EFFECTS OF MAGNESIUM, CALCIUM AND SODIUM SALTS ON GROWTH AND LUMINESCENCE OF *VIBRIO HARVEYI*

Aribah Naz[1]*, Afsheen Arif[2] and Uzma Badar[1]

[1]*Department of Genetics, University of Karachi, Karachi-75270, Pakistan.*
[2]*Dr. A.Q Khan Institute of Biology and Biotechnology (KIBGE), University of Karachi, Karachi-75270, Pakistan.*
* Corresponding author's email address: aribahnaz@uok.edu.pk

ABSTRACT

Among luminescent organisms, bacteria are most abundant, inhabiting oceans as free livings or as symbionts on the surface or inside larger marine organisms. The phenomenon of bioluminescence is well regulated and requires growth medium supplemented with various organic and inorganic salts for persistent luminescence.

To study the effects of various salts like Calcium Chloride, Magnesium Sulphate and sodium Chloride on growth and luminescence, *vibrio harveyi strain DGU300* (GenBank Accession No. KY653092) was cultured under various growth conditions. Luminescence was significantly reduced in medium deficient in essential inorganic compounds. When cultured in enriched medium, considerable luminescence was recorded even when the cells were in stationary phase. However when the strain was cultured in ASW (Artificial Sea Water) + 1 % glycerol lacking in any of the selected organic compounds displayed no luminescence even in log phase, suggesting these compounds should be an integral part of the media for culturing luminescent bacteria.

Keywords: Bioluminescence, Calcium Chloride, Magnesium Sulphate, Sodium Chloride, *Vibrio harveyi.*

INTRODUCTION

Bioluminescence enhances the beauty of dark world by illuminating it, these glowing organisms are mostly distributed in deep dark oceans and fresh water while some of them are also present on land. Among these luminescent organisms, bacteria are most abundant and can be found as free-living species, as saprophytes on dead fishes, as parasites of crustacea and as symbionts in the gut and light organs of many marine fishes and squids (Meighen, 1991). The glowing feature of bacteria is an attribute of *lux* operon, which codes for substrate (Luciferin) and enzyme (luciferase) that oxidizes it in the presence of oxygen (Widder, 2010). The reduction of molecular oxygen results in the formation of intermediates of high energy, which releases the energy as light (Lin *et al*., 2004).

Luciferase genes are reviewed and documented as reporter genes for *in vivo* visualization of their expression in various transgenic organism including plants (Koncz *et al*., 1990). It is employed to monitor toxicity in water tasters obtained from various sources, considerable decrease in light production from glowing culture indicates presence of potential contaminants in a sample (Attar and Afshar, 2010).

The expression of the operon is regulated by mechanism known as Quorum sensing, a density dependent system regulated by concentration of autoinducers released from the cells for switching the operon ON (Ng and Bassler, 2009). *Lux* system is also reported to be an expensive and luxurious system which drains about 20% of the cells energy (Nackerdien *et al*., 2008; Kozakiewicz *et al*., 2005), that justifies the need of strict control system for its regulation. Studies revealed that there is continuous struggle for energy utilization between luminescence and growth known as energy sink hypothesis (Nackerdien *et al*., 2008). Considerable variability has been reported at species level in metabolic patterns of luminescent bacteria and these differences may reflect ecologically important traits for individual species. Factors like media composition, pH, oxygen content and other environmental factor are reported to play major role in regulation of bioluminescence. It was reported that the cells cultivated in an inorganic salts solution containing KCl, $CaCl_2$, $MgCl_2$, $NaHCO_3$, and $MgSO_4$ exhibits more intense luminescence (Tabei *et a*l., 2013).

The study aimed to investigate the concentration of calcium, magnesium and sodium salts required for bioluminescence. Luminescent *V. harveyi* isolated from fish gut (Accession No: KY653092) was cultured in Nutrient Medium (NM), Artificial Sea Water (ASW) supplemented with 1% glycerol and with various concentration of Calcium Chloride, Magnesium Sulphate and Sodium Chloride to monitor the alteration in growth and luminescence pattern.

