

Optimization of *Vibrio fischeri* bioluminescence property

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ABSTRACT

Vibrio fischeri is a bacterium that produces light, mainly in sea water. The glowing light from luminous bacteria is a specific function consisting of luciferase and luciferin. This ability of *V. fischeri* is a valuable application for researchers. In this study, the growth conditions were optimized for emitting light. We selected some factors can affect on bacterial growth such as temperature, pH and culture medium. 5 ml of sea water broth and 5 µl of *V. fischeri* were incubated in 15, 18, 21, 24 °C. Following that three concentrations of NaCl (1 %, 2 %, 3 %), Asp and Cys (0.005, 0.004 g) were used. The result showed that the best light emitting of marine bacteria was at 24 °C. Top relative light unit (RLU) for this optimization was 22 million. The result showed that we could use these optimized *V. fischeri* for checkup of water and food toxicity.

Keywords: *Vibrio fischeri*, quorum sensing, bioluminescence, amino acid

INTRODUCTION

Vibrio fischeri is a Gram-negative marine bacterium, a member of the family of Vibrionaceae who can live a free or symbiotic with an eukaryotic host [1]. Some species of bioluminescent vibrio have useful

relations with squid and marine fish. However, these bacteria are flexible in the metabolic pathway, and they are not restricted to the host position [1]. The bioluminescence is a Greek word composed of two parts including bio (living) and Latin lumen (light). This kind of chemical

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phenomenon is common in many types of organisms such as bacteria, fungi, fish, crabs, and dinoflagellates, and it is wandering for scientists because of beauty, physiology, biochemistry and genetics features. In the late 19th century, Raphael Dubois experimentally extracted two main enzyme combinations included luciferin and luciferase in bioluminescence response that can produce light. Many bacteria regulated Lux genes by a quorum sensing, which auto-inducer causes a certain response from the cell [2]. There is bioluminescence reaction in parasitic, saprophyte and glowing in the free life. This response is an ecological benefit for fish or squid symbiosis through luminescence bacteria. Host can spread the light to help of bacteria to capture the prey and escape the hunter.

Bioluminescence is spreading of light by way of an organism as a result of a biochemical pathway. Compared with fluorescence and phosphorescence, bioluminescence reactions do not require the early absorption of sunlight or other electromagnetic radiation by a molecule or pigment for the distribution of light. Bioluminescent systems produce light through the oxidation of a substrate which is generally called luciferin (green light) and luciferase enzyme [3,4]. The bioluminescence are extremely altered between organisms, but generally it

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could be described as a catalyst - producing luciferase from an intermediate oxygen and luciferin that emits light when returning to the original state. In addition, many of these systems contain co-factors such as FMNH₂, ATP, excess enzymes, and intermediate phases to create light [4].

The bacterial luciferase is a heterodimer enzyme, with 78 kDa molecular weight, be made up of two unequal α and β subunits with 30 % sequences similarity with LuxA and LuxB genes. Subunits equally are required for luminescence. These subunit is emitted a green-blue light by the maximum concentration of 490-480 nm. Laterally with luciferase genes, only 3 other genes (LuxC, D, and E) are required in whole Lux systems. The genes of LuxC, D, E are encoded for three proteins, which translates amino acid from the pathway of fatty acids into a long branch of aldehydes for luminescence [5].

The marine bacterium because of luminescent activity are usually used in numerous biological testing for investigation of severe toxic responses. Hence, light producing dosage is very important. In this study, we wish to optimize the growth of *Vibrio fischeri* bacteria by making changes in the medium of culture and environmental conditions. It is greatest to know that the overall factors

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for instance metabolism, bacterial growth, conditions of culture bioluminescence activity are sensitive to this choice.

MATERIALS AND METHODS

Bacterial strain

Vibrio fischeri (ATCC 7744) was prepared from the Iranian biological resource center.

