Evaluation of UV-Resistance of Epibiotic Bacteria Co-existing with the Kenyan Marine *Lyngbya majuscula*

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Abstract

The marine cyanobacterium *Lyngbya majuscula* is reported to be a source of a wide array of natural products. Some of the products exhibit photo-protective properties. L. *majuscula* has also been shown to live in association with epibiotic bacteria. However, the potency of these epibionts to withstand ultraviolet radiation is not well documented. This study focused on epibiotic bacteria isolated from the surface of *L. majuscula* from Kenya. Twelve strains or isolates were exposed to UV irradiance (365 nm, intensity of 11.6W cm^{-2}) for 15, 30 and 45 minutes. Survival curves showed that the *Bacilli strains* were the most tolerant to UV, followed by the β-*proteobacteria*, while the actinobacteria were the least resistant to UV. The observation that the actinobacteria were less resistant to UV suggested that cell wall characteristics and G+C content are not the sole determinants of UV resistance. It would be interesting to determine the compounds and/or metabolite underlying the ability of these isolates to withstand exposures of varying levels of UV-radiations.

Key words: Lyngbya majuscula, epibiotic bacteria, Ultraviolet radiation

INTRODUCTION

Cyanobacteria are Gram-negative, photolysis-mediated oxygen evolving, cosmopolitan prokaryotes that have survived and flourished on earth for over two billion years with the creation of oxygenic environment (Sergeev et al., 2002). They have become recognized as an extremely rich source of novel, bioactive secondary metabolites with approximately 700 different natural compounds having been isolated and characterized (Tidgewell et al., 2010a). Some of these compounds have gained considerable attention due to their pharmaceutical and biotechnological potential (Tan, 2007). Marine strains of the genus *Lyngbya* are the most prolific producers of these natural products with nearly 300 compounds reported from this genus (Tidgewell et al., 2010a), with 76% of these products attributed to the species *L. majuscula*. The compounds isolated from *L. majuscula* include those which exhibit biological activities which may have use in human health including anticancer, anti-inflammatory, antibacterial and anti-infective therapeutic agents (Tidgewell et al., 2010b). Additionally, the species have been reported to be potential sources for photo-protective agents such as scytonemin and mycosporine-like amino acids (MAAS) which are known to screen-out harmful effects caused by ultraviolet (UV) radiation (Cockell & Knowland, 1999). Marine *Lyngbya* isolates are found pan-tropically in shallow Coral reef environments with frequent exposure to diverse natural selection pressures such as desiccation during low tide and exposure to high fluxes of UV radiation (Gerwick et al., 2008). They are known to grow in close association with other cyanobacteria and algae, and provide an ideal substrate for a variety of heterotrophic bacteria (Simmons et al., 2008). Epibiotic bacteria in general are heterotrophic (Hempel et al., 2008; Hube et al., 2009). These heterotrophic bacteria can be found attached to cyanobacterial trichomes as well as imbedded in the mucopolysaccharide layer surrounding the trichomes (Nausch, 1996) of unicellular cyanobacteria (Brunberg, 1999). Many of the heterotrophic bacteria play an important role in nutrition (Hempel et al., 2008), and defence against predators and biofouling (Bewley et al., 1996). However, the existence of epibiotic bacteria among *L. majuscula* collected from the Kenyan Coast responses towards varying levels of Ultra-violent radiations are not well understood. The aim of this study was to evaluate UV resistance of the epibiotic bacteria co-existing with the Kenyan marine *L. majuscula.*

MATERIALS AND METHODS

Experimental Layout

The bacterial strains used in this study were previously isolated from the Kenyan marine *L. majuscula* collected from four different sites along the Kenyan Coast namely Mida (039.99505º to 039.96600ºE) and Kilifi (039.785˚E to 039.835˚E) in the North Coast and Shimoni (039.36565ºE to 039.36696ºE) and Wasini (039.35906ºE to 039.35942ºE) in the South (Table 1).

Irradiation was done by 'direct plate – kill' method following a slight modification of Kevin and Alice (2001). Cultures for irradiation were grown on nutrient agar media plates and incubated overnight at 30ºC. A suspension of the overnight culture was made using sterile distilled water and serial dilutions were prepared up to 10^{-3}. The 10^{-3} dilutions were used

for preparing the plates for irradiation by spread-plating 100µl of this dilution onto nutrient agar petri plates. After plating, the plates were irradiated in a custom-built UV analysis cabinet (ACM 82307-Delhi (India) using the long wavelength lamp (365nm-UVA, intensity 11.6W cm^{-2}). Different exposure times of 0, 15, 30 and 45 minutes were used, with the zero (0) minute exposure time being used as control. All irradiations were done in the dark to avoid photo-activation and light source was only present during the transfer of the petri plates containing the bacterial treated media into and out of the UV chamber. The lids of the treatment plates were also removed before placing the plates into the chamber to avoid shielding by the lid and replaced immediately after irradiation. The treated plates were then incubated in the dark at 30ºC for 24 to 48 hours before scoring the number of colonies. The UV dose (in W S^{-1} cm^{-2}) was calculated by multiplying the intensity by the irradiation time (in seconds). The UV intensity was considered to represent the irradiance on a flat surface.