MATERIALS AND METHODS

Growth Media and Culture Conditions

From purified bacterial culture stock, 50µL *Vibrio harveyi* was inoculated in 100mL Nutrient medium supplemented with 3% NaCl, kept at 30°C in shaking incubator at 100rpm. Growth ($O.D_{600nm}$) was recorded using Beckman Coulter DU® 730 life Science UV/Vis spectrophotometer until it reaches 0.2.

To gauge the effect of selected salts, minimal growth media was prepared with ASW (26.3gm NaCl, 0.74g KCl, 0.99g $CaCl_2$, $MgCl_2$. 6H2O, 3.94g $MgS0_4$) dissolved in one liter of distilled water followed by autoclaving at 121°C for 20 minutes and supplemented with 1% glycerol.

Quantitative Analysis

In order to perform quantitative analysis**,** 1mL bacterial culture ($O.D_{600nm}$= 0.2) was transferred to each flask containing 10mL ASW, 1% Glycerol, each flask is also supplemented with the definite concentration of any one of the given salt i.e., $CaCl_2$ ranging from 0%-5% (with the difference of 0.5%), $MgSO_4$ ranging from 0%-1% (with the difference of 0.1%) or NaCl ranging from 0%-10% (with the difference of 1%). Nutrient Medium was also used to observe growth and luminescence in rich medium. The experiment was set in triplicate, in sterile test tubes. Growth and luminescence were recorded after 24 and 48h using Beckman Coulter DU® 730 life Science UV/Vis spectrophotometer and Turner Biosystems Modulus™ Single Tube Multimode Reader, respectively.

RESULTS

Nutrient Medium

Bacterial cells when cultured in nutrient medium, highlights direct relationship of growth and luminescence (Fig.1), intense luminescence was observed after 24 h but after 48h luminescence gradually decreases. It suggests the number of living cells decline after 48 h and those alive utilizes the energy for survival rather than luminescence.

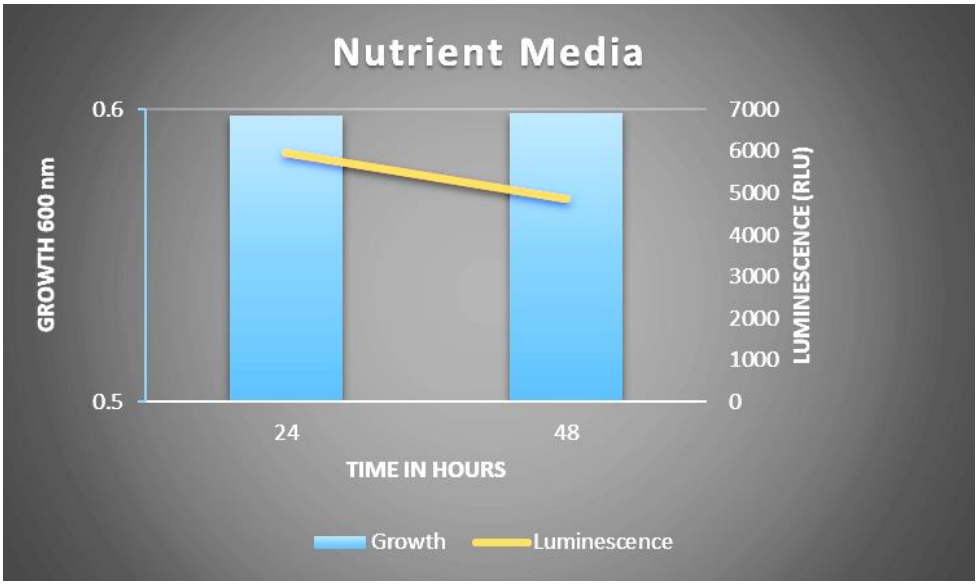

Fig.1. Relationship of growth and luminescence in Nutrient Media supplemented with 1% glycerol.

