ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 12, pp. 14722-14724, December, 2016

International Journal of Recent Scientific Research

Research Article

OPTIMIZATION OF ISOLATED BIOLUMINESCENCE BACTERIA AND ITS APPLICATION IN FABRICATING BIOLAMP

Nagamani, K*., Prabakaran, P and Vivekananth Thirunavukkarasu

Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram, Dindigul

ARTICLE INFO

Article History:

Received 05th September, 2016
Received in revised form 21st October, 2016
Accepted 06th November, 2016
Published online 28th December, 2016

Key Words:

Bioluminescence, Optimization, Spectrofluorimetry, Biolamp, Vibrio fischeri

ABSTRACT

Bioluminescence can be defined as "living lights". Bioluminescence has the potential to play a major role in influencing population dynamics in light limited environments. Bioluminescence is a widespread phenomenon in the marine world. In this research paper, the growth and the fluorescence of the luminescent bacteria Vibrio harveyi was optimized at different external factors viz pH, Temperature, Nacl and Glycerol concentration was measured using Spectrophotometer and Spectrofluorimetry. Biolamp was fabricated using Vibrio harveyi bacterium which can be used as the source of illumination.

INTRODUCTION

Bioluminescence is a type of luminescence that occurs among a variety of organisms ranging from bacteria, dinoflagellates, protozoa, sponges, molluscs, echinoderms, insects and fish (Shimomura, 2006). Bioluminescent bacteria belong to the class *Gammaproteobacteria*. The capacity for luminescence is closely related to the composition of the culture medium. In order for the bacteria to generate light the presence and access to oxygen are required. The end product of bacterial luminescence, which is frequently compared to respiration, is not adenosine triphosphate (ATP) these bacteria have unique trait of the ability to emit light (Harvey, 1952).

Quorum sensing phenomenon is known as auto induction phenomenon. (Nunes-Halldorson *et al*., 2010). The emission of light controlled by the expression of genes which activated by the auto induction and bacterial density. Increased luminescence is seen due to high metabolic activity which builds up the energy levels i.e. ATP in the cells (Gokhale *et al*., 2012). The excess of ATP is diverted to the emission of light in the luminescence. However, as the level of ATP in the cell decreases, the light reaction does not take place resulting in lowered luminescence observed as a sharp drop. As the ATP builds again, the light reaction takes place exhibiting increased luminescence.

Bioluminescence generated by bacteria cultivated in the light organ of juvenile squid provides the squid with protection from predators by providing counter illumination (Visick and Ruby, 2006). This symbiosis is maintained throughout the life of the squid, and is highly specific. Other marine bacteria, even luminescent organisms such as *Vibrio harveyi* are pumped through the body of the squid as it swims, but yet not cultivated by the squid. *Vibrio fischeri* benefits from the symbiosis through nutrients provided by the squid.

The energy generated is more than sufficient to provide the 60 Kcal mol^{-1} necessary for light emission (Meighen, 1993). This is, however, an energetically costly process. Hastings and Nealson estimated that light emission represents an energy expenditure of approximately six ATP molecules for each photon, assuming 100% efficiency for the reaction. The energy is conserved in bioluminescent organisms and expressed only when physiologically necessary (Hastings, 1981).

Light, temperature and salinity are interrelated factors which affect the distribution, growth and luminescence of marine luminescent bacteria. Light is one of the major factors that affect the morphology of any organism, directly or indirectly. This phenomenon has also been observed in microorganisms and light is known to inhibit and delay their growth (Idnurm, 2005; Vescovi, 2012). Salts do not have any impact on growth, but combine with certain organic molecule complexes that have

Corresponding author:* ***Nagamani, K*
Department of Biology, Gandhigram Rural Institute – Deemed University, Gandhigram, Dindigul

an important role in controlling luminescence (Zirpolo, 1972). Factors such as chemical (Fidopiastis, 1999), mechanical (Ismail, 1981) and physical (Czyz, 2002) also found to control luminescence. As no other stimulus is required to produce the light, one possible application of bacterial bioluminescence is as a source of illumination. The electronics company Philips is currently exploring the possibility for bioluminescent home lighting in an experimental project called the Microbial Home System. Part of this project includes a 'Bio-light' design, consisting of glass cells containing live bacterial cultures that emit soft green bioluminescent light in the home.

