolerant mechanism that involves DNA repair represents 20% of the total resistant mechanism. Meanwhile, the other 80% comes from antioxidants. Since *D. radiodurans* R1 was discovered in 1956, the whole radio-resistant mechanism is not yet fully understood. Mycosporine-like amino acids (MAAs) are a group of 40 or more compounds that have antioxidant, growth stimulation and UV protective properties found in many microorganisms. In *D. radiodurans* R1, 3-dehydroquinate synthase (DHQ) gene annotated in chromosome 1 encodes the precursor for all MAAs. In this study, a significant amount of MAAs was found in *D. radiodurans* R1 after treatment with a different type of UV radiations, namely; UVA (360 nm) 6W and 100 W, and UVC (254 nm) 6W at a period of 12 to 48 hours. The RNA and MAAs were isolated from the UV-treated *D. radiodurans* R1. RT-qPCR experiment of the DHQ gene resulted in a significant increase in the number of expression fold from 4 to 9273 fold. 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 for its expression at every UV treatment. This study may well help to understand radiation resistance mechanism further, and it is potential to be utilized as human protective compound against radiation risk.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGENALPASTIAN MIKOSPORIN SEPERTI ASID AMINO DAN GEN 3-DEHIDROQUINAT SINTASE PADA *Deinococcus radiodurans* R1 YANG DIDEDAHKAN KEPADA SINAR RADIASI UV

Oleh

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Deinococcus radiodurans R1 adalah bakteria heterotrophik yang terkenal dengan daya ketahannya yang kuat terhadap sinaran radioaktif. Ia mampu bertahan terhadap radiasi sehingga 15,000 Gy sementara masih mampu tumbuhbiak pada 60 Gy/h. Mekanisme anti-radiasi yang melibatkan pengaktifan gen untuk pembaikan DNA mewakili 20% daripada jumlah keseluruhan mekanisme ketahanan radiasi. Selebihnya, 80% diwakili oleh antioksidan. Asid amino seperti mikosporin (MAAs) adalah sekumpulan daripada 40 atau lebih molekul terkandung di dalam banyak mikroorganisma yang mempunyai sifat antioksidan, rangsangan pertumbuhan dan perlindungan UV. Gen penghasilan MAAs disusun pada kromosom 1 *D. radiodurans* R1 di dalam gen 3-dehidroquinate sintase (DHQ). Di dalam kajian ini, sejumlah MAAs telah dikenalpasti terdapat di dalam *D. radiodurans* R1 selepas didedahkan dengan radiasi UV daripada jenis yang berlainan, iaitu UVA (360 nm) 6W dan 100 W, dan UVC (254 nm) 6W selama 12 ke 48 jam. RNA dan MAAs telah berjaya diasingkan daripada spesimen bakteria. Eksperimen RT-qPCR gen DHQ menghasilkan peningkatan ketara dalam bilangan fold ekspresi dari 4 hingga 9273 fold. Sekumpulan MAAs juga telah dapat dikenalpasti dengan menggunakan time-of-flight mass spectrometry (TOF-MS). Ianya terdiri daripada mikosporin-taurina, mikosporin-glutamina, mikosporin-glutaminol, mikosporin-glutaminol-glukosida, mikosporin-glisina, mikosporin-2-glisina, mikosporin-glisina: asid glutamik, shinorina, mikosporin-metilamin:serina, palitin-serina, dan palitinol. Hasil kajian ini mencadangkan bahawa kesemua MAAs terutamanya mikosporin-metilamin:serina dan palitin-serina memainkan peranan yang penting dalam toleransi radiasi dalam setiap pendedahan UV terhadap *D. radiodurans* R1. Kajian ini mencadangkan bahawa kedua-dua kompaun mikosporin-metilamin:serina dan palitin-serina memainkan peranan penting dalam toleransi sinaran pada *D. radiodurans* R1 kerana terdapat pada setiap dedahan UV. Kajian ini juga membantu untuk memahami dengan lebih lanjut berkenaan mekanisme rintangan

radiasi, dan potensi untuk ia digunakan sebagai kompaun perlindungan manusia terhadap risiko radiasi.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

RT-qPCR	Real-Time Quantification Polymerase Chain Reaction
PCR	Polymerase Chain Reaction
cDNA	Complementary DNA
TOF-MS	Time of Flight Mass Spectrometry
MAAs	Mycosporine-Like Amino Acids
ROS	Reactive oxygen species
CPD	Cyclobutane Pyrimidine Dimers
ºC	Degree Celsius
UV-A	Ultraviolet A
UV-B	Ultraviolet B
UV-C	Ultraviolet C
PDR	Post-desiccation recovery
DHQ	3-dehydroquinate synthase
M-tau	Mycosporine-taurine
M-Gln	Mycosporine-glutamine
M-Gln(OH)	Mycosporine-glutaminol
MGG	Mycosporine-Glutaminol-Glucoside
M-Gly	Mycosporine-glycine
M-2-G	Mycosporine-2-glycine
M-Gly:Glu	Mycosporine-Glycine:Glutamic Acid
SH	Shinorine
MMS	Mycosporine-methylamine:serine
PS	Palythine-serine
M+H	M+1 Hydrogen
M+K	M+39 Potassium
M+Na	M+23 Sodium

