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Antibiotic Production in Co-Cultures of DNA (Streptomyces) Crown Cells (Artificial Cells) and Yeast (Beer)

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ABSTRACT

DNA crown cells are artificial cells which possess outer membranes covered in DNA. Various types of DNA crown cells (artificial cells) are easily prepared by incubation in egg white. However, the practical applications of such DNA Crown Cells remain unclear

Previously, I synthesized DNA crown cells using DNA from the antibiotic-producing Streptomyces griseus and noted that the antibiotic was produced in a co-culture with yeast (beer) and egg white containing DNA crown cells.

However, many questions remain. In particular, it was unclear whether DNA crown cells themselves are associated with antibiotic production, as egg white was present with the DNA crown cells. Thus, to eliminate the effects of egg white, DNA crown cells were recovered from egg white and we then examined whether antibiotic production was observed in co-culture with DNA crown cells and Yeast.

I confirmed antibiotic production in Yeast and DNA crown cell co-culture. I also discussed the potential applications of co-cultures of DNA crown cells and yeast, as different types of DNA crown cell may yield useful results for other applied fields.

Keywords

Antibiotic, DNA crown cells, Sphingosine-DNA, Streptomyces.

Introduction

There has been significant progress in the generation of artificial cells since the 1960s [1,2]. Recently, several approaches for generating fully operational (self-replicating) artificial cells have been reported [3,4]. Such cells are covered with DNA (known as DNA crown cells) [5], and are generated by incubation with egg white. Thus, the mechanisms of formation of DNA crown cells are now quite clear and a wide range of DNA crown cells can now theoretically be obtained [6-10].

On the other hand, it is unclear whether DNA crown cells can contribute to applied fields, such as biomedicine or bio-industry. DNA crown cells using DNA from Streptomyces griseus, which produces several types of antibiotic, were prepared, and antibiotic was produced in co-cultures with egg white containing DNA crown cells and yeast (beer) [11]. However, there were several problems in these experiments. Antibiotic production was observed after about 5 weeks in co-culture, and the rate of success was very low. In addition, the experiment was carried out using egg white containing DNA crown cells. Therefore, it is unclear whether the antibiotic was produced in the relation to the egg white or the cells. To clarify such problems, the present experiments were carried out using DNA crown cells that were collected from egg white.

The present report shows that antibiotic production was observed after about 17 days in co-culture with DNA crown cells and yeast. Moreover, the rate of success was very high. These findings suggest that antibiotic was produced as a result of the relationship between DNA crown cells and yeast.

Many types of DNA crown cell can be obtained, and thus, the

present co-culture system of DNA crown cells and yeast may be applied to different DNA crown cells and is expected to contribute to various bio-industrial fields.

Materials and Methods

Materials and methods are largely as described previously [11], with some modification.

Materials

DNA (Streptomyces griseus) crown cells, DNA (*Streptomyces kanamyceticus*) crown cells. Edible white leghorn eggs were purchased from a market. Albumin (egg) (Wako, Japan), Dulbecco's minimal Essential Eagle's Medium (MEM) (Sigma, USA) and Bovine serum (Sigma, USA) were obtained. Dry Ale Yeast (Safale S-04) (Formentiis Bergy) and Black Rock PISENER medium (New Zealand) were obtained from a Handmade-beer kit (AUBERCRAFT, Okazaki, Japan) and were used in accordance with the manufacturer's instructions.

Medium: Potato Dextrose Agar (kyodo- nyugyo Tokyo, Japan).
Tested bacteria: Dry Bacillus subutils natto (Daikokuya, Nagoya, Japan),
Bacteria were suspended in distilled water (10 mg/ml).

Methods

DNA (*S. griseus*) crown cells and DNA (*S. kanamyceticus*) crown cells. Both cells were obtained as described previously [11,12]. In this experiment, cells were stored in a freezer until use.

First, egg white was prepared from two eggs. DNA crown cells recovered from the egg white of one egg were designated strain 1, and cells from the other egg were designated strain 2. Therefore, in this experiment, 4 strains were used in total: Strains 1 and 3 were DNA (*S. griseus*) crown cells, and strains 2 and 4 were DNA (*S. kanamyceticus*) crown cells, respectively. These 4 strains were transplanted by inoculating the sample into fresh eggs. In all experiments, these strains were used after 2~3 generations.

