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Isolation of Antibiotic-Producing Cells from Plate Culture of Egg Powder Enclosing DNA (*Bovine Meat*) Crown Cells and Beef Extract

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Abstract

Synthesized DNA crown cells can be synthesized using sphingosine (Sph)-DNA-adenosine-monolaurin compounds within cultures of egg white. Numerous DNA crown cells have been prepared to date. In previous experiments, it was demonstrated that antibiotics could be produced in co-cultures of yeast and DNA (*Streptomyces*) crown cells and antibiotic-producing cells were separated from the beer that were produced in cultures of DNA (*Streptomyces*) crown cells and yeast and from the egg white powders enclosing. DNA (*Streptomyces*) crown cells and yeast, as well as DNA (*HepG2*) crown cells and yeast. It is suggested that the production of antibiotics or antibiotic-producing cells was not attributed to DNA (*Storeptmyces*) crown cells, and the role of yeast in antibiotic production was unclear. It is important to clarify whether the yeast needs to be viable in order to produce antibiotics or antibiotic-producing cells. Here, it was examined whether antibiotic-producing DNA crown cells could be produced using a partner cell other than yeast. The results showed that antibiotic-producing cells could be produced in the combination of beef extract with DNA (*Bovine meat*) crown cells. The resulting cells was named antibiotic producing Crown-Bovine-beef-ex- cells-p.

Keywords: DNA (Bovine) crown cells; Sphingosine-DNA; Antibiotic producing Crown-Bovine –beef-ex -cells; Monolaurin

Abbreviations: Sph: Sphingosine; PDA: Potato dextrose agar; A-M: adenosine-monolaurin; SAM: Standard agar medium; DMEA: Dulbecco's modified eagle's medium

Introduction

Self-replicating artificial cells were first produced in 2012 [1] and the principal methods to prepare the artificial cells were reported in 2016 [2]. These cells were classified as DNA crown cells in 2016 by the present author [3]. The exterior of these cells consists of DNA. Synthetic DNA crown cells were produced using four commercially available compounds: Sphingosine (Sph), DNA, adenosine and monolaurin. The produced cells developed into fully self-replicating DNA crown cells by incubation in egg white. Numerous kinds of DNA crown cells, including DNA (*Bovine meat*) crown cells, have been produced to date [4-8], and strains of these cells have been prepared by the author [9-14]. These cells can be stored in egg white at 4°C until such time as they are required. In addition, antibiotic-producing cells have been prepared from beer. These cells were produced in co-cultures of DNA crown cells with yeast [15]. Antibiotic-producing cells can be separated from the egg white powder used to enclose DNA (*Streptomyces*) crown cells [16] and DNA (*HepG2*) crown cells in the presence of yeast [17]. These results suggest that the production of antibiotics or antibiotic-producing cells is a characteristic of DNA crown cells. On the other hand, the role of the partner, i.e., yeast in this case, on the production of these crown cells is unclear. Specifically, the question of whether a partner must be viable in order to produce antibiotics or antibiotic-producing cells has not yet been clarified. This study therefore examined whether antibiotic production occurs when DNA (*Bovine meat*) crown cells were co-cultured in the presence of a non-living, beef extract. It was found that antibiotic-producing cells could be separated from the egg-white powder that enclosed DNA (*Bovine meat*) crown cells with beef extract. These cells are named antibiotic producing Crown Bovine-beef-ex-cells-p

Materials and Methods

Materials

DNA (*bovine meat*) crown cells were prepared as described previously and refrigerated at approximately 4°C [4]. However, the methods are provided here again for clarity. The materials used were the same as those employed in previous studies [4,18,19]: 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. 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, Japan). Beef meat (Sendai Beef, Japan,) was obtained from local market. Food colorant (Crown Foods, Japan), potato dextrose agar (PDA; Kyodo Nyugiou, Tokyo Japan), *Bacillus subtilis* (Daikokuya, Nagoya, Japan), Dulbecco's modified eagle's medium (DMEM; Sigma, USA), and bovine serum (Sigma) were also used.

Methods

Preparation of DNA (*Bovine meat*) crown cells [4,18,19].

Step 1: A total of 90 µl of Sph (10 mM) and 40 µL of DNA (1.7 µg/µl) were combined, and the mixture was heated and cooled twice.

Step 2: A-M solution (50 µl) was added and the mixture was incubated at 37°C for 15 min.

