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Separation of Antibiotic-Producing Cells from a Plate Culture of Egg White Powder-Enclosed DNA (Bovine Meat) Crown Cells and Kaiware-Seed Extract

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ABSTRACT

Many kinds of DNA crown cells can be prepared, using sphingosine (Sph)-DNA-adenosine-monolaurin within egg white. Previously, antibiotics or antibiotic-producing cells were produced from the fluids obtained from the culture of egg white powder with DNA crown cells and various partner (e.g., yeast, salmon roe, meat extract, etc.). This study examined whether antibiotic-producing cells could be produced using DNA crown cells and plant-seed extract. The results showed that antibiotic-producing cells could be produced using DNA (Bovine) crown cells in conjunction with Kaiware-seed extract. The resulting powder was named antibiotic Crown- Bovine meat-kaiware seeds -ex- cells-p.

Keywords

DNA (Bovine) crown cells, Sphingosine-DNA, Antibiotic Crown-Bovine meat–Kaiware 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 common commercial substances (sphingosine (Sph), DNA, adenosine, and monolaurin), developed into fully self-replicating DNA crown cells after incubation in egg white and were referred to as DNA crown cells in 2016 by the present author [3]. The exterior of these cells consists of DNA. Numerous kinds of DNA crown cells, including the 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, various 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].

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 in conjunction 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 been clarified.

This study examined whether antibiotic production was possible using a combination of DNA (Bovine) crown cells and of Kaiware-seed extract. The antibiotic-producing cells were separated from egg white powder, which was used to enclose DNA (Bovine) crown cells with the Kaiware-seed extract, and the cells could be cultured in milk. These cells were named antibiotic Crown Bovine meat-Kaiware 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 [4,29,30]: 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 [29]. 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 Eagle Minimal Essential Medium (D-MEM) (Sigma, USA), and bovine serum (Sigma, USA) were also used in the study.

Methods

Preparation of synthetic DNA (Bovine meat) crown cells [4,29,30].

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 a DNA (Bovine meat) crown cells.

Preparation of Kaiware -seed extract

Seeds (~50 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-Kaiware seeds-ex-P, was stored at room temperature until se.

Preparation of Antibiotic Assay Samples

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

Objects within the frame shown in Figure 6 that were grown on the plate 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.

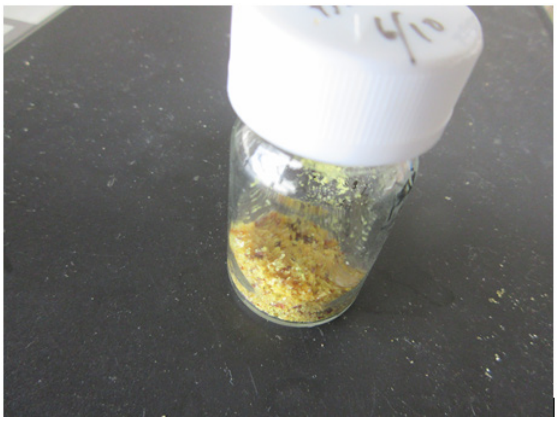

Figure 1: Powder used in experiment.

Objects (200 µL) within the frame shown in Figure 6 that had been suspended in distilled water were poured onto an agar plate and incubated at 37°C for 2 days.

Objects that were grown on the agar plate (Figure 9) 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.

Preparation of Plates for Antibiotic Assay

The antibiotic assay was carried out using the agar well method, as described previously [20]. The test bacteria **(*Bacillus subtilis*)** were 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 plates, which were 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

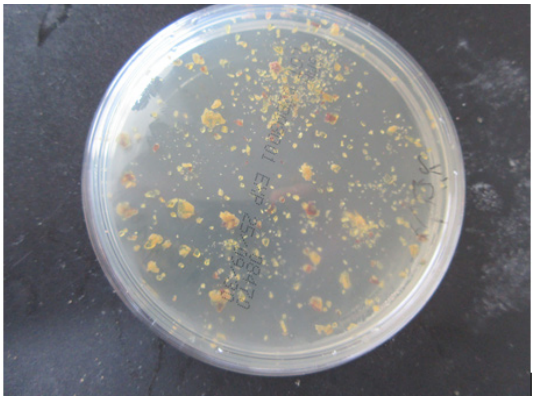

Figure 2: Shows a photograph of an agar plate **immediately after the start of powder culture** (Crown Bovine meat-Kaiware-seeds-ex-P). Powder particles of various sizes were observed covering the entire Petri dish.

