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Isolation of Antibiotic-Producing Cells from Plate Cultures of Egg Powder Enclosing DNA (Hepg2) Crown Cells and Salmon Roe

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ABSTRACT

DNA crown cells can be prepared using sphingosine (Sph)-DNA-adenosine-monolaurin compounds and egg white. Numerous types of DNA crown cells have been prepared to date. In previous experiments, it was demonstrated that antibiotic-producing cells were isolated from beer that was inoculated with DNA (Streptomyces) crown cells and yeast, enclosed in egg white powder, DNA (Streptomyces) crown cells and yeast, or DNA (HepG2) crown cells and yeast. Moreover, antibiotic-producing DNA crown cells could be produced using beef extract instead of yeast. The present study examined whether antibiotics or antibiotic-producing cells could be produced using salmon roe, a type of animal cell. The results showed that antibiotic-producing cells could be produced using salmon roe in combination with DNA (HepG2) crown cells. The resulting cells were named antibiotic-producing Crown-HepG2-Salmon Roe-cells.

Keywords

DNA (HepG2) crown cells, Sphingosine-DNA, Antibiotic-producing Crown-HepG2-Salmon Roe-cells, Monolaurin.

Introduction

Self-replicating artificial cells were first produced in 2012 [1] and the principal methods to prepare them were reported in 2016 [2]. These cells were subsequently classified as DNA crown cells by the present author in 2016 [3]. The exterior surface of DNA crown cells consists of DNA. These cells are produced using four commercially available compounds: Sphingosine (Sph), DNA, adenosine, and monolaurin. They are then incubated in egg white. Numerous kinds of DNA crown cells have been produced to date [4-8], and several strains of these cells have been prepared by the author [9-14]. DNA crown cells have several promising applications in biotechnology and medicine.

In the medical field, DNA crown cells could potentially be used in infection prevention, antibiotic production, drug delivery systems, and other areas. It was demonstrated that antibiotics or antibiotic-producing cells can be produced with DNA crown cells using methods described previously [15].

For example, antibiotic-producing cells have been prepared from beer using co-cultures of DNA crown cells with yeast [16]. Several other cases in which antibiotic-producing cells have been produced have been described [17-18].

Antibiotic-producing cells could be separated from the egg white powder used to enclose DNA (***Streptomyces***) crown cells [17] and DNA (***HepG2***) crown cells in the presence of yeast [18]. Thus, antibiotic-producing cells could be separated using both DNA crown cells, suggesting that the production of antibiotics or antibiotic-producing cells is a characteristic of DNA crown cells. On the other hand, antibiotics and antibiotic-producing cells can also be produced when DNA (***Bovine meat***) crown cells are co-cultured in the presence of a non-living beef extract in addition to yeast [15]. It was demonstrated that micro-organisms and tissue extract were effective for producing antibiotics or antibiotic-producing cells with DNA crown cells The present study examined whether salmon roe, a type of animal cell, was effective when used in combination with DNA crown cells.

It was found that antibiotic-producing cells could be separated from the egg-white powder that enclosed DNA (***HepG2)*** crown cells with salmon roe. These cells are named antibiotic-producing Crown *HepG2-Salmon Roe*-cells.

Materials and Methods

Materials

DNA (***HepG2***) crown cells were prepared as described previously and refrigerated at approximately 4°C [14]. However, the methods are provided here again for clarity. The materials used were the same as those employed in previous studies [14,19,20]: Sph (Tokyo Kasei, Japan), DNA (from HepG2 cell lines), adenosine (Sigma-Aldrich; Wako, Japan), monolaurin (Tokyo Kasei), and adenosine-monolaurin (A-M), a compound synthesized from a mixture of adenosine and monolaurin. Monolaurin solution was prepared to a final concentration of 0.1 M in distilled water. Agar plates were prepared using standard agar medium (SAM) (AS ONE, Osaka, Japan). Salmon roe was obtained from a local market. Potato dextrose agar (PDA; Kyodo Nyugiou, Tokyo, Japan), ***Bacillus subtilis*** (Daikokuya, Nagoya, Japan), Dulbecco's modified eagle's medium (DMEM; Sigma, Burlington, MA, USA), and bovine serum (Sigma) were also used.

Methods

Preparation of DNA (HepG2) Crown Cells [14,19,20]

Step 1: A total of 180 µl of Sph (10 mM) and 90 µL of DNA (0.3 µg/µl) were combined, and the mixture was heated and cooled twice.

Step 2: A-M solution (100 µl) was added and the mixture was incubated at 37°C for 15 min.

Step 3: A total of 30 µl of monolaurin solution was added, and the mixture was incubated at 37°C for another 5 min.

Step 4: The suspension (Synthetic DNA (HepG2) cells) was injected into egg white and incubated for 7 days at 37°C. The egg white was then recovered and used as DNA (***HepG2***) crown cells.

