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Preservation and utilization of luminous bacteria as a light source

Yata HANEDA*

(With 3 text-figures)

発光細菌の光の利用と最も簡単な発光細菌培養基

羽根田弥太*

著者は1943年シンガポールの昭南博物館に勤務中，旧南方総軍より夜間シグナル用に発光細菌の一年間ほど光の持続する小発光体を作るよう要請された。試験的に10 mlの小アンプルに発光細菌を培養し火炎でアンプルを密封したところ，発光細菌はアンプル内の空気中の酸素を消費し，発光は4日後に消えた。この消光した密封アンプルを室温 (26～28°C) 中に放置し，6ケ月後にアンプルを切って空気を入れたところ，10時間後に新しい培養基に接種したと同様，細菌は増殖し，発光を回復した。防疫給水部隊は200名の兵にアンプルを持たせ，夜間演習の朝，アンプルを兵自身の手で開封させて，各自の帽子につけて演習を行った。アンプルは同夜あたかも螢の大群が移動するような壮観を呈した。軍はこの密封アンプルの実用性を認め，防疫給水部隊で大量製造をはじめたが，1945年8月15日日本軍降服のため，全てのアンプルは同部隊の手で破壊され，実戦には使用されなかった。

著者は1946年に復員後，この封入アンプルが何年間，発光を回復するかを知るために，いくらかの封入アンプルを作成し，4年以内であれば100% 発光を回復することを認めた (Figs. 1, 2)。4年以上を経過すると，発光の回復する割合は徐々に落ちたが，アンプル内容を新しい培養基に接種すると，また発光した。そのうち，1949年7月に培養封入したアンプルは28年後の1976年6月に開封し，新しい培養基に接種した20例中の4例は発育発光をみた。電子顕微鏡下で細菌体内に微粒子が認められた。それは発光細菌が胞子を持たない菌であり，無酸素状態で微粒子の形で生き残ったと考えられる (Fig. 3)。この発光細菌培養基は，3% 食塩水を加えたイカ煮汁の寒天培養基で，pH 修正の必要もなく，いつ，どこでも簡単に作れ，発光・生長ともに従来の培養基と比較してすぐれていると考え，羽根田発光細菌培養基と命名する。

Introduction

Man is able to culture large quantities of luminous bacteria using artificial media. The possibility that luminous bacteria can be used as a source of light has been examined and tested thoroughly by many people. The main difficulty has been the relatively short time, perhaps several days or a week, that luminous bacteria are luminescent. Thus, it has been difficult to make any practical use of bacterial light.

During the War from 1942–1945, I was appointed curator of the Raffles Museum and Library (now Singapore National Museum) by the Japanese military authorities in Singapore. At that time, I was studying luminous bacteria

* 横須賀市博物館 Yokosuka City Museum, Yokosuka 238, Japan.
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and other luminous organisms, and the Japanese military asked me to make cultures of luminous bacteria that could be used as a source of light for at least a month or a year. I replied that this would be impossible, but it occurred to me that if I could culture large quantities of luminous bacteria by some simple method, the psychological effect would be great during "black-outs" and "brown-outs," even if the light lasted for only a short duration.

Although it is almost impossible to culture luminous bacteria that would produce long-lasting light, it is conceivable that if we could maintain them in a living condition for long periods of time so that their light-emitting ability can be easily restored, they can be of value in time of war. The Military Research Institute in Japan had been planning to use cultures of luminous bacteria and they prepared some samples for this purpose. In March, 1943, I saw some of the samples in Singapore. They consisted of dried culture media and some strains of luminous bacteria. However, the tropical climate caused the samples to putrify rapidly and they could not be used for preparing cultures. Thus, the experiment for producing light failed.