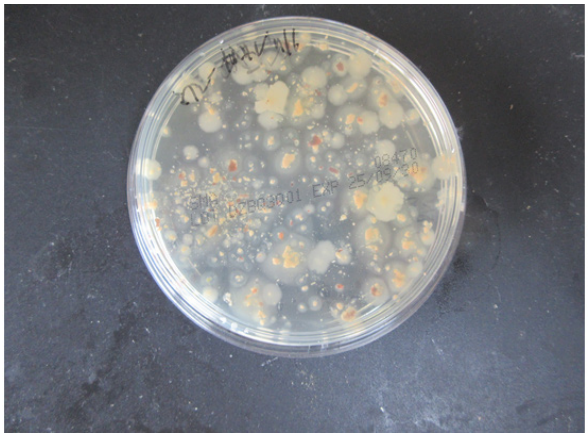

Figure 3: Shows a photograph of an agar plate after 1 day of powder culture. Large objects of various sizes and shapes were observed on the Petri dish.

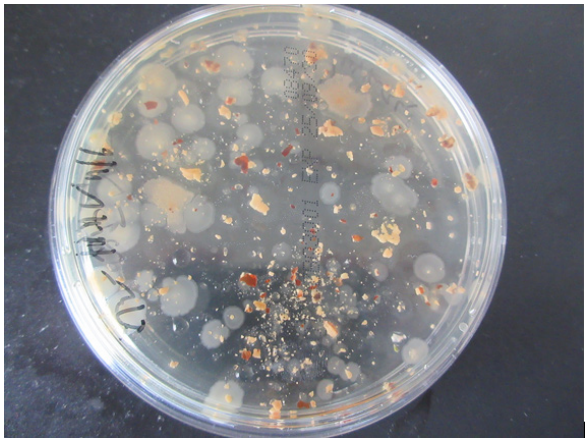

Figure 4: Shows a photograph of an agar plate after 2 days of culture using the powder.

The appearance was similar to that observed after 1 day.

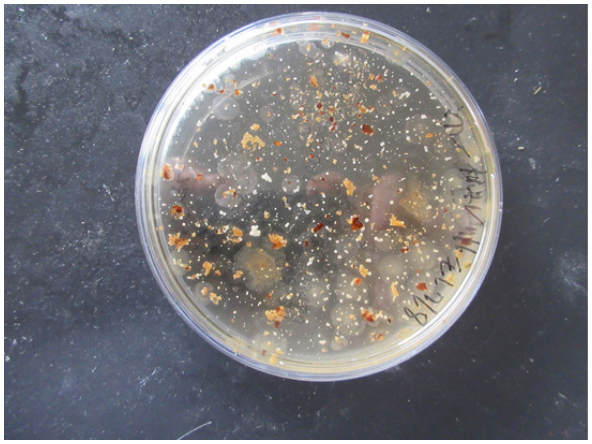

Figure 5: Shows a photograph of an agar plate with powder cultures at 1 day after the addition of monolaurin. Brown objects were observed.

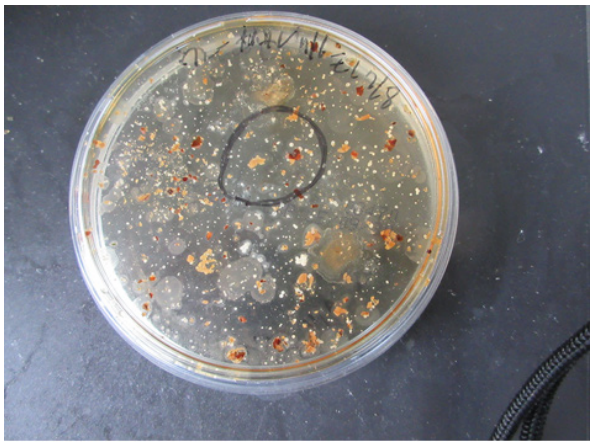

Figure 6: Shows a photograph of an agar plate at 2 days after the addition of monolaurin to the powder. Various brown objects of different sizes and shapes were observed. Objects within the frame were collected and cultured with D-MEM containing 10% bovine serum (Figure 7), milk (Figure 8), and agar plate (Figure 9). The antibiotic assay was carried out using fluids obtained from each culture.

