Unique Colony Housing the Coexisting *Escherichia coli* and *Dictyostelium discoideum*

M. TODORIKI¹, S. OKI¹, S.-I. MATSUYAMA¹, I. URABE¹ and T. YOMO^{1,2,3,*}

¹Department of Biotechnology, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita City, Japan

²PRESTO, JST, 2-1 Yamadaoka, Suita City, Osaka 565-0871, Japan

³Department of Pure and Applied Sciences, University of Tokyo, Komaba, Meguro-ku, Tokyo 153-8902, Japan

(*Author for correspondence, e-mail: yomo@bio.eng.osaka-u.ac.jp)

Abstract. Two well-characterized and phylogenetically diverse species, *Escherichia coli* and *Dictyostelium discoideum*, were used as the model organisms. When the two species were mixed and allowed to grow on minimal agar plates at 22 °C, instead of the predator *Dictyostelium* exterminating *E. coli*, the two species remarkably achieved a state of stable coexistence in about two weeks. In addition, the emerged colonies housing the coexisting species have a mucoidal nature that is distinctive from its origin. The simplicity of the system and the short time span for the two species to develop the coexistence state, that is proven stable and reproducible on laboratory conditions, hence, provides a new model system for the study of symbiosis, particularly with reference to the initial stages.

Key words: coexistence, *Dictyostelium*, *E. coli*, initial stage, mucoidal colony

1. Introduction

Mechanism of how organisms of different species develop symbiotic relationship remains elusive, although analysis of symbionts are well underway in determining factors or genes effecting symbiosis [1–5]. Reconstitution experiments from existing symbionts have been reported in an attempt to scrutinize how such relationship was developed [6–8]. However, the results gained from the constituent species of the symbionts could be misleading, since it is likely that the species isolated from the symbionts no longer possess the initial nature of their origin. Although much has been revealed about the cell and molecular biology of the amoeba/X-bacteria symbiosis [9, 10], yet how the origin of the two species established their symbiotic relationship remains unanswered, because of the facts that X-bacteria do not grow outside the amoebae and that the origin of the X-bacteria remains unknown [9].

In an attempt to establish a model system for symbiosis studies, we made use of the well-characterized *Escherichia coli* and *Dictyostelium discoideum* as the model microorganism. *Dictyostelium* is a known natural predator of bacteria, and any relationship that could develop between the two species may provide new per-

spective on the issues of symbiosis. Indeed, allowing the two species to grow under the conditions established in this study resulted to the coexistence of both species within colonies having discrete mucoidal nature totally different from its origin. Accordingly, we were able to observe some of the initial stages of a relationship developed between two different species that could eventually lead to symbiotic one.

2. Materials and Methods

2.1. MATERIALS

Escherichia coli strain YMC21 ($\Delta(glnA-glnG)2000 \Delta lacU169 endA1 hsdR17 thi-1 supE44$) lacking glutamine synthetase gene [11] was kindly provided by Dr. Boris Magasanik (Massachusetts Institute of Technology). Slime mold *Dictyostelium discoideum* strain HS175(*erkB*⁻) aggregation-defective mutant [12] was kindly provided by Dr. Mineko Maeda (Osaka University). Plasmid pKEN1-GFPmut2 [13], a derivative of pKEN1 containing mutant *gfp*, was kindly provided by Dr. Brendan Cormack (Stanford University). GFPmut2, encoded in pKEN1-GFPmut2, exhibits higher fluorescent intensity than the wild type and is employed as a detector for *E. coli* cells. Transformation of *E. coli* YMC21 cells with pKEN1-GFPmut2 was carried out as described by Maniatis et al. [14].

