Conference Paper

# The Effect of Light Treatment and Media Combination on Luminescence Endurance of Bioluminescent Bacteria Isolated from Squid [*Loligo duvacelli* (D'Orbigny, 1835)]

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## Abstract

Some bacteria emits light in the dark. The aim of this research is to find lighting duration and medium composition which produce the most enduring luminescence. The results showed that one of the bacteria isolated from light organ of *Loligo duvacelli* (D'Orbigny, 1835) squid are able to emit light. The isolation use trisalt solution and cultured into nutrient agar with addition of NaCl emits the longest blue-green light duration under a 1 : 1 dark-light shift incubation 8.3 d. Meanwhile under a total light, total dark, and in the oven incubator it illuminate for 4.2 d, 3.6 d and 2.6 d, respectively. The combination of beef extract + peptone + a commercial agar + NaCl generate the longest duration of luminescence when incubate in the oven incubator (3,2 d). Meanwhile it illuminated for 2.6 d and 1.7 d on NA + NaCl and NC + NaCl media, respectively, but it failed to illuminate on PCA, NA, and green beans extract + commercial agar + NaCl media. Based on this research we conclude that a commercial agar is potential to replace a technical bacterial agar function.

**Keywords:** bioluminescence; commercial agar; light treatments; *Loligo duvaceli* (D'Orbigny, 1835); medium treatment.

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## 1. Introduction

Bioluminescence is a chemical reaction that takes place in an organism and produces detectable light. These organisms use a variety of body parts to emit light in different colors and for different purposes. This chemical process is different from fluorescence, another process that can cause things to emit light. In a few organisms, bioluminescence and fluorescence both occur [1].

Bioluminescence typically requires at least three components: a light-emitting organic molecule known as a luciferin; a source of oxygen (may be $O_2$, but could also be hydrogen peroxide or a similar compound); and a protein catalyst known as luciferase. In some organisms, these three components are bound together in a complex called photoprotein. Light production may be triggered by the presence of

ions (often calcium) or other chemicals. Some bioluminescent systems also contain a fluorescent protein that absorbs the light energy produced by the photoprotein, and re-emit this energy as light at longer wavelength. Several different luciferins have been found in marine organisms, suggesting that bioluminescence may have evolved many times in the sea among different taxonomic groups. Despite these differences, most marine bioluminescence is green to blue in color. These colors travel farther through seawater than warmer colors. In fact, most marine organisms are sensitive only to blue light [2].

Squid in Indonesia are known to emit light, helping the squid to find for food in the dark water, as well as a tool disguise from predators. Light emitted by a squid is due to the symbiotic relationship between squid species *Loligo duvaucelli* with bacteria *Photobacterium phosphoreum* that live in it [3]. Luinescent bacteria light transmission is catalyzed by an enzyme called luciferase. The enzyme catalyzes the three substrates which reduced flavinmononucleotide ($FMNH_2$), oxygen ($O_2$) and long-chain aldehyde (RCOH). That reaction frees flavin (FMN), long chain fatty acids (RCOOH), and water ($H_2O$) while emitting visible light [4]. The aim of this research is to find lighting duration and medium composition which produce the most enduring luminescence.

## 2. Material and Method

### 2.1. Source

Squid [*Loligo duvacelli* (D'Orbigny, 1835)] were obtained from traditional markets in Malang, East Java. Ink organ of squid were separated from the body and crushed using mortar and pistil until homogeneous.

### 2.2. Dilution

Dilution of sample was made by adding 1 mL sample into 99 mL trisalt solution. [3] Trisalt solution was made by diluting 0.75 g KCl, 6.94 g $MgSO_4$ and 23.4 g NaCl up to $1 \times 10^3$ mL aquadest [5].

### 2.3. Medium

Technical medium was made by diluting 20 g NA instant, 30 g NaCl and 3 mL glycerol up to $1 \times 10^3$ mL of aquadest. NA liquid (LN) medium was made by diluting 3 g beef extract and 5 g peptone up to $1 \times 10^3$ mL of aquadest.

### 2.4. Artificial medium

#### 2.4.1. Technical agar substitution

Diluting 8 g of commercial agar, 3 g beef extract and 5 g peptone up to $1 \times 10^3$ mL aquadest.

#### 2.4.2. Addition of green beans extract, substitution of beef extract

About 1 kg of green beans were washed with water, peels are removed, then dried in the open air and boiled with a ratio of $1 \times 10^3$ g green beans: $2 \times 10^3$ mL of water, blended, filtered and reboiled. 8 g of commercial agar (plain) were added in 20% of green bean extract and 3 g NaCl up to $1 \times 10^3$ mL aquadest.

### 2.5. Sterilization

All mediums were sterilized using an autoclave for 15 min at 121 °C/15 PSI temperature (1 pascal is equal to 0.000145037738007 PSI, or 0.001 kPa).