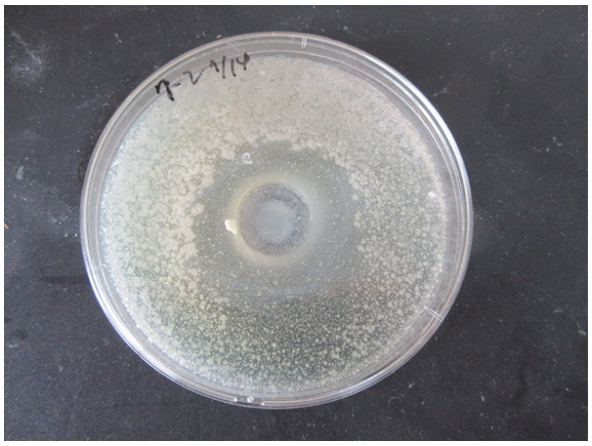

Figure 7: Shows a photograph of the antibiotic assay of the fluids obtained from culture of the objects within the frame in Figure 6 and D-MEM containing 10% bovine serum.

A clear zone of inhibition was observed.

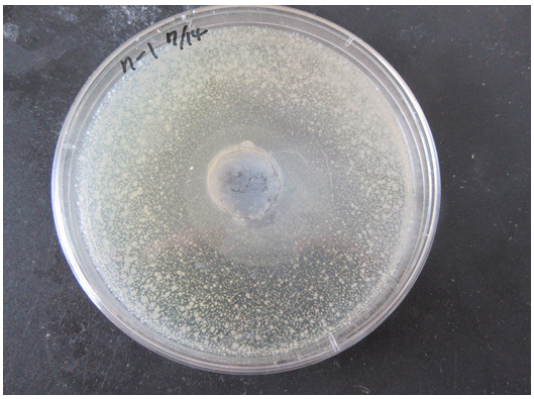

Figure 8: Shows a photograph of an antibiotic assay using milk cultures of the objects within the frame in Figure 6. A clear zone inhibition was observed.

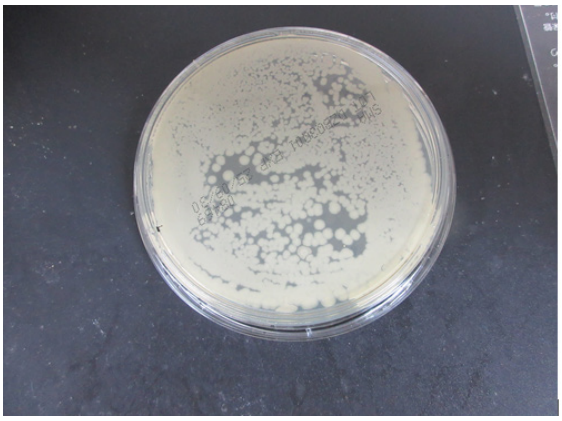

Figure 9: Shows a photograph of objects grown on an agar plate from culture of the objects within the frame in Figure 6. Microorganism-like objects were observed over the entire plate.

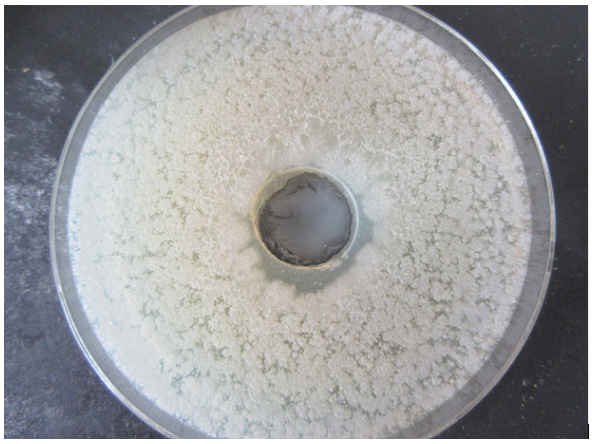

Figure 10: Shows a photograph of the plate used for the antibiotic assay of the microorganism-like objects shown in Figure 9 cultured in D-MEM containing 10% bovine serum. A clear zone of inhibition was observed around the edge.