Step 3: A total of 50 µl of monolaurin solution was added, and the mixture was incubated at 37°C for another 5 min.

Step 5: The whole suspension was injected into egg white and incubated for 7 days at 37°C. The egg white was then recovered and used as a DNA (*Bovine meat*) crown cells.

Preparation of meat extract

Meat was homogenized in 10% (w/v) in distilled water and the extract was prepared. Some extract was colored with food coloring to facilitate observations.

Preparation of powder

i. First, 3 ml of meat extract was mixed with 3 ml of egg white

ii. Mixtures were incubated for 5 h at 37°C

iii. Approximately 25 ml of fresh egg white was added to the mixture

iv. The fluid component was poured and spread onto two petri dishes and dried for 1–2 days at 37°C

v. The dried materials were collected and ground into a powder with a mortar and pestle

vi. The powder (Figure 1) were stored at room temperature before use

Figure 1: Two kinds of powder used in the experiment; right with colorant, left without colorant.

Two kinds of powder (with or without colorant) were prepared. The powder was named Crown *Bovine meat*-beef-ex-P.

Preparation of sample to assay 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, and was then incubated for 2 days at 37°C. The objects (Figure 6, within frame) that formed after the addition of monolaurin were resuspended in a small volume of distilled water (about 0.4 ml). An aliquot of this solution (200 µl) 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 antibiotic assay. Objects (Figure 7 within frame), which were grown in other plate, were suspended in a small quantity of distilled water (about 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 (about 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 antibiotics.

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Preparation of plates to measure antibiotic production: The antibody assay was carried out using an agar-well method, as described previously [15]. 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 on 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.

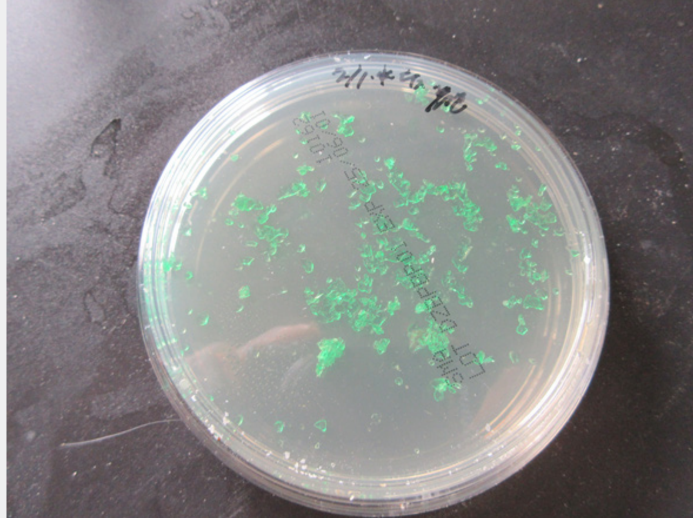

Figure 2: Photograph of an agar plate shortly after the cultures of the color powder. Colored powder was observed throughout the petri dish, which had a diameter of 8.0 cm.

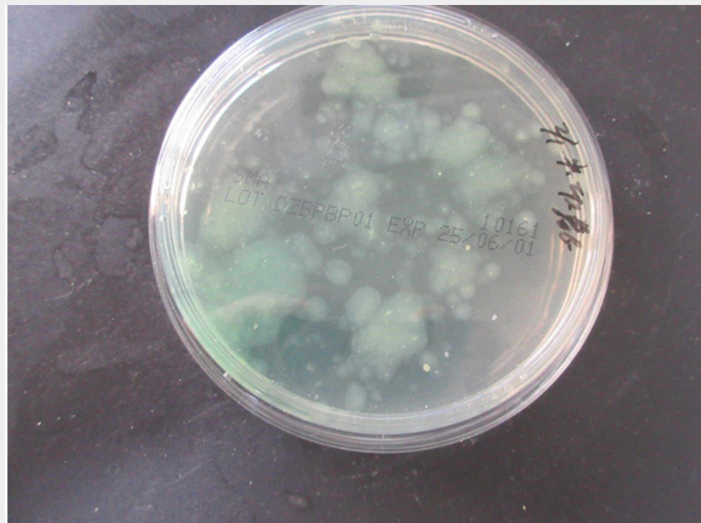

Figure 3: Photograph of an agar plate at 1 day of culture using the colored powder. Objects similar to diffused powder were visible to the naked eye throughout the petri dishes.