Preparation of Salmon Roe

Salmon roe was washed twice with distilled water before use.

Preparation of Powder

1) First, 3 ml of distilled water containing approximately 20 salmon roe was mixed with 3 ml of egg white.
2) The mixtures were then incubated for 5 h at 37°C.
3) Following the incubation, approximately 25 ml of fresh egg white was added to the mixture.
4) The fluid component was poured and spread onto two petri dishes and dried for 1–2 days at 37°C
5) The dried materials were collected and ground into a powder with a mortar and pestle.
6) The powder (Figure 1), named Crown *HepG2-Salmon Roe*-P, was then stored at room temperature until use.

Preparation of samples for assaying antibiotics

A small amount of powder (30-40 mg) was added to an agar plate and incubated for 2 days at 37°C. Then, approximately 1.5 ml of 0.1 M monolaurin solution was poured onto the plate, which was then incubated for 2 days at 37°C. The objects (Figure 7, within frame A) that formed after the addition of monolaurin were re-suspended in a small volume of distilled water (approximately 0.4 ml).

An aliquot of this solution (200 l) was then added to 5 ml of DMEM containing 10% bovine serum at 37°C for 2–7 days. The culture fluids were used as the sample (A) for the antibiotic assays. Objects (Figure 7 within frame B) were suspended in a small quantity of distilled water (approximately 0.4 ml), and 200 µl of distilled water was poured onto an agar plate and incubated at 37°C for 1–2 days.

Cultured objects (Figure 8) were then collected and placed in a small quantity of distilled water (approximately 0.4 ml). This solution was then cultured with 5 ml of DMEM containing 10% bovine serum at 37°C for 2–5 days (Figure 8; objects comprised of Sample B are shown within the frame).

Samples A and B were then tested for antibiotic activity.

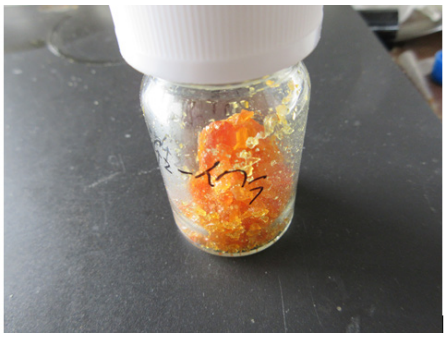

Figure 1: The powder used in this study.

Preparation of Plates for Measuring Antibiotic Production

Antibody assays were conducted using an agar-well method, as described previously [16]. The test bacterium (***Bacillus subtilis***) was mixed with 200 ml agar medium and poured into petri dishes. A well measuring approximately 2 cm in diameter was then prepared in each plate. The test fluid (approximately 400 µl) was dispensed into each plate, which was then incubated for 18 h at 37°C. After incubation, the zone of inhibition was observed.

General observations

Objects on plates were observed by the naked eye.

Results

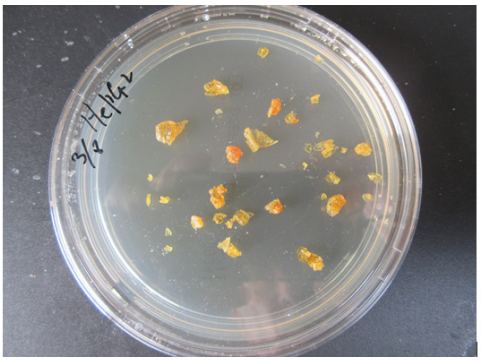

Figure 2: Shows a photograph of an agar plate shortly after inoculation with the powder. The powder or its mass was observed throughout the petri dish, which had a diameter of 8.0 cm.

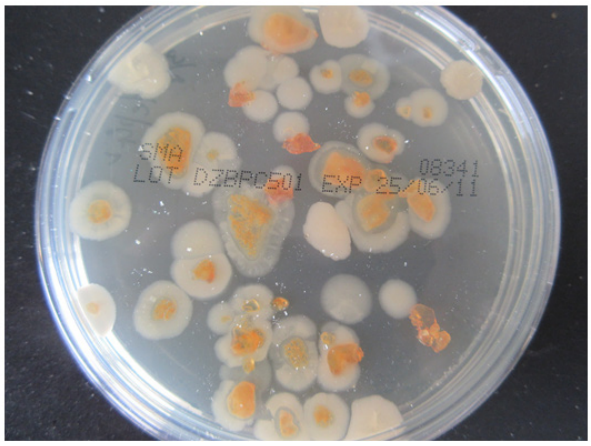

Figure 3: Shows a photograph of an agar plate at 1 day after inoculation with the powder. Objects similar to a diffusely scattered powder were visible to the naked eye over the entire petri dish