Sea water broth medium preparation

The Sea Water Broth (SWB) medium was formulated by 3 ml of glycerol, 5 g of peptone, 3 g of yeast extract, 750 ml of sea water, and 250 ml of deionized water for bacterial culture [6]. To study the water effect of different sea, three kinds of sea water used from Bushehr, Kish and synthetic sea water. In order to prepare the culture medium, we used each of these sea waters separately. For culture of *Vibrio fischeri* 50 µl of bacterial added in 5 mL of SWB medium in a 50 mL falcon, and then was incubated for different time and temperature. The quantity of reflected light was measured by a luminometer, this method is based on ATP which can be transformed from RLU / mol to ATP. The amount of light, as measured by the luminometer, was expressed in relative light unit (RLU). Therefore, there was a direct relationship between living cell levels and light measurement (figure 1).

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Temperature optimization

Temperatures of 15 °C, 18 °C, 21 °C, 24 °C and room temperature (RT) were designated and incubated bacteria by medium in overnight in 150 rpm. Then we observed the bacterial growth frequency through spectrophotometer at 600 nm and luminometer.

pH optimization

The pH of 6.5, 7, 7.5 were examined for culture medium. In order to measure the effect of pH, three pH of 6.5, 7, 7.5 selected. After preparing the growing medium, the amount of pH measured by pH-meter to mentioned values. Next, bacteria were cultured to this medium with different pH.

Optimization of NaCl, Asp and Cys

For changing in sea water, concentrations of 1 %, 2 %, 3 % NaCl, were added to 25 mL of medium, then added 50 µl of bacteria to 5 mL of culture medium. The Asp (0.004 g) and Cys (0.005 g) amino acids were dissolved in 5 mL of medium, and then added 50 µl of bacteria to it. For examination of above test, growth of the bacteria was measured and also amount of ATP.

RESULTS

After 16 h, in the SWB medium with the water of Bushehr and Kish, the bacteria were grown and produced light, but in SWB synthetic growing and light producing were weak. According to the result in table 1, the best temperature was 24°C and RT for growth and light emitting. In 15 °C the rate of RLU unreadable because all samples lost light. Next appropriate medium and optimum temperature for bacterial growth, investigated the pH 6.5, 7, 7.5 on SWB culture medium were considered in Bushehr and Kish sea water at RT and 24 °C. Evidence showed pH of culture medium has no effect in the bacterial growth and light spread (table 2). However, the results of RT were better than 24 °C, these results (figure2)

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were shown the Bushehr sea water in two temperatures. It can be obtained room temperature and 24 °C with Bushehr sea water.

Bioluminescence bacteria live in waters of the sea and ocean, so they needs salt to grow. As a result of some papers, NaCl is very necessary to prepare a medium for this bacteria. Also, the effect of amino acid in bioluminescence mechanism was examined. For this reason, two types of Asp and Cys amino acids was tested in volume of 0.004 and 0.005 g in 5 mL of the culture medium, respectively. The results (table 3) show that the NaCl 1 % and Asp 0.005 + Cys 0.005 have the best effect in light intensity (figure 3).



Figure 1. *V. fischeri* on SWA medium.

Table 1. Primary and secondary OD for investigate of different cultures and temperature

Culture medium	Temperature	RPM	initial absorbance	Second absorbance	RLU
SW Bushehr	15 °C	150	0/285	1/406	-
SW kish		150	0/190	2/850	-
SW synthetic		150	0/152	0/437	-
SW Bushehr	18 °C	150	0/285	3/135	7282730
SW kish		150	0/380	2/964	6476799
SW synthetic		150	0/171	2/983	1394508
SW Bushehr	21 °C	150	0/361	5/111	18210196
SW kish		150	0/475	6/878	-
SW synthetic		150	0/266	4/987	16790720
SW Bushehr	24 °C	150	0/437	10/526	21816836
SW kish		150	0/076	4/693	438629
SW synthetic		150	0/095	4/731	2869
SW Bushehr	RT	150	0/209	8/854	16799872
SW kish		150	0/323	9/633	20530352
SW synthetic		150	0/323	0/589	-

Table 2. Results based on the OD and RLU to investigate the effect of the pH

pH	Culture medium	Temperature	Initial OD	OD	RLU
6/5	SW bushehr	Room temperature	0/152	7/467	19322156
7/5			0/152	4/940	19895652
7/5			0/323	7/448	18562396
6/5	SW kish		0/114	3/420	17185456
7/5			0/114	3/515	21882268
7/5			0/285	3/895	22189364
6/5	SW bushehr	24°C	0/114	5/815	22330196
7/5			0/247	5/871	19845160
7/5			0/285	5/358	22309912
6/5	SW kish		0/190	3/420	13889438
7/5			0/095	3/325	5478
7/5			0/152	3/135	9775726

