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Antibiotic-Producing Cells from a Plate Culture of Egg White Powder-enclosed DNA (Bovine meat) Crown Cells and Ipomoea Nil-seed Extract

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ABSTRACT

Numerous kinds of DNA crown cells can be prepared using sphingosine (Sph)-DNA-adenosine-monolaurin in egg white. Previously, antibiotics or antibiotic-producing cells were produced from cultures prepared using egg white powder and DNA crown cells together with various partners (e.g., yeast, salmon roe, meat extract, plant-seeds extract). This study examined whether antibiotic-producing cells could be produced using DNA crown cells and flower-seed extract. The results showed that antibiotic-producing cells could be produced using DNA (Bovine) crown cells with Ipomoea Nil-seed extract. The resulting cells were named antibiotic Crown- Bovine meat-Ipomoea Nil-seeds-ex-cells.

Keywords

DNA (Bovine) crown cells, Sphingosine-DNA, Antibiotic Crown-Bovine meat–Ipomoea Nil seeds-ex-cells, Monolaurin.

Introduction

Self-replicating artificial cells were first reported in 2012 [1], and the principal methods for their preparation were reported in 2016 [2]. These cells, which were synthesized using four commercially available substances (sphingosine (Sph), DNA, adenosine, and monolaurin), developed into fully self-replicating DNA crown cells within egg white and were referred to as DNA crown cells in 2016 [3]. The exterior of these cells consists of DNA. Numerous kinds of DNA crown cells, including DNA (Bovine) crown cells [4-8] and associated cell strains [9-14] have been prepared by the author. All crown cells have been stored in egg white at approximately 4°C since production. Interestingly, numerous objects were observed when monolaurin-treated synthetic **DNA (*E. coli/Human placenta/Ascidian/HepG2/ Streptomyces*)** crown cells were cultured with or without egg white on agar plates [15-19].

Also, in a previous study, antibiotic-producing cells were separated from beer that was produced in co-cultures of DNA crown cells and yeast [20]. In addition, egg white powder was used to enclose different DNA crown cells with different substances [21-25]. These antibiotic-producing cells could be cultured using milk as a medium [26-28]. However, the use of seeds or seed extracts for co-cultures has not yet been clarified.

In previous experiments [29], it was demonstrated that antibiotic production was possible using a combination of DNA (Bovine) crown cells and of Kaiware-seed extract.

In the present study, it was examined whether the antibiotic-producing cells were separated from egg white powder, which was used to enclose DNA (Bovine) crown cells with the flower (Ipomoea Nil)-seed extract, and whether the cells could be cultured in milk. These cells were named antibiotic Crown Bovine meat-Ipomoea Nil seeds-ex-cells.

Materials and Methods

Materials

DNA (Bovine meat) crown cells that had been prepared previously and stored in a refrigerator at approximately 4°C were used in this study [4]. However, the methods are described here again for clarity.

The materials used in the present study were the same as those employed in previous studies [4,30,31]: Sph (Tokyo Kasei, Japan), DNA (from bovine meat), adenosine (Sigma-Aldrich; Wako,

Japan), monolaurin (Tokyo Kasei), and adenosine-monolaurin (A-M), a compound synthesized from a mixture of adenosine and monolaurin [30]. Monolaurin solutions were prepared to a final concentration of 0.1 M in distilled water. Agar plates were prepared using standard agar medium (SMA) (AS ONE, Japan). Beef samples (Sendai Beef), kaiware seeds, and milk were obtained from a local market.

Potato dextrose agar (PDA) (Kyodo Nyugiou, Tokyo, Japan), *Bacillus subtilis* (Daikokuya, Nagoya, Japan), Dulbecco's Modified Eagle's Medium (D-MEM) (Sigma, USA), and bovine serum (Sigma) were also used in the study.

Methods

Preparation of synthetic DNA (Bovine meat) crown cells [4,30,31]

Step 1. A total of 180 µL of Sph (10 mM) and 50 µL of DNA (1.7 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. Then, 0.3–0.5 mL of the suspension was injected into egg white and incubated for 7 days at 37°C. Egg white was subsequently recovered and used as DNA (Bovine meat) crown cells.

