Cultural and luminescent Conditions of a Marine luminous Bacterium

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Abstract：Study of marine noctilucence in marine is important to fishery, environmental monitoring and military affairs. A luminous bacterial strain D2 was isolated from the marine sediment samples collected near Donghai Island in Zhanjiang, China. The primary cultural and luminescent conditions of luminous bacterium D2 which was identified as *Vibrio* sp. were determined in liquid culture. The results showed that pH 7.0, 35 °C, with 2.0 % NaCl, were the best growth conditions, and pH5 - 6, 20 °C, OD_{600} 0.08, with 3.0 % NaCl, were the optimal luminescent conditions.

Keywords：Luminous bacterium；growth conditions；luminescent conditions；quorum sensing system

Introduction

Luminous bacteria can emit light under normal metabolic state. Changes in environmental conditions affect the intensity of bioluminescent emission which can be detected by using toxicity induced bioluminescence sensing systems. In recent years, the luminosity of the bacteria has been discovered to show special responses to the toxicity of environmental pollutants and therefore could be used as bacterial biosensors. It has the advantages of sensitivity, accuracy and easy operation.

In 1960s, the bioluminescence of a marine bacterium *Vibrio fischeri* was reported in many researches. For the first time, Nealson et al. (1970) discovered that the intensity of bioluminescent emission was positive correlated to the density of *Vibrio fischeri* individuals. And the bioluminescent emission was controlled by the quorum sensing system (QS).

The bacteria sense cell density within groups by detecting the amount of the signal molecules in order to act as multi-cellular organisms. So they have the functions which can not be obtained by single cell. The density-dependent intercellular communication was defined as bacteria quorum sensing system (Fuqua et al. 1994). Beside bioluminescence, many physiological functions and phenotype characteristics were controlled by the signal transduction induced by quorum sensing system. They include synthesis of antibiotics, regulation of nitrogen fixation gene expression, conjugative transfer of Ti plasmid, swarming, biofilm formation and pathogenic gene expression. N-acyl-homoserine lactones（AHLs）, which was defined as autoinducers (AIs), acts as signal molecules in QS of most gram-negative bacteria. The gene

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expression of bacteria was triggered as soon as the concentration of AHLs molecules exceeded the critical value with the increase of cell density within groups (Fuqua 1996).

Luminous bacteria are generally assigned to four generas, that is, *Vibrio*, *Photobacterium*, *Shewanella* and *Xenorhabdus*. Classification of luminous bacteria that can emit light continuously and effectively is one of the keys to develop bacterial biosensors. A marine luminous bacterium strain was isolated from the sediment near Donghai Island, Zhan-jiang City of China. The purpose of this research was to optimize the growth and bioluminescent emission conditions.

1 Materials and methods

1.1 Materials

1.1.1 Bacterium

Based on morphological and biochemical characteristic analysis, the marine luminous bacterium D2 was identified as *Vibrio* sp. according to Bergets's Manual of determinative bacteriology.

1.1.2 Reagents and instruments

The peptone and yeast extract were produced by OXOID Company. The natural seawater was treated in darkness for weeks before it was used to preparation for culture medium. Other reagents were analytically pure. The organism density of D2 was measured by a Model 722 spectrophotometer. The intensity of bioluminescent emission was detected using a bioluminescence sensing system made in Institute of Soil Science, Chinese Academy of Science.

1.2 Methods

1.2.1 Growth curve of marine luminous bacterium D2

Cultivation of pure bacterial colonies of D2 was conducted in 3 mL 2216E medium (5.0 g peptone, 1.0g yeast powder, 1000 mL treated seawater, pH 7.0). The liquid culture was shaken well in temperature 30 °C for 15 hours. A volume of 100μL culture was combined with 200 mL LB liquid medium. The treatments were incubated at 30 °C reelingly. OD_{600} of the treatments were measured at 2 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h and 13 h, respectively.