2.2. MIXED CULTIVATION OF THE TWO SPECIES

The cells collected from 37 °C overnight culture of *E. coli* YMC21 in 5 ml of 2 × YT medium [14] containing 50 µg/ml ampicillin were washed with medium C [15] containing 0.1 M glutamine. Medium C consists of 100 mM L-glutamate, 4 g/l glucose, 10.5 g/l K₂HPO₄, 4.5 g/l KH₂PO₄, 5 mg/l thiamine·HCl, 50 mg/l MgSO₄·7H₂O, 50 µg/ml ampicillin. The same treatment was done for the culture of *Dictyostelium* HS175 cells. The HS175 cells were grown in a petri plate containing 10ml HL5 as described by Sussman [16] with slight modification (15.4 g/l glucose, 14.3 g/l bacto peptone, 7.2 g/l yeast extract, 0.485 g/l KH₂PO₄, 1.28 g/l Na₂HPO₄·12H₂O, 50 µg/ml ampicillin) for about two weeks on standing at 22 °C.

Each of the washed cells were resuspended in medium C containing 0.1M glutamine with consecutive adjustment of concentration such that *E. coli* at OD₆₀₀ = 1.0 was about 4 × 10⁸ cells/ml and *Dictyostelium* was about 10⁶ cells/ml as determined by hemocytometer. An aliquot of 100 µl each of cell suspensions was dispersed onto medium C agar plate through the 1 ml medium C containing 0.1 M glutamine added onto the plate. After the surface of the plate was completely dried, the plate was incubated at 22 °C for about two weeks to one month. For control, *E. coli* YMC21 (pKEN1-GFPmut2) cells and *D. discoideum* HS175 cells were independently plated in the same manner described above. *D. discoideum* HS175 showed no growth even after one month of cultivation. The mixed culture was ob-

Figure 1. Mixed culture containing *E. coli* YMC21 (pKEN1-GFPmut2) with *D. discoideum* HS175. Mixed culture was grown on minimal agar plate at 22 °C. **A**, The plates were observed by the naked eye up to 2 weeks or more. A-1, one day after incubation of the plate; A-2, Fifth day; A-3, about 2 weeks of incubation. Scale bar represents 1 cm width. **B**, Observations of the events towards the coexistence of the 2 species under the phase contrast microscope ($\times 100$). See text for the detail description of each event. Scale bar represents 100 μm .

served by the naked eye and under the phase contrast microscope DIAPHOT-TMD (Nikon Inc.).

3. Results and Discussion

In an attempt to establish an experimental system that will allow the observation of the emergence of symbiotic relationship between two phylogenetically different species, we made use of the well-characterized *E. coli* and *Dictyostelium* as the model microorganisms. *E. coli* YMC21 (pKEN1-GFPmut2) was incubated with *Dictyostelium* HS175 (*erkB*⁻) aggregation-defective mutant on minimal nutrient

agar at 22 °C, a condition that slowed down the growth of YMC21 and consecutively restricting the growth of HS175, which feed on the bacteria (YMC21). After the overnight incubation, a thin lawn of bacterial cells grew on the surface of the agar plate. Scattered small clearing zones were observed on the YMC21 cell lawn upon extended incubation, indicating that HS175 are viable (Figure 1A-1). These clearings spread to an extent of almost covering the entire surface of the agar plate, leaving only a few scattered YMC21 lawns (Figure 1A-2). When the plates were incubated further for about two weeks or more, colonies resembling dewdrop of mucoidal nature developed around the clearing zones (Figure 1A-3) with a few mm to cm order sizes. The number of colonies was about tenths order per plate. It is to be noted that HS175 strain of *erkB*⁻, aggregation deficient mutant was used to eliminate occasionally observed fruiting body formation of *Dictyostelium* cells that might cause complicity in the observation. Nonetheless, the same results were obtained with the wild type strain (data not shown).