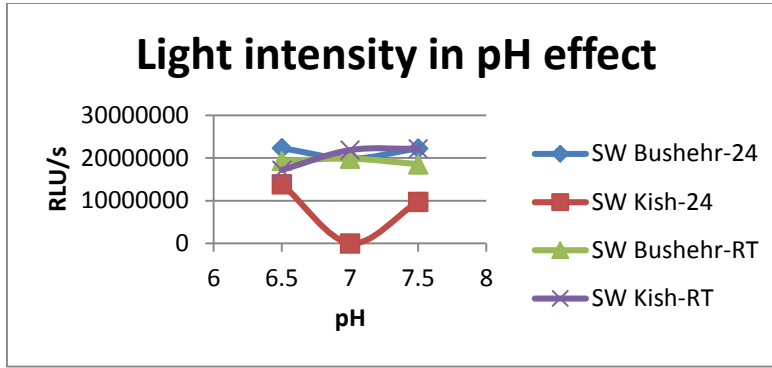


Figure 2. Light intensity result about pH test at RT and 24 °C in two selected medium. Based on graph, pH range effect on light intensity is equal. But results of Kish SW medium at 24 °C reduced.

Table 3. Examination of the effect of salt and amino acids

Amino acid volume	Absorbance OD	Second absorbance	RLU	temperature
Asp 0.005	0/342	0/247	1300	24 °C
Asp 0.004	0/209	5/928	18401760	24 °C
Cys 0.005	0/114	3/287	21933948	24 °C
Cys 0.004	0/152	4/693	21601052	24 °C
Asp 0.005 + Cys 0.005	0/285	2/603	22255092	24 °C
Asp 0.004 + Cys 0.004	0/380	4/313	21348232	24 °C
Asp 0.005 + Cys 0.004	0/304	3/078	13818432	24 °C
Asp 0.004 + Cys 0.005	0/380	3/211	954110	24°C
NaCl 1 %	0/361	3/553	RLU	24 °C
NaCl 2 %	0/266	4/389	1300	24 °C
NaCl 3 %	0/266	1/805	18401760	24 °C

Finally, after achieving optimal growth settings, we investigated 24 h in optimal

conditions of culture, pH and temperature. According to the optimal time (figure 4), the

growth of 24h between 16 and 18 h was considered.

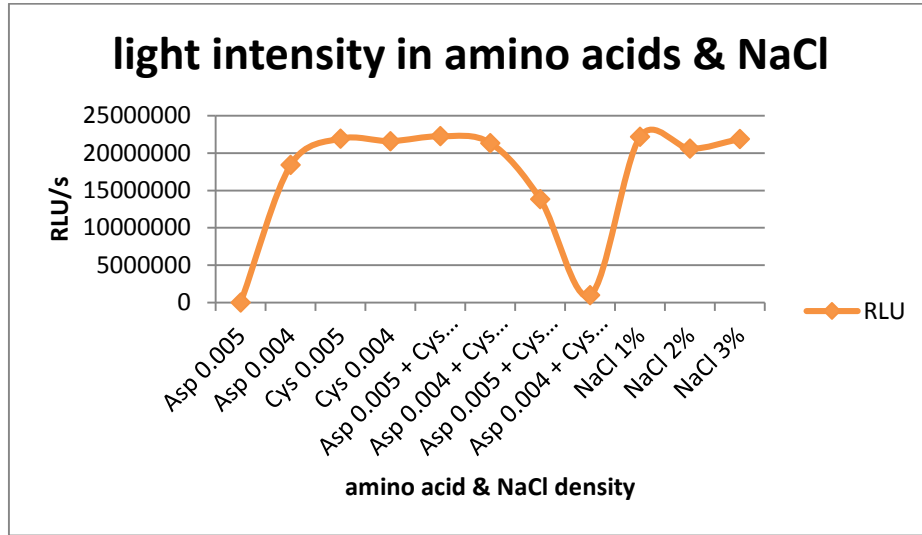


Figure 3. RLU of amino acids and NaCl examination, showed Asp 0/005 and Asp 0/004+Cys 0/005 had low light intensity and Asp 0/005+Cys 0/005 and NaCl 1 % .