These applications of bioluminescence bacteria's luminescence includes night-time road markings warning strips on flights of stairs, kerbsides etc, informational markings in low-light settings (eg. theatres, cinemas, nightclubs) and new genres of atmospheric interior lighting with, for example, possible therapeutic and mood-enhancing effects. In the field of biotechnology, bioluminescence is applied in the construction of biosensors for detection of contaminants, measurement of pollutant toxicity and monitoring of genetically engineered bacteria released into environment. Other application of biosensor is as the indicator of cellular metabolic activity and for detection of pathogen. In the food industry, the application of bioluminescence was found to have started a few centuries earlier (Griffith, 1993) where the bioluminescence were used to detect pathogens in food spoilage.

MATERIALS AND METHOD

Sampling

The squids were bought from the Tuticorin local market and they were submerged in 3% of Nacl solution and brought to Gandhigram Rural Institute, Dindigul.

Isolation of luminescent bacteria

Squids were dissected and fluids from various parts of Squid viz Skin, Eyes, Light organ and Intestinal region were swabbed on the sterile plates containing SWCA (Sea Water Complex Agar)medium and incubated at 37°C for 2 days and observed under dark condition to visualize the luminescent colonies and they were marked. The marked colonies were pricked and streaked agin to the sterile SWCA plates to get pure cultures. Eight luminescent colonies were isolated and only one colony was selected due to its bright luminescence.

Identification of bacteria

The isolated and selected colony was identified by macroscopic Characterization of colony morphology, colony form, colour and Shape and microscopic characterization of Gram's staining, morphology and structure of the bacterium by light and Scanning Electronic Microscope.

Biochemical Tests

Biochemical characterization viz., iMViC, Carbohydrate utilization test, Oxidase test, Catalase test was performed to identify the Genus of the bacterium.

Sequencing (16sRna)

The genetic level identified bacterium was given to sequencing to identify the species of the bacterium and the identified Nucleotide sequence of the bacterium was submitted to the Bankit tool.

Growth curve

Growth curve experiment was performed using SWC (Sea Water Complex) broth culture using Spectrophotometer at 600nm for 15 hours.

Optimization of Growth of Luminescent bacterium

The growth of the luminescent bacterium was optimized under different concentrations of Nacl and Glycerol viz., 1%, 2%, 3%, 4% and 5%, at various temperatures such as -4°C, 4°C, 27°C, 35°C and 45°C, at various pH viz., 3, 5, 7, 9 and 11 using Spectrophotometer at 600 nm for three days.

Effect of external factors on Fluorescence of luminescent bacteria

The fluorescence of the luminescent bacterium was analysed using Spectrofluorimetry under various concentration of Nacl and Glycerol viz., 1%, 2%, 3%, 4% and 5%, at various temperatures such as -4°C, 4°C, 27°C, 35°C and 45°C, at various pH viz., 3, 5, 7, 9 and 11.

Fabrication of Biolamp

The bio lamp was fabricated by using fused 2feet tube lights with one end closed and other end open.

RESULTS AND DISCUSSION

The Macroscopic characterization of selected colony was pale yellow, Creamy, Round and Raised. Microscopic characterization of the cell was Pink and short bent rod considered as Gram negative bacteria observed under light and Scanning Electron Microscope (SEM).

Biochemical tests viz., Indole, Methyl Red, Nitrate, Oxidase, Catalase and sugars tests such as Maltose, Glucose, Mannitol showed Positive results whereas negative results showed in Voges Proskauer, Citrate Utilization, starch hydrolysis, Casein hydrolysis and sugar tests such as Lactose, Xylose and Sorbitol.

The selected bacterium was given for sequencing and it was identified as *Vibrio harveyi* and its nucleotide sequence was submitted to Bank it tool (http://www.ncbi.nlm.nih.gov/websub/?tool=genebank) and received the accession number KX024568.