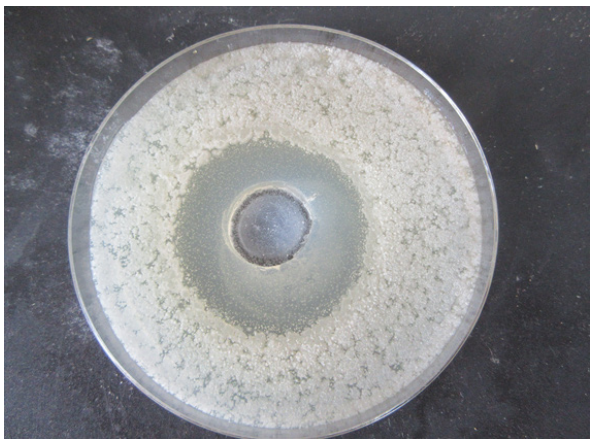

Figure 11: Shows a photograph of the plate used for the antibiotic assay of the microorganism-like objects shown in Figure 9 cultured in milk.

A clear zone of inhibition was observed.

In the present study, DNA (Bovine meat) crown cells with Kaiware-seed extract were used, and antibiotic-producing cells were produced using this combination. The results showed that antibiotic-producing cells were produced in the combination of DNA (Bovine meat) with Kaiware-seed extract together with monolaurin and suggested that plant-seed extract can be used as a partner of DNA (Bovine meat) crown cells.

Antibiotic-producing cells were produced in association with DNA crown cells, accordingly, may be associated with DNA which DNA crown cells have. Therefore, the cells may have functions in addition to antibiotic production; for example, they may have or affect the characteristics of bovine meat (e.g., taste, quality, etc.). On the other hand, they may also have or affect the characteristics of the co-culture substance (i.e., Kaiware seeds); for example, they may promote germination, growth, 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 were 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, this suggests that the antibiotics produced to date differed among cells.

On the other hand, it has already been shown that DNA crown cells can be produced yogurt [5]. This implies that the present cells, which were produced in cultures of DNA (Bovine meat) crown cells with a co-culture substance (i.e., Kaiware-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 for food was chip and stylized 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.

The findings suggested that these antibiotic-producing DNA crown cells, in addition to potential applications in the medical field, plant production, meat production, or beer production, could also potentially be applied to the milk industry, such as in cheese production [29,30].

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

The present experiments showed that antibiotic-producing cells and antibiotics were produced in the combination of DNA (Bovine meat) crown cells and Kaiware -seed extract. Moreover, in addition to being able to produce antibiotics against ***Bacillus subtilis*****,** these cells could potentially extend the functions of DNA crown cells or seeds. It is necessary to clarify the role of plant-seed extracts as co-

culture substances for crown cells in the production of antibiotics or antibiotic-producing cells.

Future studies will examine whether antibiotic-producing cells and antibiotics can be produced using various combinations of DNA crown cells with other plant-seed extracts,