1.2.2 Effects of the PH on the growth and luminescence of D2

Combination of pure bacterial colonies with 3 mL 2216E liquid medium was incubated at 30 °C and shaken for 15 hours overnight. A volume of 100μL culture was mixed with 25 mL 2216E medium in which pH varied from 5.0 to 8.0 in 1.0 steps. The treatments were vortexed and incubated at 30 °C. OD_{600} of the treatments were measured after 12 h cultivation. The luminous intensity of the combination of 100μL culture with 0.9 mL NaCl buffer (w/v 3 %) was also measured in which pH ranged from 4.0 to 8.0 in 1.0

steps.

1.2.3 Effects of culture temperature on the growth and luminescence of D2

A volume of 100 μL culture was combined with 25 mL 2216E liquid medium. The treatments were shaken and incubated at the temperature ranging from 20 ℃ to 35 ℃ in 5.0 ℃ steps. OD_{600} of the cultures were measured after 12 h cultivation. The luminous intensity of the combination of 100 μL culture with 0.9 mL NaCl buffer (w/v =3 %) was also measured at 4 ℃, 20 ℃, 30 ℃, 35 ℃ and 40 ℃.

1.2.4 Effects of the concentration of sodium chloride on the growth and luminescence of D2

NaCl solutions, whose concentrations ranged from 0 to 4 % (w/v) in 1.0 % steps, were used to prepare for 2216E liquid medium instead of seawater. A volume of 100 μL culture was combined with 25 mL 2216E medium mentioned above. OD_{600} of the treatments were measured after being incubated at 30 ℃ for 12 h. The luminous intensity of the combination of 100μL culture with 0.9 mL NaCl buffer, whose concentrations ranged from 0 to 6 %, was also measured.

1.2.5 Effect of bacteria density on the luminescence of D2

A volume of 5 mL 3 % (w/v) NaCl buffer was combined with cultures whose volumes were 5 μL, 10 μL, 20 μL, 50 μL, 100 μL and 200 μL. The luminous intensity of the treatments was measured.

2 Results

2.1 Growth curve

An exponential growth type of D2 was observed with time increased (Fig. 1). The stationary phase occurred after 11 h to 13 h incubation.

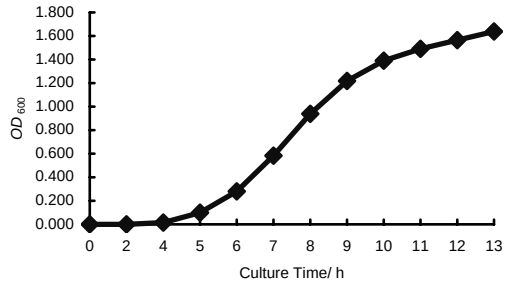
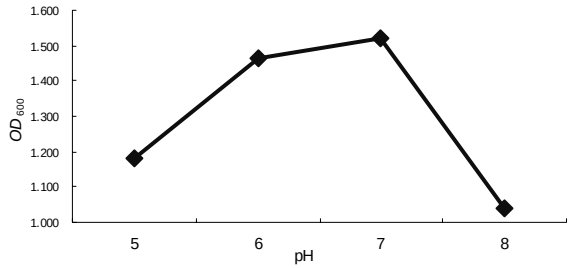


Fig. 1 Growth curve of marine luminous bacterium D2

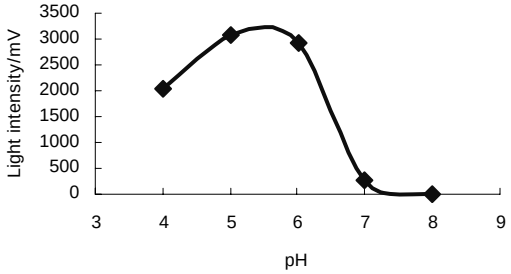
2.2 Effects of the pH on the growth and luminescence of D2

The pH at 7.0 was optimum for growth of D2 (Fig. 2A). Bioluminescence caused by D2 occurred at

pH varying from 4 to 8. The greatest intensity of bioluminescent emission was detected at pH from 5 to 6 (Fig.2B).