Preparation of Ipomoea Nil-seed extract
Seeds (~30 grains) were ground using a mortar and pestle and suspended in 3 mL of distilled water.

Preparation of Powder

1. First, 3 mL of seed extract was mixed with 3 mL of egg white.
2. The mixtures were incubated for 5 hours at 37°C.
3. Then, approximately 25 mL of fresh egg white was added to the mixture.
4. The fluids were poured into two Petri dishes and dried for 1–2 days at 37°C.
5. Dried materials were collected and powder was prepared using a mortar and pestle.
6. The powder (Figure 1), named Crown Bovine meat-Ipomoea Nil seeds-ex-P, was stored at room temperature until use.

Preparation of Antibiotic Assay Samples

A small amount (~40–50 mg) of powder (Crown Bovine meat-Ipomoea Nil seeds-ex-P) was added to an agar plate and incubated for 2 days at 37C. Then, approximately 1.5 mL of 0.1 M monolaurin was poured into a plate and incubated for 2 days at 37°C.

Objects that grew on the plate, shown within the frame in Figure 5, were suspended in approximately 1 mL of distilled water. Then, approximately 200 µL of that suspension was cultured in 5 mL of D-MEM containing 10% bovine serum and 5 ml of milk at 37°C for 2–7 days. Culture fluids were used as samples for the antibiotic assay. Objects (200 µL), shown within the frame shown in Figure 5, that had been suspended in distilled water were poured onto an agar plate and incubated at 37°C for 2 days.

The objects that were cultured on the agar plate (Figure 8) were cultured in 5 mL of D-MEM containing 10% bovine serum and 5 ml of milk at 37°C for 2–5 days. The culture fluids were used for the antibiotic assay.

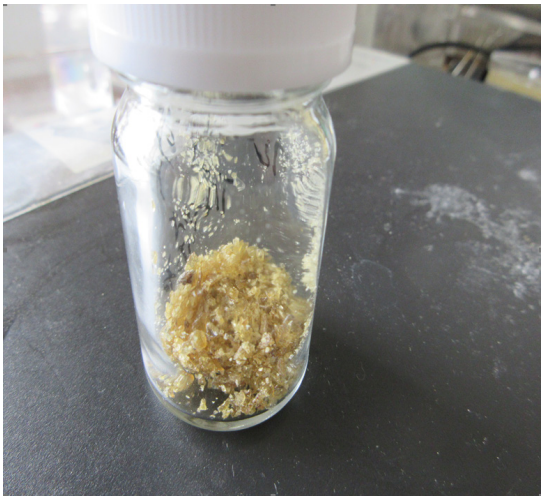

Figure 1: Powder used in the experiments performed in this study.

Preparation of Plates for Antibiotic Assays

The antibiotic assay was carried out using the agar well method, as described previously [20]. *Bacillus subtilis* was mixed with 200 mL agar medium and dispensed into Petri plates. A well measuring 2 cm in diameter was prepared in each plate. The test fluid (400 µL) was dispensed into the well of each plate, and the plates then incubated for 2–3 days at 37°C. After incubation, a clear zone of inhibition was observed.

General observations

Objects on the plates were observed directly with the naked eye.

Results and Discussion

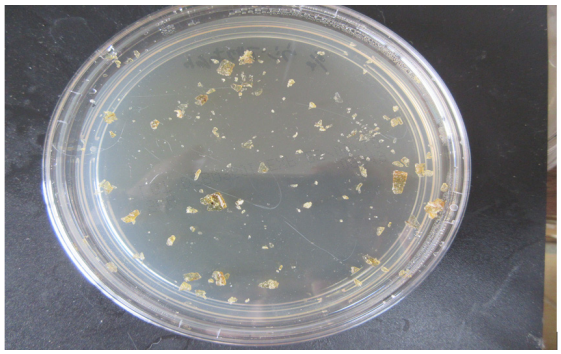

Figure 2: Photograph of an agar plate **immediately after the start of powder culture** (Crown Bovine meat-Ipomoea Nil-seeds-ex-P). Powder particles of various sizes were observed to cover the entire Petri dish.