CHAPTER 1

INTRODUCTION

Deinococcus radiodurans R1 is a well-known extreme radio-resistant and non-pathogenic gram-positive bacterium with a heterotrophic lifestyle (Makarova et al., 2001). It exhibits radiation survival trait of 15,000 Gy (1Gy=100 Rad). Besides, it can grow contiguously at 60 Gy/h (Makarova et al., 2001; Wan et al., 2013). For comparison, 2 to 5 Gy may kill a human, while *E. coli* can survive up to 200 to 800 Gy (Blasius et al., 2008; Battista, 1997; Piechura et al., 2015). Information on the mechanism of radiation resistance of the *D. radiodurans* R1 is limited since its discovery in 1956 (Wan et al., 2013). The whole genome sequencing of *D. radiodurans* R1 has been performed by White et al. (1999) reporting that the genome comprises two chromosomes (2,648,638 and 412,348 bp); a mega-plasmid (177,466 bp) and a small plasmid (45,704 bp) resulting in the overall genome size of 3.2 Mbp. 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) (Ghosal et al., 2005).

D. radiodurans R1 radiation resistance mechanism is classified into three portions; cellular cleansing, which is when oxidized nucleotide is disintegrated by hydrolases while other harmful constituents are transferred away from the cells; antioxidant defense by the ROS scavenging system that comprises superoxide dismutase (SOD), catalase, carotenoids, manganese ($Mn2^{+}$) and vitamins A, E.; and lastly DNA reparation through nucleotide excision repaired and stretched synthesis reliant strand annealing with energetic homologous rearrangement (Daly, 2004; Luan et al., 2014).

Oxidative stress is experienced through ROS that could be produced metabolically or when exposed to a physical and chemical substance such as desiccation, ionizing radiation, UV radiation, mitomycin C (MMC) and hydrogen peroxide (Slade and Radman, 2011). ROS 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 (Daly, 2009). D. radiodurans R1 exhibits significant resistance for the entire ROS-generating agents unequalled among the entire acknowledged species (Slade and Radman, 2011).

D. radiodurans R1 can breakdown its genome to many fragments (as many as 2,000 DSBs per multi-genomic cell) without producing substantial protein destruction (Daly et al., 2007). The toughness of the bacterium is based on the robust oxidative stress resistance mechanisms that shield the proteins form destruction resulted from oxidation (Daly et al., 2007) and DNA reparation that effectively completes the exact DNA fragments reassembly (Slade et al., 2009; Zahradka et al., 2006). The antioxidation protection of DNA repair and other proteins enables them to retain their catalytic activity and to provide a swift response under the conditions of oxidative stress.

Conventionally, DNA is considered the target of initial radiation. The current study in D. radiodurans R1 revealed that the bacterium is vulnerable to radiation induce DSBs like other species (Gérard et al., 2001), while its proteome is better shielded against ROS-induced oxidative destruction compared to other radiation sensitive species (Daly et al., 2007). These discoveries advocate that it is the protein destruction level that persists in radiation and not the destruction of DNA (Daly et al., 2007; Daly et al., 2010).

Mycosporine-like amino acids (MAAs) are an assemblage of 40 or higher number of colourless molecules that can dissolve in water and can take up UV-A and UV-B radiation and diffuse the energy into a moderate. MAAs have been reported in the microbial world, for example, in heterotrophic bacteria, cyanobacteria, macro, micro-algae, phytoplankton and protozoan (Sinha et al., 2007). The MAAs display a high variety of molecular arrangement, with molecular weight ranging from 188 to 1050 Daltons. The employment of extensive diversity of organisms comprising eukaryotic and prokaryotes micro-organisms which reside in the terrestrial, marine and aquatic habitat of MAAs have been done (Wada et al., 2015).

The characteristics advocate that MAAs are steady and essential molecules permitting the organisms to survive in UV radiation. Therefore, MAAs is believed to be significantly essential at an early stage in life on the globe, which functions as a principal sunscreen in reducing the impact of short wavelength light. MAAs are 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 (Sinha and Häder, 2008; Sinha et al., 2007). It has antioxidant properties, growth stimulation activity in human and UV protection role (Misonou et al., 2003; Oyamada et al., 2008).

In cyanobacteria and other organisms, the origin molecule of all MAAs 3-dehydroquinate is produced by 3-dehydroquinate synthase (DHQ) (Gabani and Singh, 2013; Wada et al., 2015). D. radiodurans R1 has the DHQ synthase gene annotated in chromosome 1 GenBank accession numbers: DR_0777 (DHQ) (White et al., 1999). Nevertheless, the association of MAAs production concerning the radiation-tolerance in D. radiodurans R1 is yet to be revealed.

1.1 Problem Statement

Since *D. radiodurans* R1 was discovered in 1956, the whole radio-resistant mechanism is not yet fully understood. About 80% of the repairing mechanism is believed to come from antioxidants such as carotenoids, $Mn2^{+}$ and vitamins A, E. Until now, there is no record of measuring DHQ gene, that produces MAAs in *D. radiodurans* R1.

1.2 Hypothesis

MAAs are predicted to have functions in *D. radiodurans* R1 in radiation tolerance. Thus during the UV-radiation induced stress, particular MAAs are expected to be synthesized.

1.3 Objectives

In response to the radiation treatment, the objectives of this study are:

1. To identify the types of MAAs in *D. radiodurans* R1 expressed under UV-radiation (different wavelengths) induced stress.
2. To measure DHQ gene expression in *D. radiodurans* R1 under UV-radiation induction.

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