General observations

Objects on plates were observed by the naked eye.

Results

Figure 2 shows a photograph of an agar plate shortly after the cultures of the color powder. Colored powder was observed throughout the petri dish, which had a diameter of 8.0 cm.

Figure 3 shows a photograph of an agar plate after 1 day of culture using the colored powder. Objects similar to diffused powder were visible to the naked eye throughout the petri dishes.

Figure 4 shows a photograph of an agar plate at 2 days using the colored powder after the addition of monolaurin.

Round objects with small cubes at their center were observed.

Figure 5 shows a photograph of a plate with colored powder at 2 days after monolaurin addition. Round objects with colored centers were observed.

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Figure 6 shows a photograph of a plate of powder cultures at 2 days after monolaurin addition. The objects within the frame were used as sample A.

Figure 7 shows a photograph of plate of powdered culture at 2 days after monolaurin addition

Objects within the frame were cultured on an agar plate.

Figure 8 shows a photograph of agar cultures at 24 days, showing the objects in Figure 8.

Two kinds of large objects were observed. Objects within the frame 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.

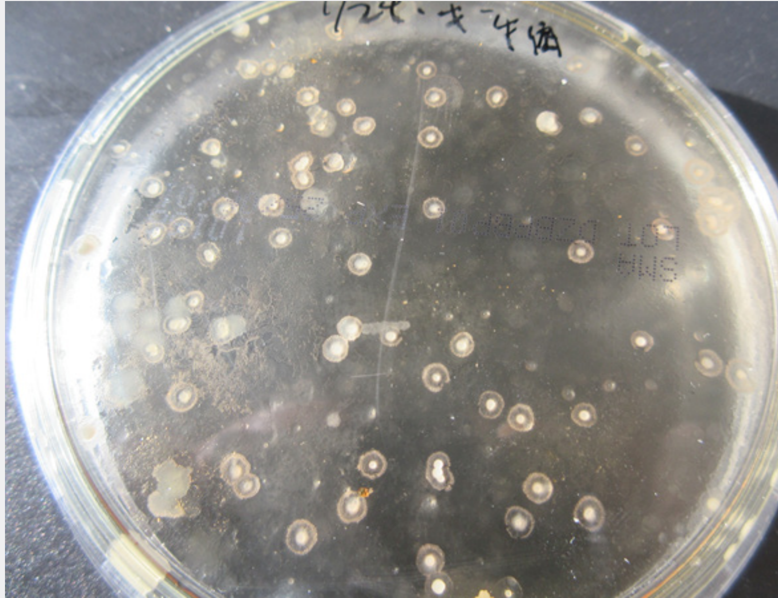

Figure 4: Photograph of an agar plate at 2 days using the powder after the addition of monolaurin. Round objects with small cubes at their center were observed.

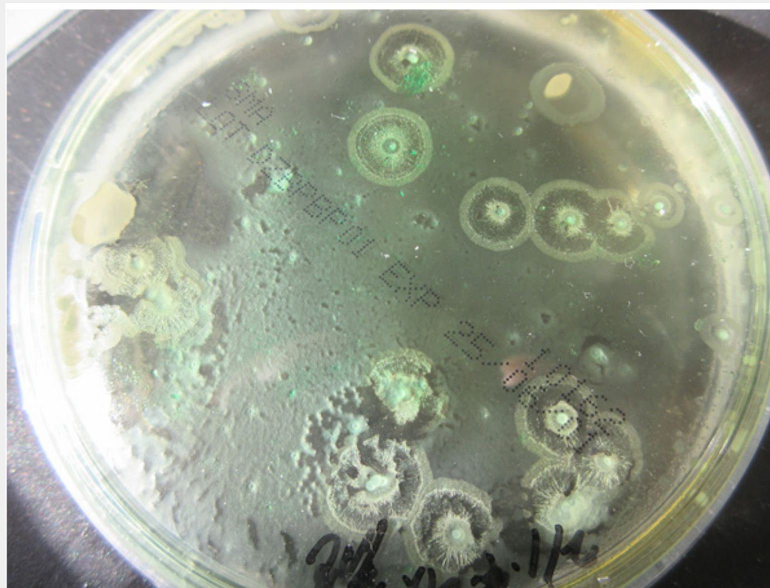

Figure 5: Photograph of a plate with colored powder at 2 days after monolaurin addition. Round objects with colored centers were observed.