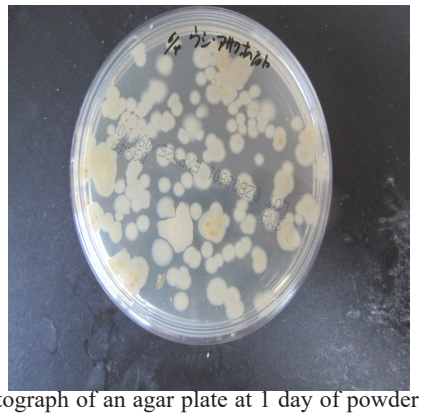

Figure 3: Photograph of an agar plate at 1 day of powder culture. Large objects of various sizes and shapes were observed on the Petri dish.

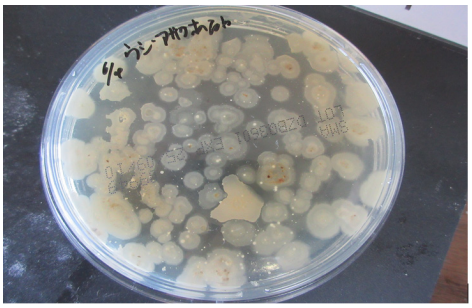

Figure 4: Photograph of an agar plate with powder cultures at 1 day after the addition of monolaurin. Brown objects were observed.

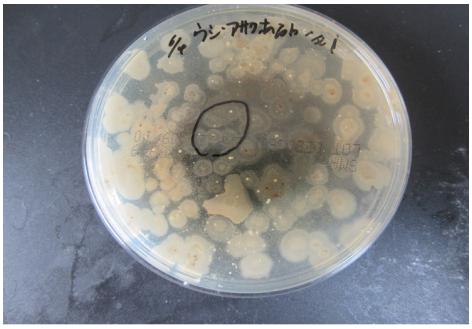

Figure 5: Photograph of an agar plate at 2 days after the addition of monolaurin to the powder. Various brown objects of different shapes and sizes were observed. Objects within the frame were collected and cultured with D-MEM containing 10% bovine serum (Figure 6), milk (Figure 7), and agar (Figure 8). The antibiotic assay was conducted using fluids obtained from each culture.

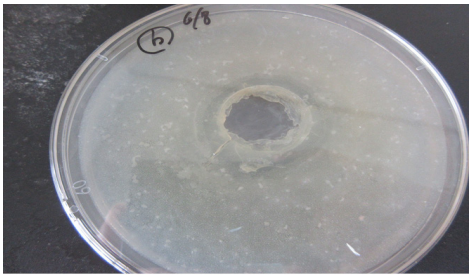

Figure 6: Photograph of the antibiotic assay of the fluids obtained from culturing the objects within the frame shown in Figure 5 and D-MEM containing 10% bovine serum. A clear zone of inhibition was observed around the well.

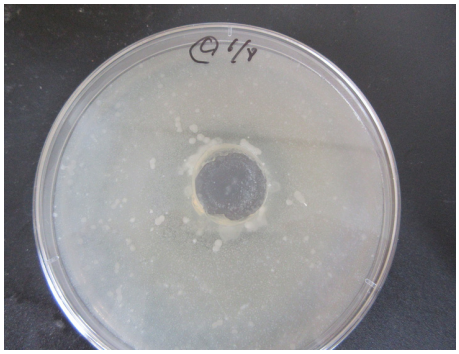

Figure 7: Photograph of an antibiotic assay using milk cultures of the objects within the frame shown in Figure 5. A clear zone inhibition was observed around the well.

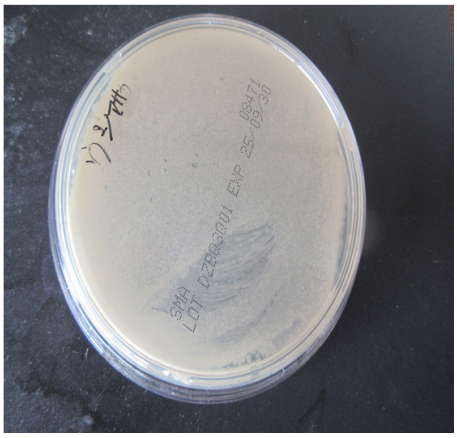

Figure 8: Photograph of objects grown on an agar plate from culture of the objects within the frame shown in Figure 5. Microorganism-like objects were observed over the entire plate.