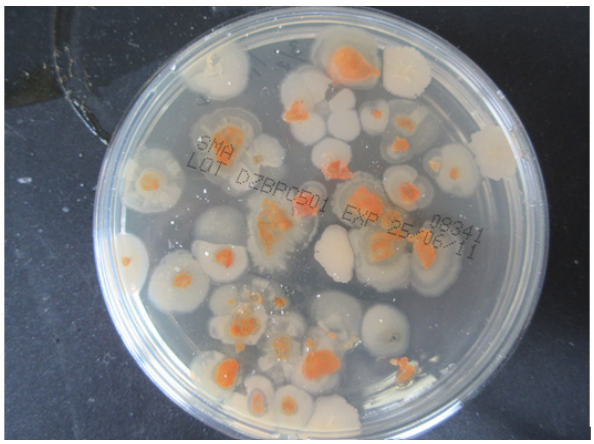

Figure 4: Shows a photograph of an agar plate at 2 days after inoculation with the powder. Objects similar in appearance to roe were observed at the center of the plates.

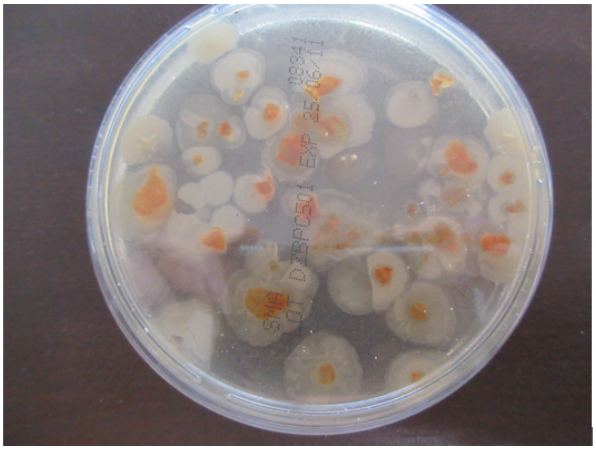

Figure 5: Shows a photograph of a plate inoculated with powder at 1 day after monolaurin addition. Round objects with or without roe-like objects at their center were observed.

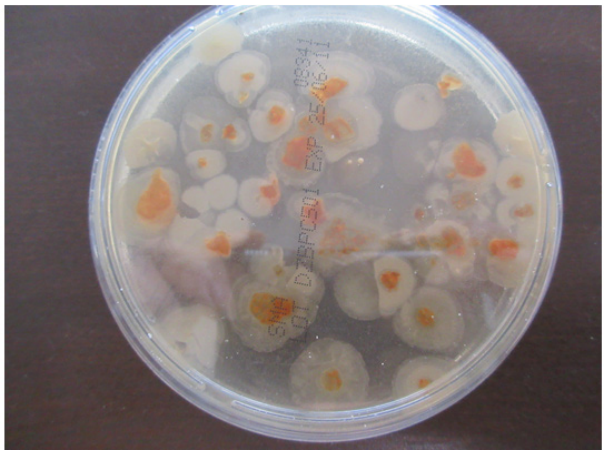

Figure 6: Shows a photograph of a plate inoculated with powder at 2 days after monolaurin addition. Round objects with or without roe-like objects at their center were observed.

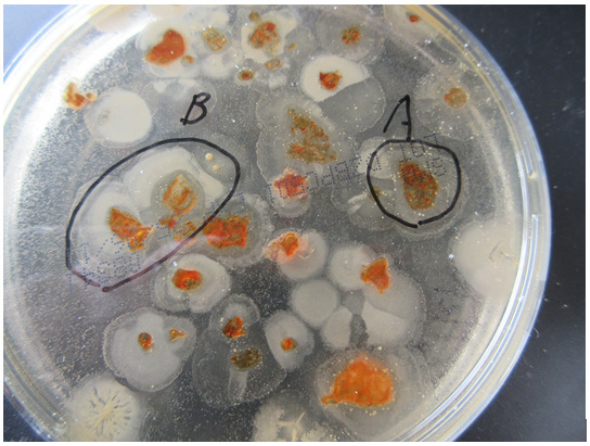

Figure 7: Shows a photograph of a plate **inoculated with powder** at 2 days after monolaurin addition. Objects within frame A were cultured on DMEM and objects within frame B were cultured on SAM. Growing objects were used as sample B.

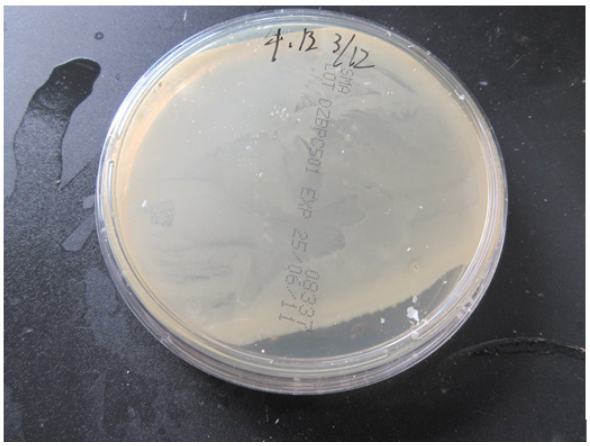

Figure 8: Shows a photograph of agar cultures at 24 days, showing the objects in Figure 7 (frame B). Microorganism-like objects were observed over the entire petri dish.