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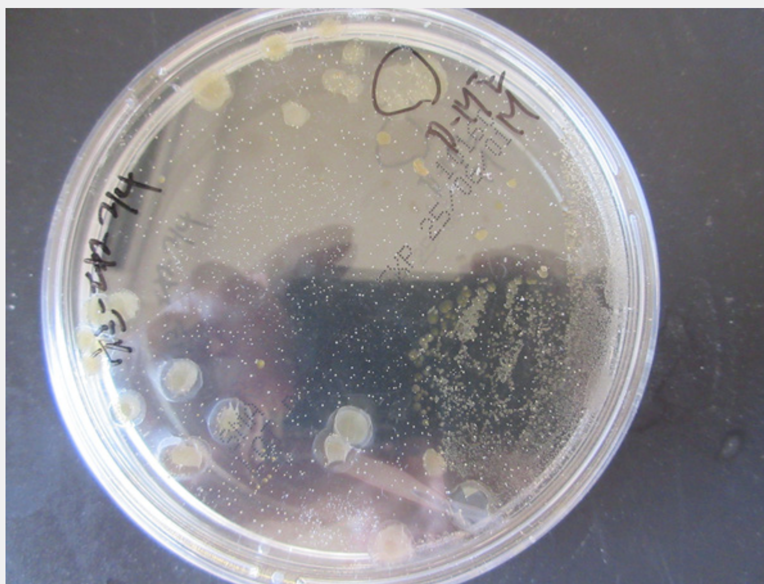

Figure 6: Photograph of a plate of powder cultures at 2 days after monolaurin addition. The objects within the frame were used as sample (A).

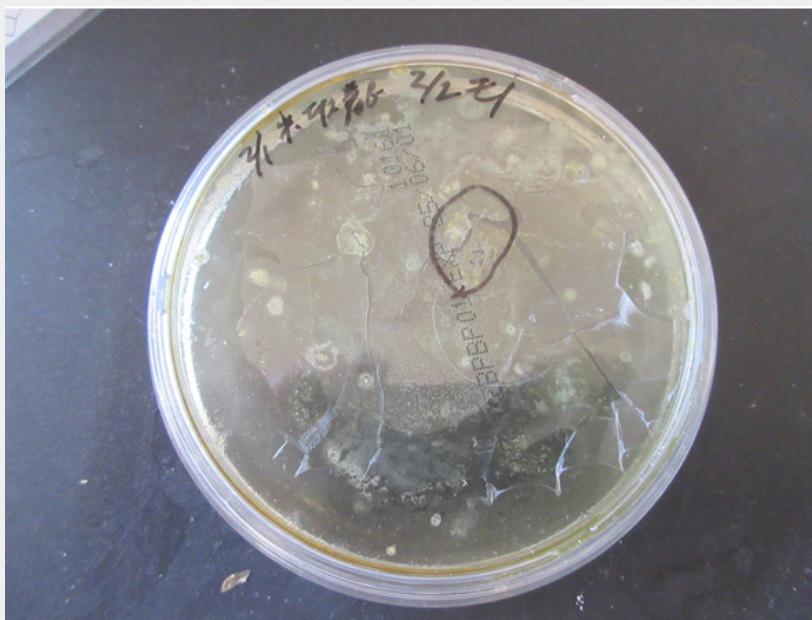

Figure 7: Photograph of a plate of powdered culture at 2 days after monolaurin addition
Objects within flame were cultured on an agar plate.

Previously, it was demonstrated that antibiotic was produced in co-cultures of yeast and DNA (*Streptomyces*) crown cells in beer production, and that antibiotic-producing cells could be separated from the beer by stimulation of egg white or monolaurin [15]. Also, 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 these powders [16,17]. Thus, antibiotic-producing cells could be separated using DNA (*Streptomyces*) crown cells and DNA (*HePG2*) crown cells. The results suggested that antibiotic-producing cells can be separated using various kinds of DNA crown cells. Namely, the results suggest that the emergence of antibiotic-producing

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cells was not due to a specific ability unique to DNA crown cells, but rather due to a common ability shared by most DNA crown cells. On the other hand, only yeast has been used as a partner for DNA crown cells to date. The present experiments were therefore conducted to clarify the precise role of partners and to determine whether partners need to be viable. It was shown that two kinds of antibiotic-producing cells could be produced using DNA (*Bovine meat*) crown cells and beef extract, which is a non-living partner. The results showed that antibiotic-producing DNA crown cells could be produced using non-living partners. However, it is not clear why multiple antibiotic-producing cells are produced. Regarding the mechanisms by which antibiotic-producing cells are produced, they may originate as part of the relationships between the DNA crown cell and their partners. It was therefore suggested that many living or non-living materials could be used as a partner, and that an unlimited number of new organisms with antibiotic-producing characteristics could potentially be created with DNA crown cells and their partners.