Material and methods

In my official residence in Singapore I was experimenting with making culture media for luminous bacteria. In time, I finally succeeded in developing the following medium, using freshly caught squid obtained at the fish market:

Remove the ink sac, intestines, and eyes; use only the muscles of the mantle, fins, tentacles, and arms, and cut them into small pieces; boil them in approximately 3% NaCl solution in water in an ordinary pot for about 20–30 minutes; add 15 grams of agar to one liter of hot squid extract and stir until the agar is completely dissolved; pour the mixture onto clean plates or dishes; allow to harden. The pH of the squid extract is about 7.0–7.5 and does not have to be corrected. The emulsion of luminous bacteria may then be poured over the hardened agar.

In the tropics, the air temperature throughout the year varies from 26–28°C. Thus, if the culture medium is inoculated in the morning, the plate will be beautifully luminous and bright by nightfall. The bright light continues for about two days. After three days, the bacterial culture shows little light and is of little use. This method of light production is very simple and not expensive. Therefore, I prepared cultures of luminous bacteria every other day and put them in every room. I found out that this worked out very well during black-outs.

As I mentioned previously, luminescent bacteria are not luminous for long periods of time. However, I imagined that luminous bacteria could live for several months or a year in an non-oxygen atmosphere, such that after a month or a year, they could be revived by simply supplying them with oxygen. With

this in mind, I prepared several samples. I cultured the luminous bacteria in small ampules, containing 10–15 ml of culture media and then immediately sealed them (the tips) with a flame. After 10–15 hours at room temperature, the sealed cultures luminesced beautifully and the luminescence continued for several days until luminescence disappeared for lack of oxygen. If a culture of luminous bacteria is to be made to luminesce again, the tip of the ampule is cut to admit oxygen. This is done 10–15 hours before the light is desired and at a temperature which will permit the bacteria to grow. I made several ampules in May, 1944, and found that they would all produce light 10 hours after the tips were broken.

These sealed ampules can be easily made in the laboratory. During wartime, if a soldier needs a small light for a signal at night or for other use during a black-out or brown-out, or in the field, the ampules offer a useful source of light. I perfected the technique for making these sealed ampules in February, 1945, and the Military Medical Institute at Singapore (Director: Dr. Yoshio HAYAMA) produced about 250,000 of them in the middle of June, 1945, to be shipped to other occupied areas such as Java, Borneo, New Guinea, and Burma. However, in the middle of August, 1945, the war came to an end and the ampules were destroyed by the Medical Institute, thus ending any practical use of them during the war.

After I was repatriated to Japan in February, 1946, I experimented with these sealed ampules again to find out how long the bacteria could survive under the oxygen-free conditions. In these experiments, I used two strains of *Photobacterium phosphoreum* (Syn. *Micrococcus physiculus*, cultured from the luminous organ of *Physiculus japonicus*, and *Photobacterium leiognathi* (Syn. *Coccobacilus equule*) cultured from the luminous organ of *Leiognathus rivulatus*). Sealed ampules of luminous bacteria were prepared between July, 1948, and June, 1952, and they were opened at different times in order to determine the viability of the bacteria. I found that the two strains of bacteria could be made to grow and luminesce again if the tips were cut before 4 years had passed, but after 4 years the percentage of ampules yielding successful growth and luminescence diminished progressively. In the tropics, the temperature is rather even, but in the temperate zones, the temperature, at times, drops sharply and this must be taken into account in carrying out these experiments.

The sealed ampule technique does permit luminous bacteria to be used for the following purposes: 1) Strains of luminous bacteria can be kept for long periods of time without periodic transfers to new culture media. 2) Pure strains of luminous bacteria can be kept without contamination for long periods of time. 3) Luminous bacteria can be kept in ampules without showing diminished luminescence capability, as is often observed with strains that are cultured repeatedly on artificial media. 4) A small light source is conveniently available

which lasts for one to one and a half days by simply cutting off the tip 10–15 hours before hand.

Results

In August, 1980, after 4 years had elapsed since the ampules were sealed, the viability of the bacteria in 10 ampules were tested by opening the ampules to air (Fig. 1). Growth or luminescence was found in any of the ampules. Once again, in June, 1976, 28 years after the ampules had been sealed, 20 ampules were opened and none was found to be luminous. However, on re-inoculating some of the contents of the ampules onto new culture media, 4 ampules (one strain of *M. physiculus* and three strains of *P. leiognathi*) yielded strains that grew and emitted light brightly (Fig. 2).