(A)



(B)

Fig. 2 Effects of the pHon the growth and luminescence of D2

2.3 Effects of culture temperature on the growth and luminescence of D2

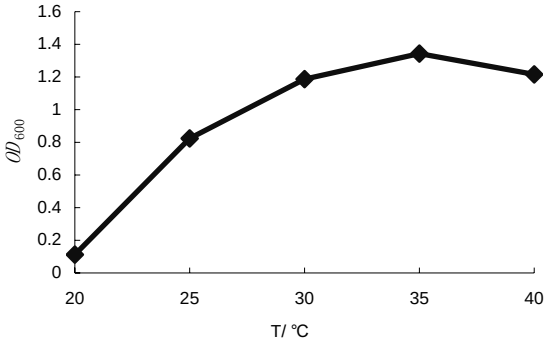
The growth of D2 was optimum at about 35 °C (Fig.3A), while the greatest intensity of bioluminescent emission was detected at about 20 °C (Fig.3B). An inhibition on the growth of D2 by temperature was observed when it exceeded 35 °C.

2.3 Effects of the concentration of sodium chloride on the growth and luminescence of D2

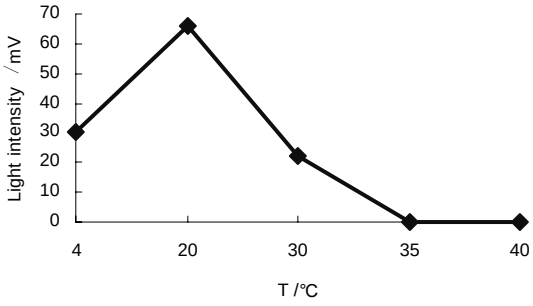
The growth of D2 was optimum at 2 % (w/v) NaCl (Fig. 4A). The significant effects on luminosity of D2 by the concentration of sodium chloride have been observed in the experiment. The greatest emitted light intensity was detected at 2 % (w/v) NaCl (Fig.4B).

2.5 Effects of bacteria density on the luminescence of D2

The intensity of bioluminescent emission was significantly correlated to bacteria density. Little light was emitted when the cells were under the critical density. A burst-mode of luminosity emitted by D2 was observed as soon as the OD_{600} of the treatments reached 0.08 with a density of about 10^5 / mL.



(A)



(B)

Fig. 3 Effects of culture temperature on the (A) growth and (B) luminescence of D2

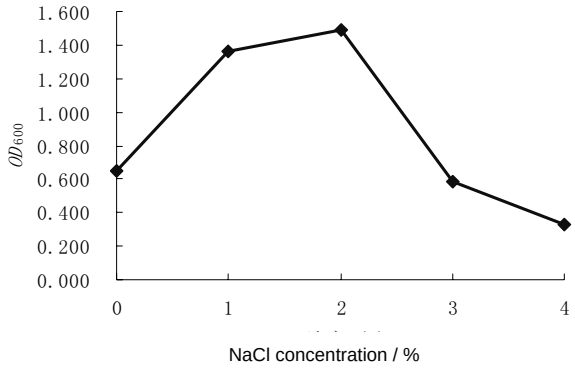
3 Discussion

Marine bacteria, which were rich in environment, have great value in medicine development, ecological research and environmental protection. Marine luminous bacteria are so common that they are isolated from environment for toxicity assessment.

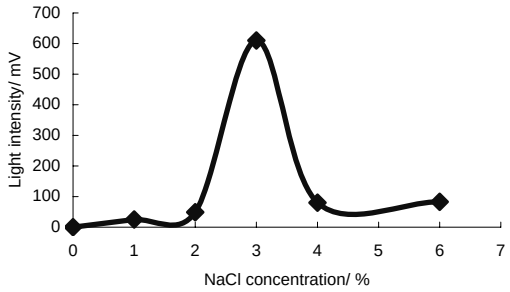
The marine luminous bacteria strain D2, identified as *Vibrio* genus, were isolated from the sediment near Donghai Island at Zhanjiang City. An experiment was conducted to optimize the growth and bioluminescence conditions for D2. The results showed that the optimum conditions for growing D2 were at pH 7.0, 35 ℃ and 2.0 % (w/v) NaCl, while the optimum conditions for bioluminescence were at pH 5 to 6, 20 ℃ and 3.0 % (w/v) NaCl.

A density-dependent reaction was discovered in the bioluminescence of D2. The light emission increased significantly as soon as the cell density exceeded the critical density. In contrast, none of light

emission was detected because the cell density was under the critical density. The results indicated that the critical density of D2 was OD_{600}=0.08. This suggested that the bioluminescence of D2 was also regulated by the quorum sensing system (QS).