### 2.6. Light provision treatment

The experiment was conducted in Microbiology Laboratory, State University of Malang. Dark treatments were done by putting the culture in a closet and light treatment was done by placing the cultures in a constantly lighted room. Dark-light shift incubations were done by putting the cultured bacteria in a glass in front of the room as it followed the day-cycle.

## 3. Results and Discussions

### 3.1. Light treatment duration

Table 1 and Figure 1 showed the highest to lowest average lighting duration in the Dark-Light Shift Room (8.3 d), Light Room (4.2 d), and Dark Room (3.6 d), and Incubator (2.6 d). The data as analyzed using SPSS 16 as shown in Table 2.

Results showed that incubation treatment was not significantly different from dark room treatment but significantly different from light room treatment, light room treatments significantly different from the dark-light shift room treatment. Therefore, dark-light shift treatments is the most effective treatment for the longest lighting duration of the bacteria.

| Treatments | Lighting Duration (d)* | | | |
|---|---|---|---|---|
| | Incubator | Dark Room | Light Room | Dark-Light Shift Room |
| Repetition 1 | 2 | 4 | 5 | 8 |
| Repetition 2 | 2 | 4 | 6 | 7 |
| Repetition 3 | 3 | 3 | 5 | 8 |
| Repetition 4 | 2 | 5 | 4 | 9 |
| Repetition 5 | 3 | 4 | 5 | 10 |
| Repetition 6 | 3 | 2 | 3 | 8 |
| Repetition 7 | 2 | 3 | 5 | 7 |
| Repetition 8 | 4 | 4 | 0 | 9 |
| Repetition 9 | 3 | 4 | 5 | 9 |
| Repetition 10 | 2 | 3 | 4 | 8 |
| Average | 2,6 | 3,6 | 4,2 | 8,3 |

*: Data were taken starting from the day after inoculation.

Table 1: Light treatment duration.

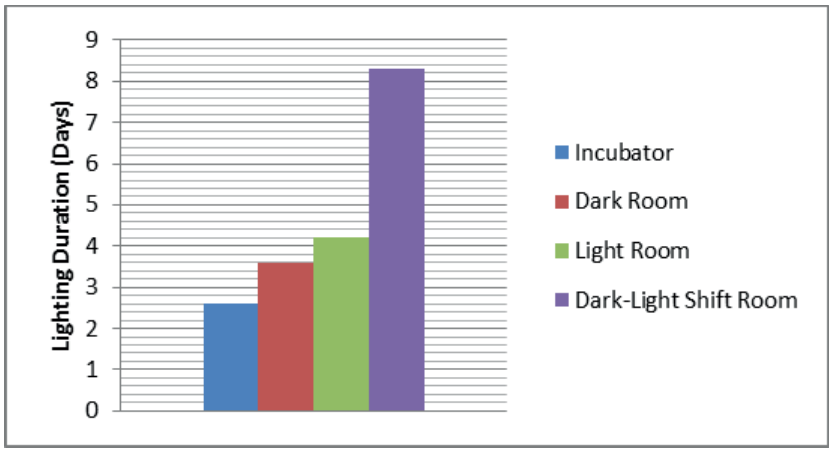


**Figure** 1: The average of Lighting duration under provision light treatment.

## 3.2. Lighting duration under different medium treatments

The combination of beef extract + peptone + a commercial agar + NaCl showed the longest lighting duration of bacteria when incubated in the incubator (3.2 d). It illuminated for 2.6 d and 1.7 d on NA + NaCl and NC + NaCl media, respectively, but it did not illuminate on PCA, NA, and green bean extract + commercial agar + NaCl media. The data was analyze using SPSS 16 as shown in Table 4.

The results indicate that a significance of anova is 0.000 smaller than count significance 0.05. It means that type of medium affect the lighting duration of the bacteria. From the statement, the test were continued using Duncan test to determine which

| Lighting Duration (d) | | | | | |
|---|---|---|---|---|---|
| | Treatments | N | Subset for alpha = 0.05 | | |
| | | | 1 | 2 | 3 |
| Duncan[a] | Incubator | 10 | 2.60 | | |
| | Dark Room | 10 | 3.60 | 3.60 | |
| | Light Room | 10 | | 4.20 | |
| | Dark-Light Shift Room | 10 | | | 8.30 |
| | Sig. | | .052 | .235 | 1.000 |
| Means for groups in homogeneous subsets are displayed. | | | | | |
| a. Uses Harmonic Mean Sample Size = 10,000. | | | | | |

Table 2: Analysis of light teatment duration using SPSS 16.