Figure 8: Photograph of agar cultures at 24 days, showing the objects in Figure 8. Two kinds of large objects were observed. Objects within frame were cultured and used for the antibiotic assay (Sample B).

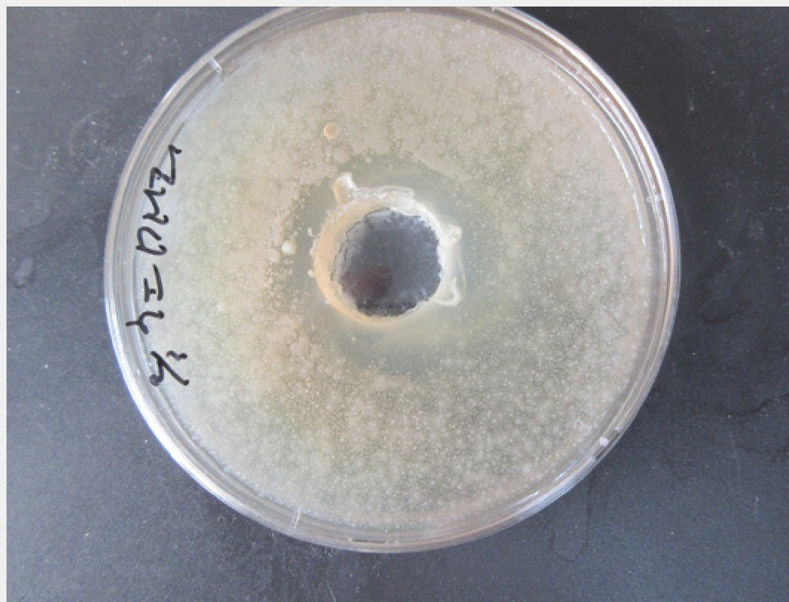

Figure 9: Photograph of an antibiotic assay of sample A. A clear zone was observed around the well.

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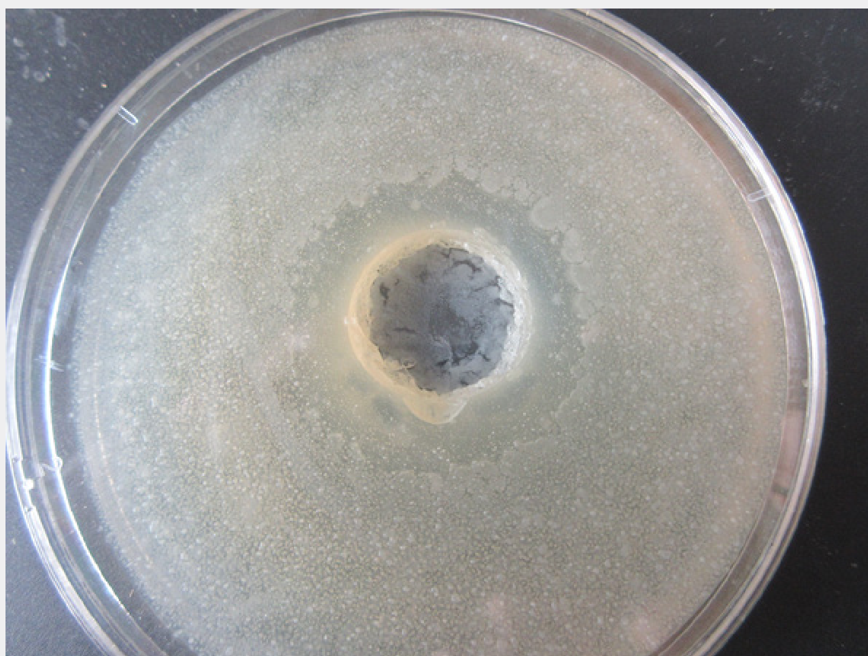

Figure 10: Photograph of an antibiotic assay of sample B. A clear zone was observed around the well.