In order to document this surprising and wonderful phenomenon, the contents of the 4 ampules were studied by electron microscopy (Fig. 3). It is well

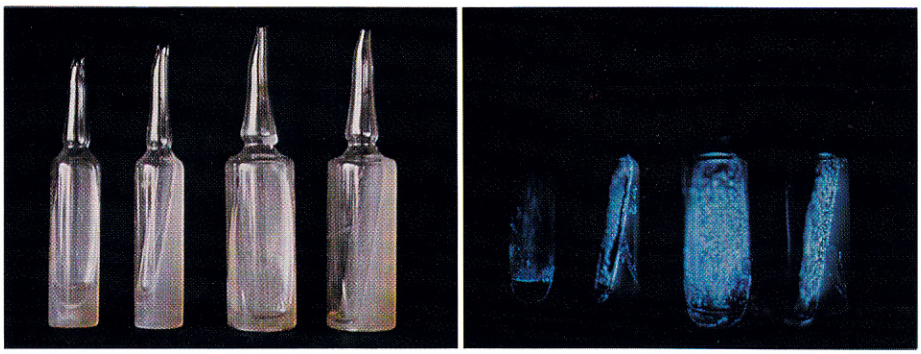

Fig. 1. Left: ampules constaining culture media innoculated with luminous bacteria which had been sealed 4 years previously and the tips subsequently cut and the ampules allowed to stand for 12 hours at 28°C.
Right: the amples photographed in the dark showing luminescence of newly grown bacteria.
Photo by F2-1ends. ASA400 Ektachrome film. 3 minutes exposure

known that *M. physiculus* and *P. leiognathi* do not form spores, but it appears that the 4 strains of luminous bacteria may have survived such a long period (28 years) in a non-oxygen atmosphere by forming spore-like particles as shown in figure 3. It is from these particles that the bacteria appear to have originated. Thus, it seems that bacteria can survive for long periods in an oxygen-free, sterile environment by forming such particles.

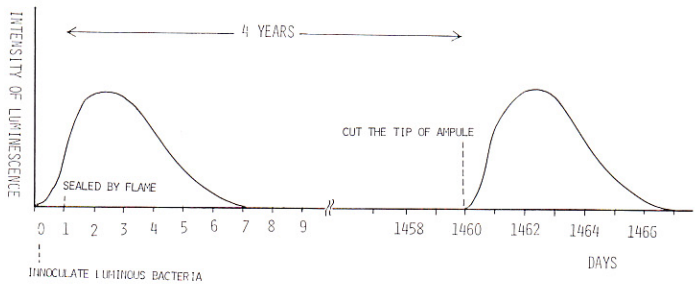

Fig. 2. Luminescence of sealed luminous ampule.
Luminescent curve of sealed ample with luminous bacteria 4 years when the tip of the ampule is cut to admit air.

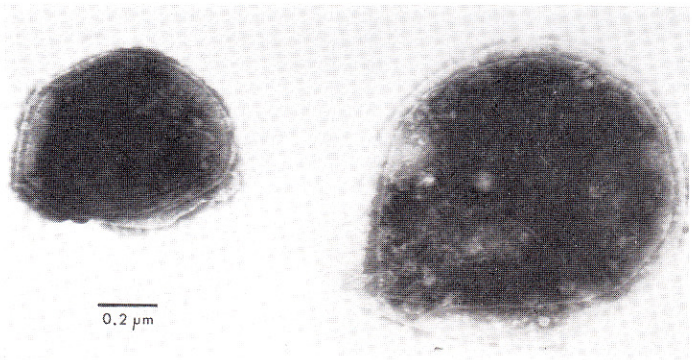

Fig. 3. Electron micrograph of the content of ampule which innoculated and sealed 28 years ago. Date of innoculated and sealed: July 7, 1949, cut the tip of ampule: June 2, 1976.

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