| Treatments | Lighting Duration (d) | | | | | |
|---|---|---|---|---|---|---|
| | PCA | NA | NA + NaCl | LN + NaCl | Beef extract + Pepton + Commercial Agar + NaCl | Green Bean Extract + Commercial Agar + NaCl |
| Repetition 1 | 0 | 0 | 2 | 2 | 3 | 0 |
| Repetition 2 | 0 | 0 | 2 | 2 | 2 | 0 |
| Repetition 3 | 0 | 0 | 3 | 2 | 2 | 0 |
| Repetition 4 | 0 | 0 | 2 | 1 | 4 | 0 |
| Repetition 5 | 0 | 0 | 3 | 1 | 3 | 0 |
| Repetition 6 | 0 | 0 | 3 | 2 | 5 | 0 |
| Repetition 7 | 0 | 0 | 2 | 2 | 4 | 0 |
| Repetition 8 | 0 | 0 | 4 | 2 | 3 | 0 |
| Repetition 9 | 0 | 0 | 3 | 1 | 2 | 0 |
| Repetition 10 | 0 | 0 | 2 | 2 | 4 | 0 |
| Average | 0 | 0 | 2,6 | 1,7 | 3,2 | 0 |

Table 3: Lighting duration under different medium treatments (incubated 37°C).

| Lighting duration (d) | | | | | |
|---|---|---|---|---|---|
| | Sum of Squares | df | Mean Square | F | Sig. |
| Between Groups | 105.150 | 5 | 21.030 | 70.535 | .000 |
| Within Groups | 16.100 | 54 | .298 | | |
| Total | 121.250 | 59 | | | |

Table 4: Analysis of lighting duration of different medium treatment using SPSS 16.

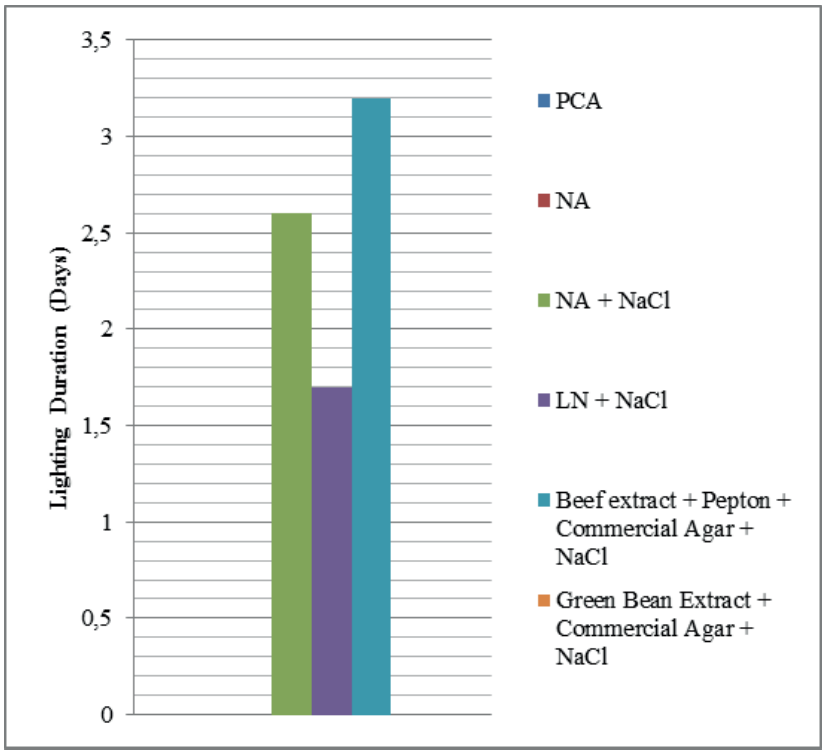


**Figure** 2: Average value of lighting duration during different medium treatments.

| Lighting Duration (d) | | | | | | |
|---|---|---|---|---|---|---|
| | Medium | N | Subset for alpha = 0.05 | | | |
| | | | 1 | 2 | 3 | 4 |
| Duncan[a] | PCA | 10 | .00 | | | |
| | NA | 10 | .00 | | | |
| | Green pea extract + commercial agar + NaCl | 10 | .00 | | | |
| | NC + NaCl | 10 | | 1.70 | | |
| | NA + NaCl | 10 | | | 2.60 | |
| | Beef extract + Pepton + Commercial Agar + NaCl | 10 | | | | 3.20 |
| | Sig. | | 1.000 | 1.000 | 1.000 | 1.000 |
| Means for groups in homogeneous subsets are displayed. | | | | | | |
| a. Uses Harmonic Mean Sample Size = 10,000. | | | | | | |

Table 5: Duncan test of lighting duration of different medium treatment.

type of medium that causing the longest lighting duration and significantly different as shown in Table 5.

The result showed that the use of PCA medium, NA and Green Bean Extract + Commercial agar + NaCl were not significantly different, but significantly different from the use of LN + NaCl medium. The use of medium LN + NaCl significantly different from the

use of NA + NaCl medium and medium NA + NaCl significantly different from the use of medium Beef extract + Peptone + Commercial agar + NaCl. The use of last medium is the most effective.

## 4. Conclusions

From the study we conclude that dark-light shift treatment is the most effective way to extend the lighting duration. A commercial agar is potential to replace a technical bacterial agar function. It is proved from the data that the replacement show the ability to grow the bacteria even a bit longer than technical agar usage.

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