Table 1: Bacterial isolates used in the experiment

	Isolate	Closest relative	% similarity	Group	Site isolated
1	79T	*Alcaligenes faecalis* strain IAM 12369	87	β-proteobacteria	Wasini
2	75W	*Bacillus anthracis* str. Ames strain	85	Bacilli	Shimoni
3	74O	*Alcaligenes faecalis* subsp. phenolicus strain J	90	β-proteobacteria	Shimoni
4	85CW	*Bacillus aerius* strain 24K	93	Bacilli	Wasini
5	62M	*Microbacterium koreense* strain JS53-2	97	Actinobacteridae	Mida
6	70CW	*Paenibacillus taichungensis* strain BCRC 17757	99	Bacilli	Shimoni
7	70W	*Alcaligenes faecalis* strain NBRC 13111 16S	91	β-proteobacteria	Shimoni
8	72T	Bacillus anthracis str. Ames strain Ames	87	Bacilli	Shimoni
9	84CY	*Bacillus marisflavi* strain TF-11	99	Bacilli	Wasini
10	72CW	*Bacillus aerius* strain 24K	96	Bacilli	Shimoni
11	KO	*Exiguobacterium* sp. AT1b strain AT1b	99	Bacilli	Kilifi
12	82W	*Bacillus anthracis* str. Ames strain Ames	88	Bacilli	Wasini

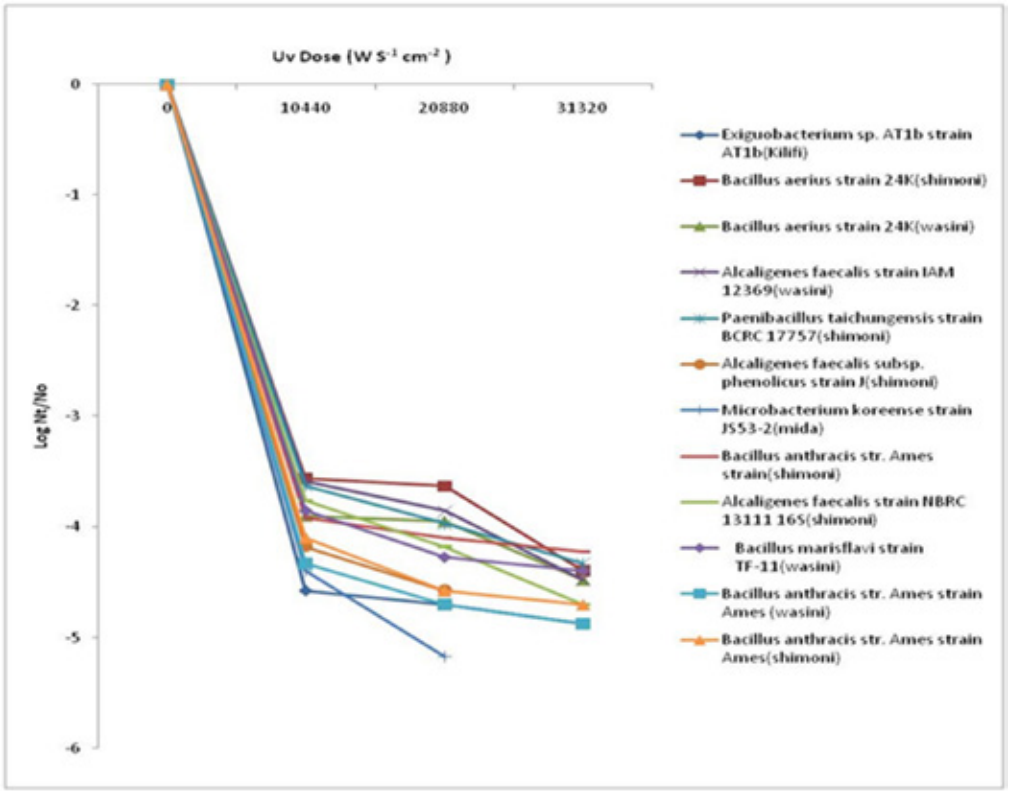

Fig. 1: UV sensitivity curves for the bacterial isolates. Nt number of CFU at time t, No is number of CFU at time 0.