Cell collection from egg white containing DNA crown cells

Methods of collection were as described previously [12]. Briefly, 7 ml of distilled water was added to a sample of egg white (2.0 ml) containing cells stored at 4°C. After mixing, 1 ml of egg albumin solution (10%) was added. These solutions were mixed and kept for 2 hours at room temperature (approximately 20°C). Precipitates were then collected and suspended in 2 ml of MEM containing 10% bovine serum. This cell suspension was used in experiments.

Preparation of antibiotic co-culture system

Dry yeast (about 0.5 g) and cell suspension (2 ml) containing DNA (Str. griseus, kanamyceticus) crown cells were mixed and incubated for 5 hours at 37°C. Then, 30 ml of beer molts were added and incubated for 17 days in room temperature. During incubation, repeated sampling of culture medium (molt) was carried out.

Preparation of plates for antibiotic assay

Assays were carried out with the agar method as described previously [11]. In short, tested bacteria (5 ml) were added to agar (200 ml) and mixed. Then, about 15 ml of agar was poured into a dish. After fixing, a well of about 2 cm in diameter was prepared in each dish. Tested fluid (400 µl) was then poured into each dish followed by incubation for 18 hours at 37°C. After incubation, the inhibition zone was observed.

Results and Discussion

Cell collection from egg white containing DNA (*S. griseus*, *S. kanamyceticus*) Crown Cells

7 ml of distilled water was added to 2 ml egg white for each strain. Then, 1 ml of egg albumin solution (10%) was added to the mixtures up to a concentration of 1% albumin. Mixtures were then kept for about 2 hours at room temperature. Figure 1 shows the precipitates indicating that the cells aggregate.

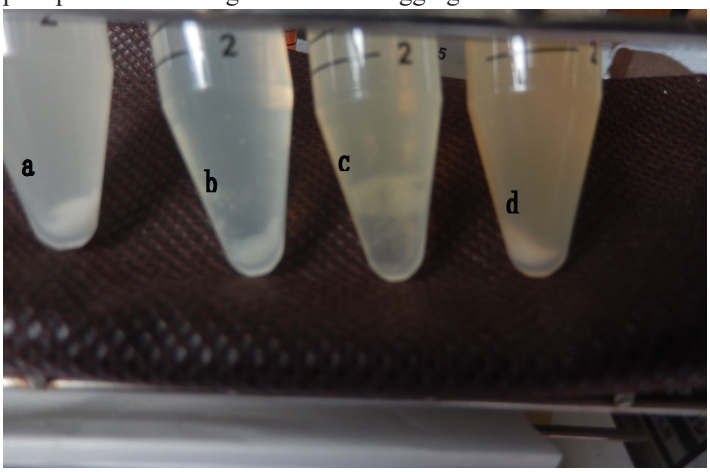
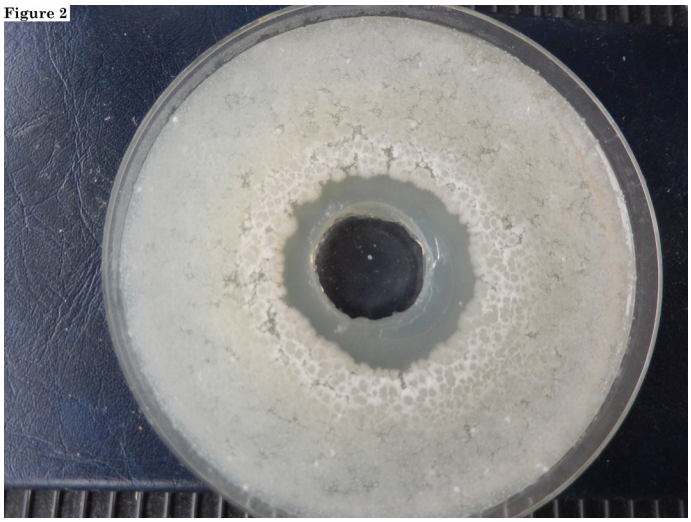


Figure 1a: strain 1; Figure 1b: strain 2; Figure 1c: strain 3; and Figure 1d: strain 4.