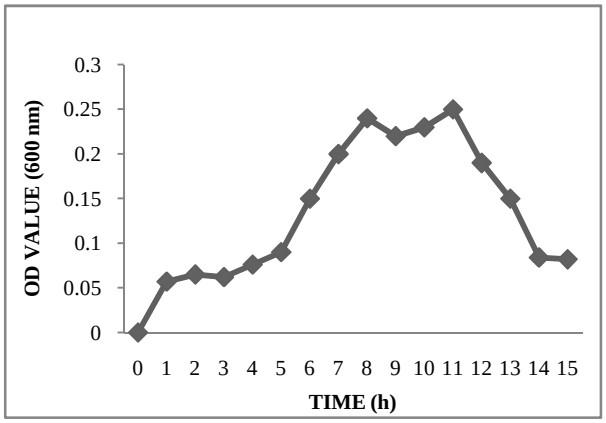

Chart 1 Growth Curve

In Growth curve experiment maximum growth was attained at the eighth hour and reached the declined phase 15 th hour **(Chart 1)**.

External factors such as pH 7, gave the maximum growth whereas other pH gives minimum growth. 1% Glycerol concentration gave the best growth as the concentration increases the growth decreases, 3% Nacl gave best growth and the temperature 30°C and 35°C showed maximum growth than low (-4°C and 4°C) and (45°C) temperature.

Fluorescence of the bacterium at 1% of Glycerol concentration was maximum and the temperature which influenced the fluorescence was 30°C. 3% of Nacl concentration and pH 7 fluoresces brightly.

Biolamp was fabricated by molding the glassware that fits the growth of *Vibrio harveyi*. Thus the lamp gave enough light which can be used as warning light.

CONCLUSION

In this research paper, the growth curve of the bacterium was measured and their growth and Fluorescence was optimized for various external factors at different pH, Temperature, Nacl and Glycerol concentration. Biolamp also fabricated using fused tubelights which gave us enough light to be used as the warning light.

Reference

1. Shimomura, O. 2006. Bioluminescence: chemical principles and methods. World Scientific Publishing Co. Pvt. Ltd., Singapore.
2. Gokhale, T., Wal, A., Parikh, S., Sood, N., 2012. Research of Marine Isolates in Development of Biosensor for environmental Pollutants. Engineering Review. 32(1): 17-22.
3. Nunes-Halldorson, V.D.S., Duran, N.L., 2003. Bioluminescent Bacteria: *Lux* genes as an environmental biosensors. *Brazilian Journal of Microbiology*. 34: 91-96,
4. Hastings, J.W., Nealson, K.H., 1981. The symbiotic luminous bacteria. *In*: Starr, M.P.; Stolp, H.; Truper, H.G.; Balows, A.; Schkegel, H.G. (eds.). Prokaryotes:A handbook of habitats, isolation and identification bacteria. Springer-Verlag, New York., 1332-1345.
5. Visick, K.L., Ruby, E.G. 1999. The emergent properties of quorum sensing: consequences to bacteria of autoinducer signaling in their natural environment. In: Cell-Cell Signaling in Bacteria (Dunny, G.M. and Winans, S.C., Eds.), Press, Washington, DC. 333-352.
6. Idnurm, A., Heitman, J., 2005. PLoS Biol., 3(95).
7. Zirpolo, G., 1972. Studies on bacteriological bioluminescence, action of magnesium salt. NASA Washington, DC. 13.
8. Vescovi, P., Lasers Med. Sci., e-pub ahead of print 29 September 2012doi:10.1007/s10103-012-1197-x.
9. Meighen, E., Dunlap, P.V., 1993. Physiological, biochemical and genetic control of bacterial luminscence. Adv. Microb. Physiol, 34: 1-67.
10. Czyz, A., Plata, K., Wegrzyn, G., 2002. *J. Appl. Genet*. 43:377–389.
11. Fidopiastis, P. M., Sorum, H., Ruby, E. G., 1999. Arch. Microbiol. 171: 205–209.
12. Griffith, M.W., 1993. Applications of Bioluminescence in the Dairy Industry. *Journal of Dairy Science*. 76(10): 34-45.
13. Ismail, S. A., Kaleemurrahman, M., 1981. Curr. Sci. 50: 555.
14. Harvey, E.N., 1952. The nature of animal light. (Philadelphia: J.P. Lippincott Company).
15. Ruby, E. G., and J. G. Morin. 2006. Specificity of symbiosis between deep-sea fish and psychrotrophic luminous bacteria. Deep-Sea Res. 25:161–171.

****

How to cite this article:

Nagamani, K and Prabakaran, P.2016, Optimization of Isolated Bioluminescence Bacteria and Its Application in Fabricating Biolamp. *Int J Recent Sci Res.* 7(12), pp. 14722-14724.