RESULTS AND DISCUSSION

The responses of the isolates towards UV exposures varied greatly as shown in **Fig. 1**. Of the tested isolates, the bacilli group was the most resistant to UV. All the isolates were gram positive. This finding corroborates with the suggestion that Gram-positive bacteria are better adapted to UV stress because their cell walls screen out a considerable fraction of UV radiation (Jagger, 1985). The resistance of these isolates varied with site of isolation as those from Shimoni were more resistant, followed by Wasini, and the least were from Kilifi. The variation in resistance amongst the sites could have been contributed to other factors such as human activities closer to the collection site .

The actinobacterium tested in this study (*Microbacterium koreense* strain JS53-2 was the least resistant to the UV, which is in agreement with observations by Ordon˜ez et al. (2009), demonstrating that cell wall characteristics and G+C content are not the sole determinants of UV resistance. However, this observation was in contrast to that made by Warnecke et al. (2005) where the high G+C content of Actinobacteria is proposed to confer protective adaptation against UV radiation by minimizing the formation of cyclobutane dimmers.

In the present study, beta-proteobacteria tested exhibited medium resistance to UV compared to the other groups. These finding corroborates with Ordon˜ez et al. (2009), where the beta-proteobacteria studied had medium resistance to UV-B exposure.

CONCLUSION

The results of this study have revealed that different epibiotic bacteria found on the surface of the Kenyan marine *L. majuscula* exhibit varied responses to UV exposure. There is need to analyze and understand the different compounds/metabolites associated with resistance to UV and also the mechanisms of UV tolerance.

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REFERENCES

Bewley, C. A., Holland, N. D., & Faulkner, D. J. (1996). Two classes of metabolites fromTheonella swinhoei are localized in distinct populations of bacterial symbionts. *Experientia*, *52*(7), 716–722.

Brunberg, A.-K. (1999). Contribution of bacteria in the mucilage of Microcystis spp.(Cyanobacteria) to benthic and pelagic bacterial production in a hypereutrophic lake. *FEMS Microbiology Ecology*, *29*(1), 13–22.

Cockell, C. S., & Knowland, J. (1999). Ultraviolet radiation screening compounds. *Biological Reviews*, *74*(3), 311–345.

Gerwick, W. H., Coates, R. C., Engene, N., Gerwick, L., Grindberg, R. V., Jones, A. C., & Sorrels, C. M. (2008). Giant marine cyanobacteria produce exciting potential pharmaceuticals. *Microbe-American Society for Microbiology*, *3*(6), 277.

Hempel, M., Blume, M., Blindow, I., & Gross, E. M. (2008). Epiphytic bacterial community composition on two common submerged macrophytes in brackish water and freshwater. *BMC Microbiology*, *8*(1), 58.

Hube, A. E., Heyduck-Söller, B., & Fischer, U. (2009). Phylogenetic classification of heterotrophic bacteria associated with filamentous marine cyanobacteria in culture. *Systematic and Applied Microbiology*, *32*(4), 256–265.

Jagger, J. (1985). Solar-UV actions on living cells.

Lin, K., & Wang, A. (2001). UV mutagenesis in Escherichia coli K-12: Cell survival and mutation frequency of the chromosomal genes lacZ, rpoB, ompF, and ampA. *J Exp Microbiol Immunol*, *1*, 32–46.

Nausch, M. (1996). Microbial activities on Trichodesmium colonies. *Marine Ecology Progress Series*, *141*, 173–181.

Ordoñez, O. F., Flores, M. R., Dib, J. R., Paz, A., & Farías, M. E. (2009). Extremophile culture collection from Andean lakes: extreme pristine environments that host a wide diversity of microorganisms with tolerance to UV radiation. *Microbial Ecology*, *58*(3), 461–473.

Sergeev, V. N., Gerasimenko, L. M., & Zavarzin, G. A. (2002). The proterozoic history and present state of cyanobacteria. *Microbiology*, *71*(6), 623–637.

Simmons, T. L., Coates, R. C., Clark, B. R., Engene, N., Gonzalez, D., Esquenazi, E., … Gerwick, W. H. (2008). Biosynthetic origin of natural products isolated from marine microorganism–invertebrate assemblages. *Proceedings of the National Academy of Sciences*, *105*(12), 4587–4594.

Tan, L. T. (2007). Bioactive natural products from marine cyanobacteria for drug discovery. *Phytochemistry*, *68*(7), 954–979.

Tidgewell, K., Clark, B. R., & Gerwick, W. H. (2010). The natural products chemistry of cyanobacteria.

Tidgewell, K., Engene, N., Byrum, T., Media, J., Doi, T., Valeriote, F. A., & Gerwick, W. H. (2010). Evolved

diversification of a modular natural product pathway: apratoxins F and G, two cytotoxic cyclic depsipeptides from a Palmyra collection of Lyngbya bouillonii. *ChemBioChem*, *11*(10), 1458–1466.

Warnecke, F., Sommaruga, R., Sekar, R., Hofer, J. S., & Pernthaler, J. (2005). Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. Applied and Environmental Microbiology, 71(9), 5551–5559.