These new organisms may contribute to the development of therapies for incurable diseases, especially those related to cellular disorders. Moreover, the antibiotic-producing cells born here may possess quality abilities than natural cells and may be able to be used as an effective cell with various functions. Thus, in addition to the medical field, DNA crown cells may contribute to various fields such as biotechnology, agriculture and so on. The methods for producing antibiotic and antibiotic producing cells with DNA crown cells have been established, and it is expected that these methods can be applied to create new effective organisms that could potentially benefit human welfare. As described previously [15], the cells developed in this study are named Antibiotic producing Crown-Bovine-meat-ex cells p. Antibiotic-producing DNA (Bovine-meat) Crown cells with meat extract, where P indicates powder (culture source). As a powder was used to produce the cells, the cells are named Crown Bovine-meat-meat extract-P, where P indicates powder derived from egg white. The antibiotic produced in the present experiments is the fifth such finding, so it is named crown antibiotic-5-1 and 5-2.

References

1. Inooka S (2012) Preparation and cultivation of artificial cells. App Cell Biol 25: 13-18.
2. Inooka S (2016) Preparation of Artificial Cells Using Eggs with Sphingosine-DNA. J Chem Eng Process Technol l7: 277.
3. Inooka S (2016) Aggregation of sphingosine-DNA and cell construction using components from egg white. Integrative Molecular Medicine 3(6): 1-5.
4. Inooka S (2017) Systematic Preparation of Bovine meat DNA Crown Cells. App Cell Biol. Japan 30: 13-16.
5. Inooka S (2013) Preparation of Artificial Cells for Yogut Production. App Cell Biol 26: 13-17.
6. Inooka S (2014) Preparation of Artificial Placental Cells. App Cell Biol 27: 4-49.
7. Inooka S (2019) Prepaation of DNA (Nannochloropsis species) crown cells (artificial cells) using eggs, sphingoshin, and DNA, and subsequent cell recovery. App Cell Biol 32: 55-64.
8. Inooka S (2018) Systematic Preparation of Genaraated DNA (Akoya pear oyster) Crown Cells. App Cell Biol Japan 31: 21-34.
9. Inooka S (2022) Preparation of a DNA (E. coli) Crown Cell line in Vitro-Microscopic Appearance of Cells. Annals of Reviews and Research 8(1): ARR.MS.ID.555728.
10. Inooka S (2023) Preparation and Microscopic Appearance of a DNA (Human Placenta) Crown Cell Line. Journal of Biotechnology & Bioresearch 5(1).
11. Inooka S (2023) Preparation of a DNA (Akoya pearl oyster) crown cell line. Applied Cell Biology Japan 36: 2023.
12. Inooka S (2022) Microscopic appearance of synthetic DNA (E. coli) crown cells in primary culture. App Cell Biol Japan 35: 71-98.
13. Inooka S (2022) Microscopic Appearance of Synthetic DNA (E. coli) Crown Cells in Secondary Cultures. Novel Research in Science 12(3) NRS.000791.
14. Inooka S (2023) Preparation of a DNA (Hepatoblastoma-Deraived Cell line HepG2) Crown Cell line Journal of Tumor Medicine & Prevence 4(2).
15. Inooka S (2025) Separation of Antibiotic-Producing Cells from Beer Produced in Co-cultures of DNA (Streptomyces) Crown Cells with Yeast Annals of Reviews and Research 12(3): ARR.MS.ID.5558399.

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16. Inooka S (2025) Separation of antibiotic producing cells from culture fluids of egg white powder enclosed DNA(Streptomyces) crown cells and yeast. International Journal of Bioprocess & Biotechnological Advanccments 10(1): 499-504.

17. Inooka S (2025) Separation of Antibiotic-Producing Cells frfom Agar Cultures of Egg Powder-Enclosed DNA (hepatoblastoma cell Line: HepG2) Crown Cell and Yeast. American Journal of Biomedical Science & Research 26(2).

18. Inooka S (2017) Biochemical and Systematic Preparation of Artificial Cells the Global Journal of Research in Engineering 17: (C1): 1-10.

19. Inooka S (2017) Systematic Preparation of Artificial Cells (DNA Crown Cells). J Chem Eng Procces Tecnolo 8: 327.

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