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

References

1. Inooka S. Preparation and cultivation of artificial cells. App. Cell Biol. 2012; 25: 13-18.
2. Inooka S. Preparation of Artificial Cells Using Eggs with Sphingosine-DNA. J Chem Eng Process Technol. 2016; l7: 277.
3. Inooka S. Aggregation of sphingosine-DNA and cell construction using components from egg white. Integr Mol Med. 2016; 3: 1-5.
4. Inooka S. Systematic Preparation of Bovine meat DNA Crown Cells. App Cell Biol. 2017; 30: 13-16.
5. Inooka S. Preparation of Artificial Cells for Yogut Production. App Cell Biol. 2013; 26: 13-17.
6. Inooka S. Preparation of Artificial Placental Cells. App Cell Biol. 2014; 27: 4-49.
7. Inooka S. Prepaation of DNA (Nannochloropsis species) crown cells (artificial cells) using eggs, sphingoshin, and DNA, and subsequent cell recovery. App Cell Biol. 2019; 32: 55-64.
8. Inooka S. Systematic Preparation of Generated DNA (Akoya pear oyster) Crown Cells. App Cell Biol. 2018; 31: 21-34.
9. Inooka S. Preparation of a DNA E. coli Crown Cell line in Vitro-Microscopic Appearance of Cells. Ann Rev Resear. 2022; 8: 555728.
10. Inooka S. Preparation and Microscopic Appearance of a DNA (Human Placenta) Crown Cell Line. Journal of Biotechnology & Bioresearch. 2023; 5: 00603.
11. Inooka S. Preparation of a DNA Akoya pearl oyster crown cell line. Applied Cell Biology. 2023; 36.
12. Inooka S. Microscopic appearance of synthetic DNA E. coli crown cells in primary culture. App Cell Biol. 2022; 35: 71-98.
13. Inooka S. Microscopic Appearance of Synthetic DNA E. coli Crown Cells in Secondary Cultures. Novel Research in Science. 2022; 12: 000791.
14. Inooka S. Preparation of a DNA Hepato-blastoma-Deraived cell line, HepG2 crown cell line. J Tumor Med Prev. 2023; 4.
15. Inooka S. Synthesis and Microscopic Appearance of Thalus-like Objects in Synthetic DNA E. coli Crown Cells Created Using Monolaurin and Egg white. Open Acc J Repro & Sexual Disord. 2024; 3.
16. Inooka S. Double Cell-Like Objects Created from synthetic DNA Human Placenta Crown Cells using Monolaurin. Am J Med Clin Sci. 2024; 9: 1-3.
17. Inooka S. Morning glory-and Dandelion-like Objects Created in Agar Cultures of Synthetic DNA Ascidian Crown Cells with Monolaurin and Egg white. Ann Rev Resear. 2024; 10: 06.
18. Inooka S. Cell proliferation and Objects Such as Fig Leaf and Chaysanthemum Generated in cultures of Eggs-prepared DNA Heepg2 cells Crown Cells with monolaurin. J Tumor Med Prev. 2024; 4.
19. Inooka S. Cell ring like objects creted in agar culture of synthetic DNA Streptomyces crown cells with monolaurin. Clinical Cardiologyand. 2024; 7.
20. Inooka S. Separation of Antibiotic-Producing Cells from Beer Produced in Co-cultures of DNA Streptomyces Crown Cells with Yeast. Ann Rev Resear. 2025; 12: 5558399.
21. Inooka S. Separation of antibiotic producing cells from culture fluids of egg white powder enclosed DNA Streptomyces crown cells and yeast. Int J Biopro Biotechol Advance. 2025; 10: 499-504.
22. Inooka S. Separation of Antibiotic-Producing Cells from Agar Cultures of Egg Powder-Enclosed DNA hepato-blastoma cell Line: HepG2 Crown Cell and Yeast. American Journal of Biomedical Science & Research. 2025; 26.
23. Inooka S. Isolation of Antibiotic-Producing Cells from Cultures of Egg Cultures of Egg Powder Enclosing DNA (Hepg2) Crown Cells and Salmon Roe. Trends Gen Med. 2025; 3: 1-5.
24. Inooka S. Antibiotic-Producing Cells Produced from A Plate Culture of Egg Powder Enclosing DNA Nannochloropsis Species Crown Cells and Yeast. Scientific Journal Biology & Life Sciences. 2025; 2.
25. Inooka S. Separation of Antibiotic Producing Cells from Plate Culture of Egg Powder Enclosing DNA Bovine Meat Crown Cells and Beef Extract. Annals Reviews Research. 2025; 12: 555850.
26. Inooka S. The Production of Yogrut Containing Antibiotics from a Milk culture of Egg Powder Enclosing DNA Lactobacilis species Crown Cells and Bacillus subtilis and an Attempt to Extract Antibiotics from the Yogurt. Advance Research in Sciences. 2025; 3.
27. Inooka S. Antibiotic Production in A Milk Cultures of Egg White Powder Enclosing DNA Hepg2 Crown Cells and Salmon Roe. Archives in Biomedical Engineering & Biotechnology. 2025; 8.
28. Inooka S. The production of Antibiotic Using Milk with Egg Powder Enclosing DNA Bovine Meat Crown Cells with Meat Extracts. Academ.J.Plym Sci. 2025; 6: 555696.
29. Inooka S. Biotechnical and Systematic Preparation of Artificial Cells. Global Journal s Inc. 2017: 17.
30. Inooka S. Systematic Preparation of Artificial Cells DNA Crown Cells. J Chem Eng Procces Technol. 2017; 8.