Some of the objects were cultured and used for the antibiotic assay (Sample B).

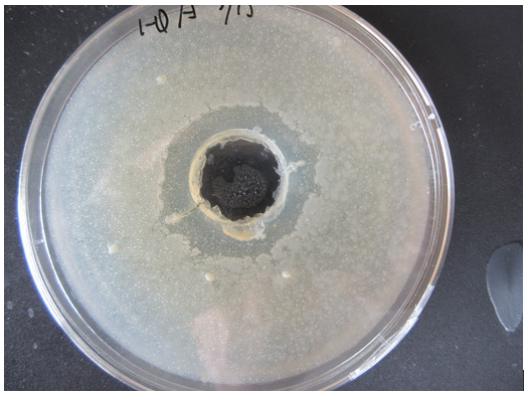

Figure 9: Shows a photograph of an antibiotic assay of sample A. A clear zone was observed around the well.

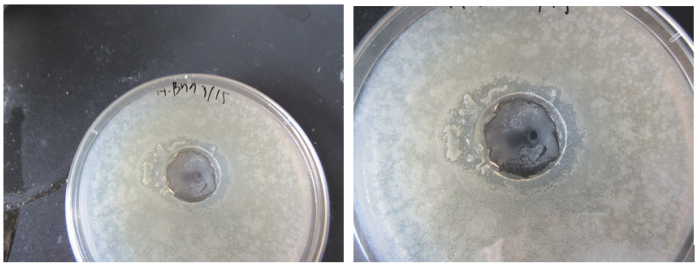

Figure 10: Shows a photograph of an antibiotic assay of sample B.

A clear zone was observed around the well.
DNA crown cells have several promising applications in biotechnology and medicine.

However, actual applications have not been developed to date. Recently, the methods which DNA crown cells may put into practical have been described by the present author [15]. Specifically, it was demonstrated that antibiotic or antibiotic-producing cells could be produced in co-cultures of DNA crown cells and another substance (partner), such as yeast.

The procedure for producing antibiotics or antibiotic-producing cells was described previously [15]. When DNA **(*Streptomyces*)** crown cells and DNA (***HepG2***) crown cells were mixed with yeast and powdered with egg white, antibiotic-producing cells were separated from the culture fluids of these powders [17,18]. Thus, antibiotic-producing cells could be separated using DNA (***Streptomyces***) crown cells and DNA (***HepG2***) crown cells. The results of those studies suggested that antibiotic-producing cells can be produced using different kinds of DNA crown cells. Taken together, the results suggest that the development of antibiotic-producing cells was due to a common ability shared by most DNA crown cells.

It was previously shown that yeast and tissue extracts could be used in conjunction with DNA crown cells [15-18]. Moreover, salmon roe, a type of animal cell, was also effective when used with DNA crown cells. Thus, antibiotic-producing cells can be produced with DNA crown cells using micro-organisms, tissue extracts, and cells. It was therefore suggested that many living or non-living materials could be used in conjunction with DNA crown cells to potentially create an unlimited number of new organisms with antibiotic-producing characteristics or cells with other functions. Thus, methods for producing antibiotic or antibiotic-producing cells were established. However, it is not clear whether the production of antibiotics by these new synthetic cells can be attributed to DNA crown cells or a partner such as tissue extracts or cells. In the present study, if antibiotic-producing cells develop due to the influence of DNA (***HepG2***) crown cells, then it is possible that they may have information derived from HepG2 (liver cancer) and that they could be applied to cancer therapies such as the production of new vaccines. To clarify this point, it will be necessary to produce more antibiotic-producing cells using various combinations of DNA crown cells and partner (tissue extracts or cells). These new organisms may contribute to the development of therapies for incurable diseases, especially those related to cellular disorders which have proved challenging to treat.

In conclusion, the methods described here for producing antibiotics and antibiotic-producing cells with DNA crown cells in combination with other cells (salmon roe) have been described. It is expected that these methods can be applied to create new organisms that could potentially benefit human welfare. Using the naming convention described previously [16], the cells developed in this study are named Antibiotic-producing Crown-*HepG2* Salmon Roe cells -p, as they are antibiotic-producing DNA (***HepG2***) Crown cells produced using salmon roe, where P indicates powder derived from egg white (culture source).

The antibiotic produced in the present experiments is the sixth such finding, so it is named crown antibiotic-6-1 and 6-2.

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