Effect of Calcium Chloride

Growth and luminescence for the culture grown with different concentration of calcium chloride (Fig. 2), found to be directly correlated with the concentration of calcium. Considerable luminescence was recorded in the samples supplemented with $CaCl_2 \geq 1.5\%$, from this point, luminescence was found to be increased with the concentration of $CaCl_2$.

Effect of Magnesium sulphate

Initially growth and luminescence increase as the concentration of magnesium sulphate increased, until 0.8% $MgSO_4$. At any further increase, growth as well luminescence was decreased (Fig. 3).

Effect of Sodium Chloride

Data for growth and luminescence suggested that the samples with 4% - 6% NaCl showed better luminescence, with maximum at 5% (Fig. 4); thereof, a decrease in luminescence was observed.

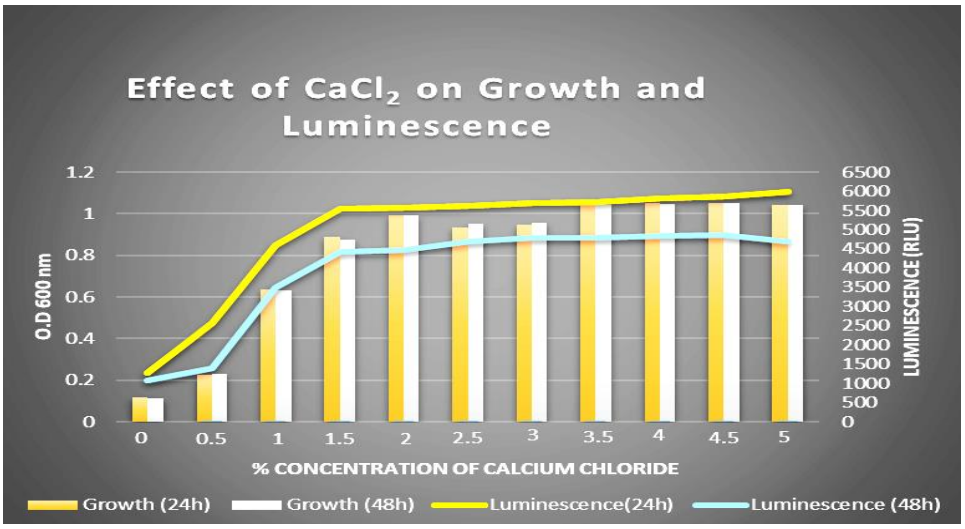

Fig. 2. Relationship of growth and luminescence in ASW supplemented with 1% glycerol, dosed with different concentration of $CaCl_2$.

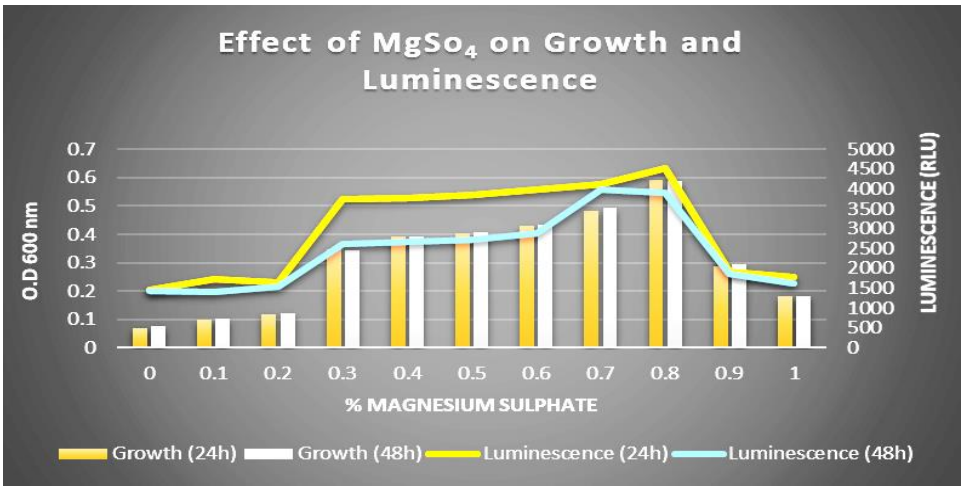

Fig. 3. Relationship of growth and luminescence in ASW supplemented with 1% glycerol, dosed with different concentration of $MgS0_4$.