(A)



(B)

Fig. 4 Effects of the concentration of sodium chloride on the (A) growth and (B) luminescence of D2

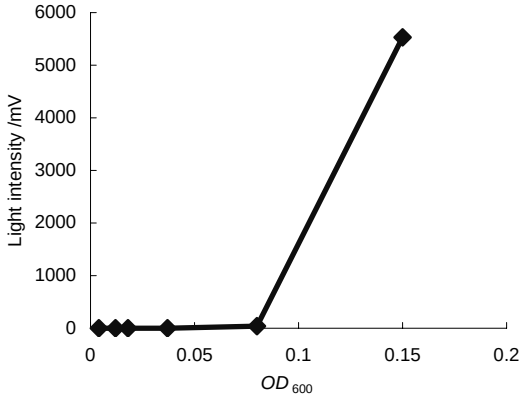


Fig. 5 Effect of bacteria density on the luminescence of D2

In the natural environment, the external cellular signal molecules were often degraded biologically or physically. Factors affecting the light emission of luminous bacteria become more complex. The external cellular signal molecules seldom reached the critical density in marine environment. In contrast, it was more practicable for the bacteria to exceed the critical density as they inhabited in the organs. For example, *Vibrio fischeri* inhabited in the organs could not compose a fluorescein enzyme to emit light until the cells reach the critical density to release enough AHLs signal molecules. Such course was not observed when *Vibrio fischeri* was free in the seawater (Engebrecht et al. 1983, 1984, 1987). Some of luminous bacteria quit light emission as soon as they left the surface of animal skins. It suggested that the bioluminescence was not only controlled by the cells density but also environmental factors such as pH which made it quite different from the niches on the surface of animal skins.

The effects on the growth and bioluminescence of D2 by environmental factors were demonstrated by the experiments. The optimal conditions for growth were different from those for bioluminescence which needs further research on whether it was specie-specific.

In summary, the researches on marine bioluminescence were of great potential for practice. The results on specie-specific spectrum are very important for developing in situ sensing systems and fast indentification kits for luminous bacteria.

Reference

[1] John G Holt, Noel R Krieg, Peter H A Sneath, et al. Bergey's Manual of Determinative Bacteriology (Ninth Edition) [M]. Williams &Wilkins, Baltimore, USA , 1994: 71-377.

[2] Nealson K H, Platt T, Hastings J W. Cellular control of the synthesis and activity of the bacterial luminescent system [J]. J Bacteriol, 1970, 104:313-322.

[3] Fuqua W C, Winans S C, Greenberg E P. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators [J]. J Bacteriol, 1994, 176:269-275.

[4] Fuqua W C, Winans S C. Conserved cis-acting promoter elements are required for density-dependent transcription of Agrobacterium tumefaciens conjugal transfer genes [J]. J. Bacteriol, 1996,178:435-440

[5] Engebrecht J, Nealson K, Silverman M. Bacterial bioluminescence: Isolation and genetic analysis of functions from *Vibrio fischer* [J]. Cell, 1983, 32:773-781.

[6] Engedrecht J, Silverman M. Identification of genes and gene products necessary for bacterial bioluminescence [J]. Proc Natl Acad Sci, 1984, 81: 4154-4158.

[7] Engebrecht J, Silverman M. Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence [J]. Nucleic Acids Res, 1987, 15:10 455-10 467.

海洋发光弧菌的生长和发光条件的研究

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摘　要：海洋生物发光研究对渔业、环境监测等有着举足轻重的意义。本文从湛江东海岛海域沉积物样品中分离出一株海洋发光弧菌（*Vibrio* sp.），本研究中编号为 D2，对这株菌的生长和发光条件进行了初步研究。实验结果表明，海洋发光弧菌 D2 在 pH 7.0、温度 35 ℃、NaCl 浓度为 2.0 %时，生长状态最好；在 pH5 - 6、温度 20 ℃、NaCl 浓度为 3.0 %、细菌密度 OD_{600} 达 0.08 时，发光强度最高。

关键词：发光细菌；生长条件；发光强度；细菌群体感应系统