When the plates containing both the *E. coli* and *Dictyostelium* were examined under the phase contrast microscope, it was found that after YMC21 cells were consumed by the *Dictyostelium*, YMC21 again propagated in a colony, as shown in Figure 1B-1. The availability of the YMC21 cells seems to dispatch a signal that HS175 cells recognize, because the *Dictyostelium* cells regain proliferation activity, as clearly indicated by their advancement towards the *E. coli* cells (Figure 1B-2). After some time, HS175 cells were dispersed among with the *E. coli* inside a colony having mucoidal nature (Figure 1B-3). The emerged mucoidal colony has translucent dome shape with glossy surface, while its origin of pure YMC21 grown under the same experimental conditions are yellowish flat shape colonies with dull surface. These observations, hence, are some of the initial events or stages that may convene two different species to develop a symbiotic relationship. Sub-culturing the mucoidal colonies proved not only that the colonies are stable in the matter of morphology, but also that the two species continued to resides within the enclosure of the mucoidal colony. It is also noteworthy that the mucoidal colonies took a shorter time span to grow (5–10 days of cultivation) as compared to its initial emergence.

Bacteria are expected to be eliminated when mixed with *Dictyostelium*, as the latter is known as a natural predator of bacteria. However, we showed here that the *E. coli* could actually coexist with the *Dictyostelium*. In addition, the coexistence of the species was observed only within the enclosure of the emerged mucoidal colonies, indicating that the mucoidal nature of the colonies might contribute to the coexistence of the two species. Accordingly, this study provide a good model system for studying the development of symbiotic relationship among species, as not only do we know the origin of the microorganisms involved, the experimental protocol is simple and replicable. In addition, as the *E. coli* genome sequence is already known [17] and the *Dictyostelium* genome project is fast progressing [18], our system can provide a useful tool for investigating the genetic network dynamics and genome structural changes during the process of symbiotic transition.

Acknowledgements

This work was supported by Grant 11CE2006 from the Ministry of Education, Science, Sports and Culture, Japan.

References

1. Jeon, K.W.: *J. Euk. Microbiol.* **42** (1995), 1–7.
2. Choi, J.Y., Lee, T.W., Jeon, K.W. and Ahn, T.I.: *J. Eukaryot. Microbiol.* **44** (1997), 412–419.
3. Radeva, G., Jurgens, G., Niemi, M., Nick, G., Suominen, L. and Lindstrom, K.: *Syst. Appl. Microbiol.* **24** (2001), 192–205.
4. Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L. and Hedges, S.B.: *Science* **293** (2001), 1129–1133.
5. Uheda, E. and Silvester, W.B.: *Plant Cell Physiol.* **42** (2001), 780–783.
6. Bomford, R.: *J. Protozool.* **12** (1965), 221–224.
7. Hale, M.E.: *The biology of lichens* Edward Arnold Ltd., London, 1967.
8. Muscatine, L., Cook, C.B., Pardy, R.L. and Pool, R.R.: *Symp. Soc. Exp. Biol.* **29** (1975), 175–203.
9. Jeon, K.W.: *Trends Cell Biol.* **5** (1995), 137–140.
10. Jeon, K.W.: *Jap. J. Protozool.* **29** (1996), 11–19.
11. Chen, Y.-M., Bachman, K. and Magasanik, B.: *J. Bacteriol.* **150** (1982), 214–220.
12. Segall, J.E., Kuspa, A., Shaulsky, G., Ecke, M., Maeda, M., Gaskins, C., Firtel, R.A. and Loomis, W.F.: *J. Cell Biol.* **128** (1995), 405–413.
13. Cormack, B.P., Valdivia, R.H. and Falkow, S.: *Gene* **173** (1996), 33–38.
14. Maniatis, T., Fritsch, E.F. and Sambrook, J.: *Molecular cloning: a laboratory manual*, Cold Spring Harbor, New York, 1982.
15. Kashiwagi, A., Noumachi, W., Katsuno, M., Alam, M.T., Urabe, I. and Yomo, T.: *J. Mol. Evol.* **52** (2001), 502–509.
16. Sussman, M.: *Methods Cell Biol.* **28** (1987), 9–29.
17. Blattner, F.R., Plunkett, G.3rd, Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Callado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F., Gregor, J., Davis, N.W., Kirkpatrick, H.A., Goeden, M.A., Rose, D.J., Mau, B. and Shao, Y.: *Science* **277** (1997), 1453–1474.
18. Kay, R.R., Williams, J.G.: *Trends Genet.* **15** (1999), 294–297.