The formation of precipitates varied in each strain. For example, the speed of precipitate formation was slower with strain 3 (Figure 1c). However, cell precipitates were observed in all samples tested (Figure 1). The upper fluids were removed and the precipitates were suspended in 2 ml of MEM containing 10% bovine serum. Cells were used in co-cultures with yeast.



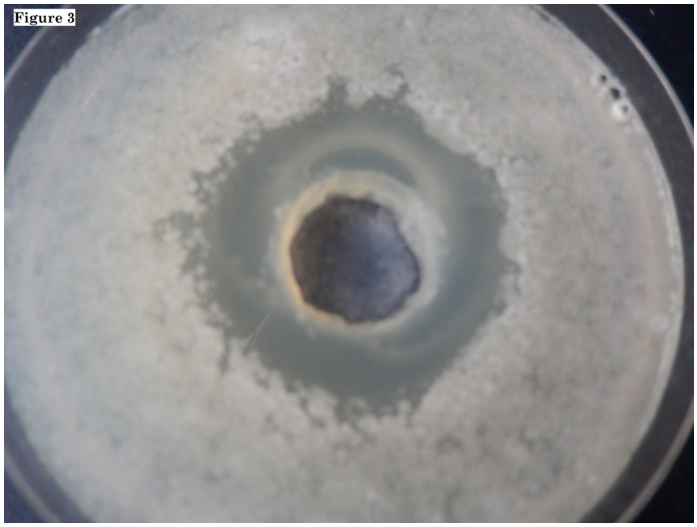


Figure 3

Antibiotic production in co-cultures

To research the practical applications of DNA crown cells, DNA (*S. griseus*) crown cells (using DNA from Streptomyces griseus), which produced Chromomycin or Streptomycin, and DNA (*S. kanamyceticus*) crown cells (using DNA from *Streptomyces kanamyceticus*), which produced Kanamycin, were prepared.

I then examined whether antibiotics were produced in the co-cultures of Yeast and DNA (*S. griseus, S. kanamyceticus*) crown cells.

The methods were as follows.
1: Yeast (about 0.5 g) was mixed with 2 ml of cell suspension, as described above.
Mixtures were then incubated for 5 hours at 37°C.
2: After incubation, 30 ml of molt was added to the mixtures, followed by incubation at room temperature (approximately 20°C).
3: During incubation, culture medium (molt) was sampled several times.
4: Antibiotic production was confirmed based on whether a clear zone was observed.

No antibiotic was found in any samples tested until 15 days of incubation (Data not shown).

Antibiotic was found in samples that were incubated for 17 days. Clear zones were observed in at least three strains tested. Figures 2 and 3 show clear zones for strain 1 (*S. griseus*) and strain 3 (*S. kanamyceticus*), respectively, indicating that antibiotic was produced.

The results indicate that antibiotic was produced in co-cultures of yeast and both DNA (Streptomyces) crown cells.

In previous experiments [3], co-cultures were carried out with yeast and egg white containing cells. The method is simple because it does not require cell collection.

However, there remain many problems: for example, it takes for a long time (over 5 weeks) until antibiotic was observed and the success rate (antibiotic production) was very low.

These problems may be resolved by the present method. Antibiotic production was observed within 17 days and the rate at which antibiotic is produced was over 75% (3 of 4 strains). In the present experiments, testing was carried out after 17 days of incubation, and longer incubation periods may improve the success rate further. On the other hand, the characteristics of the antibiotic remain unclear.

In previous experiments using TLC [11], the bands of samples did not correspond to the RF values of Chromomycin and Streptomycin (data not shown), suggesting that the antibiotics produced differed from Chromomycin and Streptomycin. In the present experiments, antibiotic production was observed in both strains. Therefore, different types of antibiotic may be produced by different strains.

On the other hand, a very wide range of DNA crown cells can be obtained. Such cells would possess characteristic functions. If the present co-culture system could be applied to such cells, numerous applications would be expected in the various fields of biotechnology.

Conclusion

Here, I first noted that DNA (Streptomyces) crown cells can be collected from egg albumin. Second, I demonstrated that antibiotic is produced in co-cultures of DNA crown cells and yeast (beer) within 17 days and with a 75% success rate. The mechanisms of antibiotic production remain unclear.. However, it is expected that these systems could be applied to various DNA crown cells and may be useful for a wide range of bio-industrial fields.

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