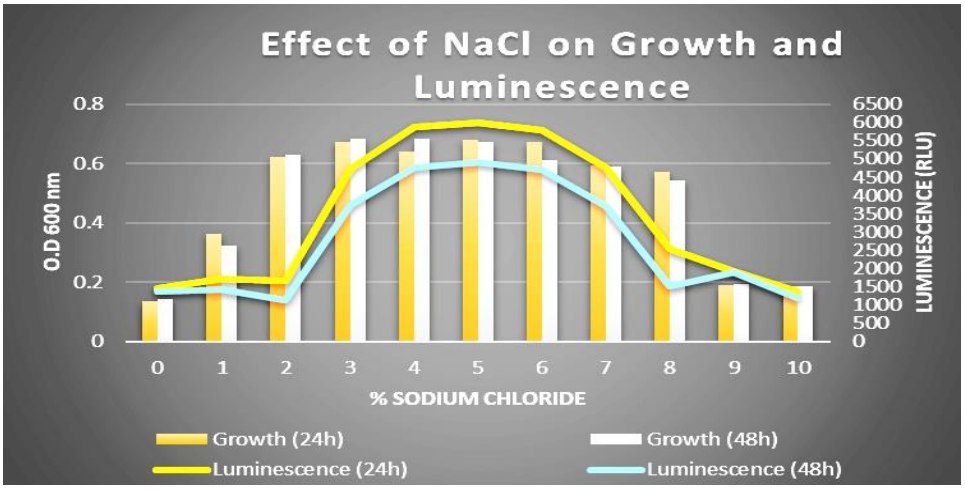

Fig. 4. Relationship of growth and luminescence in ASW supplemented with 1% glycerol, dosed with different concentration of NaCl.

DISCUSSION

Every activity taking place inside cell requires energy and so the luminescence. Huge amount of the cell's energy is consumed for luminescence and growth. The gene expression responsible for luminescence is highly regulated and it may alter under any extreme and rapidly changing growth or environmental conditions (Boor, 2006).

Calcium being an essential cation in various cellular processes has always been extensively studied, its effect has been monitored on various physiological processes including bioluminescence. Our study revealed that for considerable growth and luminescence, the concentration of calcium chloride should be ≥1.5%.

Considering the importance of Magnesium as co-factor in regulating various biochemical reactions, luminescence was also thought to be in its control. Quantitative analysis suggests that luminescence increases with increased concentration of Magnesium sulphate until 0.8%. It was reported earlier that $MgCl_2$ starvation had little effect on luminescence intensity; it was evident that magnesium was less important instead these are sulphur ions that played a crucial role in emission of luminescence under nutrient-starved conditions (Tabei *et al.*, 2011).

Marine environment is rich in various types of salt where NaCl is in highest quantity i.e., 3%. It has been concluded that about 4% to 6% NaCl is ideal for luminescence. Concentration greater than 6% is found to be toxic for cell growth as well as luminescence. A similar study was conducted by Thomulka and Lange (1997) to evaluate the optimal range of NaCl in a soil and water mixture using a bioluminescent marine bacterium *V. harveyi*, for toxicity testing. Their results suggest that the salt range for the toxicity test with soil is between 7 and 11 percent with the greatest bioluminescence at 9 and 10%. The increase in luminescence at such a high concentration can be explained by the phenomenon that bacteria mutate under stress conditions (Dunlap *et al*., 1995) and could possibly glow more when the stress increases until it reaches certain concentration (toxic).

In nut shell these studies on bioluminescence are of great potential for developing reliable reporter system for many future studies including cytotoxicity and genotoxicity testing.

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(*Accepted for publication January 2020*)