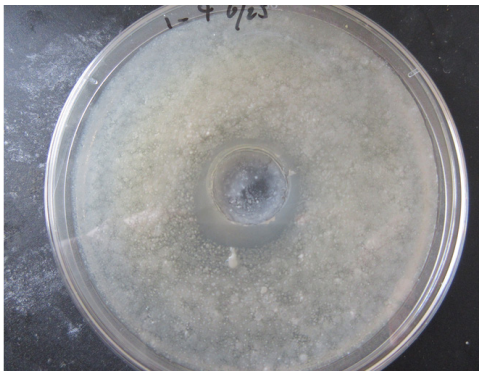

Figure 9: Photograph of the plate used for the antibiotic assay of the microorganism-like objects shown in Figure 8 that were cultured in milk. A clear zone of inhibition was observed around the edge of the well.

Figure 10: Photograph of the clear zone shown in Figure 9. A clear zone of inhibition was observed around the well.

In previous study [29], DNA (Bovine meat) crown cells with Kaiware-seed extract were used, and antibiotic-producing cells were produced using this combination. In the present experiment, antibiotic-producing cells were produced in combination of DNA (Bovine meat) with flower Ipomoea Nil-seed extract and suggested that flower-seed extract can be also used as a partner of DNA (Bovine meat) crown cells.

Antibiotic-producing cells were produced in association with DNA crown cells and may be associated with the DNA from the DNA crown cells. The cells may therefore possess functions in addition to antibiotic production; for example, they may affect the characteristics of bovine meat (e.g., taste, quality, etc.). On the other hand, they may also affect the characteristics of partner substance (i.e., Ipomoea Nil seeds); for example, they may promote germination, growth, color of flower, etc.

However, it was not clear whether these cells have additional functions. Furthermore, these antibiotic-producing cells could be cultured in both D-MEM containing 10% bovine serum and milk. Most of the antibiotics produced using crown cells have been obtained using milk as the culture medium [26-28]. Since the present antibiotics were produced in both D-MEM containing 10% bovine serum and milk, it is considered that the antibiotics produced to date differed among cells.

In addition, it has already been shown that DNA crown cells can produce yogurt [5]. This implies that the present cells, which were produced in cultures of DNA (Bovine meat) crown cells with a partner (i.e., Ipomoea Nil-seed extract), could be cultured in milk and produced yogurt [26-28]. Using milk has many benefits for antibiotic recovery. As described previously [26], milk is a cost effective and sterile culture medium and obtaining large amounts of antibiotic was straightforward. In addition, some of the yogurt that was produced with antibiotic-producing cells does not smell unpleasant, implying that new dairy products may be produced.

Another potential application of DNA crown cells is the investigation of whether they are associated with stem cells, particularly with processes such as stem cell formation, proliferation, or even the origin of cells.

Though unique objects were created with synthetic DNA crown cells and monolaurin [15-19], however, the mechanisms underlying the biosynthesis of these subjects has not yet been clarified.

The findings suggest that these antibiotic-producing DNA crown cells, in addition to potential applications in the medical field, plant production, meat production, beer production, and the dairy industry, could also potentially be applied to the life sciences, such as the proliferation of stem cells or the origin of cells.

Although these cells consist of Sph, DNA, adenosine, and monolaurin (i.e., common components of food and food-related materials) and can be synthesized and cultured using egg white, in utilization of these cells, further research is needed to assess the safety of these cells in terms of human physiology and environmental safety.

The present experiments showed that antibiotic-producing cells and antibiotics were produced using DNA (Bovine meat) crown cells in combination with Ipomoea Nil-seed extract.

Future studies will examine on the characteristics of antibiotic-producing cells and the antibiotics that were produced using DNA (Bovine meat) crown cells and Ipomoea Nil-seed extracts.

As in previous studies, the author used the same convention for naming the cells. These cells are therefore named Antibiotic Crown-Bovine meat-Ipomoea Nil seed extract cells. The cells were produced using antibiotic-producing DNA (Bovine meat) Crown cells with Ipomoea Nil-seed extract as a partner.

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