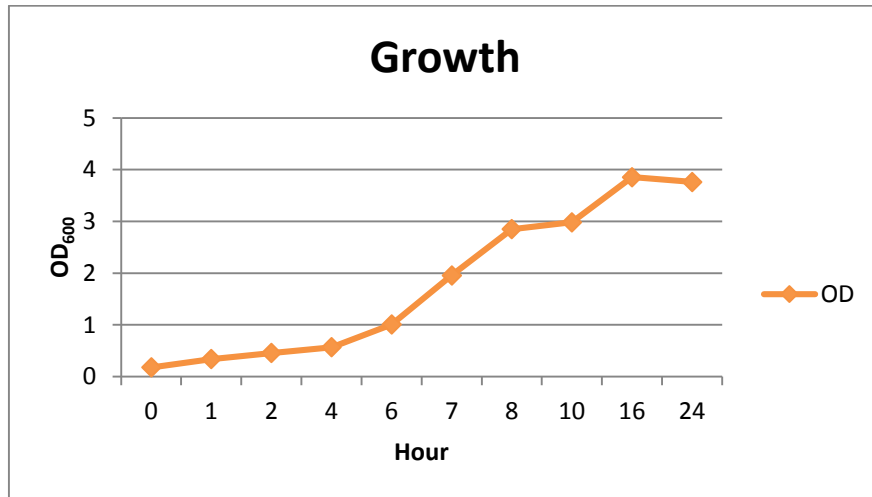


Figure 4. Check of OD in 600 nm in optimal conditions of bacterial growth, this graph show log phase started at 5 h and 16 h.

DISCUSSION

Vibrio fischeri bacterium is a marine bacterium, capable of producing light in the salty water conditions in the sea. The medium which was prepared for bacteria, using seawater, showed better results. Using change in salt and amino acid, ambient conditions such as temperature and other factors investigated the optimum condition for standard strain bacteria. By achieving these optimum conditions, we could use *Vibrio fischeri* bacteria to use different diagnostic and warning tests.

Due to the results in examination the effect of temperature and culture medium, based on OD the number of bacteria and luminescence amount has increased (table 1). It can be considered that the influence of two factors examined in addition of growth and number of bacteria that are effective in the quorum sensing process and light producing.

Different concentrations of Asp and Cys that are not affected the bacterial growth rate as high as previous phases, but so effective on luminescence intensity in bacteria, the effect of the amino acid has not been associated with growth rate increasing (table 3).

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In the case of studying salt concentration at 1 % and 3 % of both bacterial light is high, but in both of them bacterial growth was lower than the previous levels (figure 4).

In research by Hongda and et al [7], the optimum temperature for growth 35°C and temperature for optimal production of light was 20°C. Hassan and coworkers [3] studies, temperature has been set an optimum temperature of 30°C, but in our study was RT and 24°C the best one for the growth and production of light.

Hassan[3] and Hongda [7] said the optimal pH was 7 and 9 and 5-6 have the highest RLU. In our study, pH is in the range of 6.5-7.5 and does not require acidity or excessive alkalization of the culture medium. According to Cook [8] and Hassan [3] and Hongda [7] study, the optimum NaCl is 2 %, and the amount of light is increased. But in the studies showed, NaCl 1 % and 3 % have the highest RLU [9].

Vibrio fischeri, commonly used in various biological tests protocols, are used to investigate highly toxic responses. The tests of bioluminescence bacteria based on EN ISO 11348 are commercially operated by Modern Water and used to represent toxic responses from contaminants. The other techniques that are based on this study are LUMISax, tax screen, Tox Alert. The short

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time of limited tests to bioluminescence is a useful tool to evaluate. The standard Microtox protocol is simple and very cheap, but it is limited to the preparation of the specimen and is required by the relevant expensive equipment.

CONCLUSION

In summary, the optimization in *Vibrio fischeri* is depend on where sea water was collected. Small amount of pH variation is not important but amino acid and Nacl effect on bacterial for light emitting are considered. Further studies need to be undertaken to use this bioluminescence activity in detection of contamination in food and water.

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