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Research article

Construction of bacteria–eukaryote synthetic mutualism☆

Isao Kubo[a], Kazufumi Hosoda[a], Shingo Suzuki[a], Kayo Yamamoto[a], Kumiko Kihara[b], Kotaro Mori[b], Tetsuya Yomo[a,b,c,*]

[a] *Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, 1-5 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b] *Department of Frontier Biosciences, Graduate School of Frontier Biosciences, Osaka University, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan*
[c] *Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology Agency, 1-5 Yamadaoka, Suita, Osaka 565-0871, Japan*

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ABSTRACT

Mutualism is ubiquitous in nature but is known to be intrinsically vulnerable with regard to both population dynamics and evolution. Synthetic ecology has indicated that it is feasible for organisms to establish novel mutualism merely through encountering each other by showing that it is feasible to construct synthetic mutualism between organisms. However, bacteria–eukaryote mutualism, which is ecologically important, has not yet been constructed. In this study, we synthetically constructed mutualism between a bacterium and a eukaryote by using two model organisms. We mixed a bacterium, *Escherichia coli* (a genetically engineered glutamine auxotroph), and an amoeba, *Dictyostelium discoideum*, in 14 sets of conditions in which each species could not grow in monoculture but potentially could grow in coculture. Under a single condition in which the bacterium and amoeba mutually compensated for the lack of required nutrients (lipoic acid and glutamine, respectively), both species grew continuously through several subcultures, essentially establishing mutualism. Our results shed light on the establishment of bacteria–eukaryote mutualism and indicate that a bacterium and eukaryote pair in nature also has a non-negligible possibility of establishing novel mutualism if the organisms are potentially mutualistic.

1. Introduction

Organisms rarely live alone in nature, and interactions between different species are common (Begon et al., 1996). These interactions are often mutually beneficial, a state known as mutualism, and such relationships are ecologically important (Boucher, 1985; Boucher et al., 1982; Herre et al., 1999). In particular, the establishment and evolution of mutualism are important issues in ecology because mutualism is well known to be vulnerable with regard to both population dynamics and evolution (Ferriere et al., 2002; Herre et al., 1999; Sachs and Simms, 2006). For example, the organisms involved in obligate mutualism can become extinct from lack of interaction because of a decrease in population density, and mutualism can shift to parasitism through the emergence of a cheater during evolution. The histories of the establishment and evolution of natural mutualism have been estimated by analyzing extant organisms using molecular biology and bioinformatics techniques, including phylogenetic analyses (Althoff et al., 2012; Backhed et al., 2005; Bascompte et al., 2006; Cook and Rasplus, 2003; Merckx and Bidartondo, 2008; Ramirez et al., 2011; Schardl and Craven, 2003). However, it is difficult to understand the processes that drove the extinction of organisms (Sachs and Simms, 2006) such as those that encountered a potentially mutualistic partner but failed to establish mutualism or those that once established mutualism but subsequently became extinct through the emergence of cheaters. Thus, our knowledge of mutualism is inevitably biased toward successful examples. To truly understand the difficulty that organisms experience in establishing and maintaining mutualism, the experimental reconstruction of these processes is a useful strategy.

Experimental studies using synthetic ecosystems have suggested that it is feasible for organisms to establish novel mutualism. By constructing synthetic ecosystems that are analogous to mutualism through the use of two species or strains that do not naturally interact, it is possible to simulate their encounter, establishment and maintenance of their mutualism, and the breakdown of their mutualism (Mee and Wang, 2012; Momeni et al., 2011; Tanouchi et al., 2012; Wintermute and Silver, 2010a). Several studies using synthetic mutualism have indicated the difficulties in constructing mutualism in defined cases. This information may be useful

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* Corresponding author at: 1-5 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 4171; fax: +81 6 6879 7433.
E-mail address: yomo@ist.osaka-u.ac.jp (T. Yomo).

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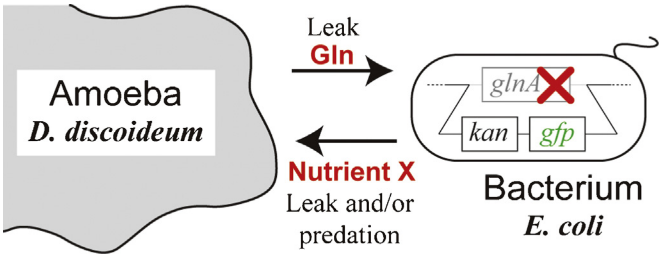

Fig. 1. A scheme of our SOBEM. Both the amoeba and the bacterium can grow in coculture only when they supply a sufficient amount of Gln and nutrient X, respectively (or substitutes that complement the lack of those nutrients).

in determining how difficult it is for organisms to establish mutualism. Shou et al. (2007) pioneered the construction of synthetic mutualism; this interaction was constructed between two genetically engineered auxotrophic yeasts, each lacking a gene essential for the biosynthesis of a nutrient. The study showed that a simple genetic modification resulting in the overexpression of a gene leads to the establishment of synthetic mutualism. In another mutualistic interaction between two different bacterial species studied by Harcombe (2010), the enhancement of the nutrient supply capacity of both bacteria was also necessary for the establishment of mutualism. By mixing 2 of 46 auxotrophic strains of *Escherichia coli*, Wintermute and Silver (2010b) showed that 17% of the 1035 tested pairs increased in population because of the interaction. Using one of those pairs of nutrient auxotrophs, we have shown that the *E. coli* populations grew continuously, rapidly changing to more cooperative phenotype (Hosoda et al., 2011; Hosoda and Yomo, 2011). Although the generality has not been sufficiently confirmed, these studies suggest that (i) certain enhancements of cooperative behavior allow the construction of novel mutualism and (ii) novel mutualism can be constructed merely by mixing two populations if approximately one dozen pairs are tested. These results suggest that organisms can with a non-negligible possibility establish mutualism in nature merely through encountering each other. However, mutualism between bacteria and eukaryotes, which is ecologically important, has not yet been constructed.

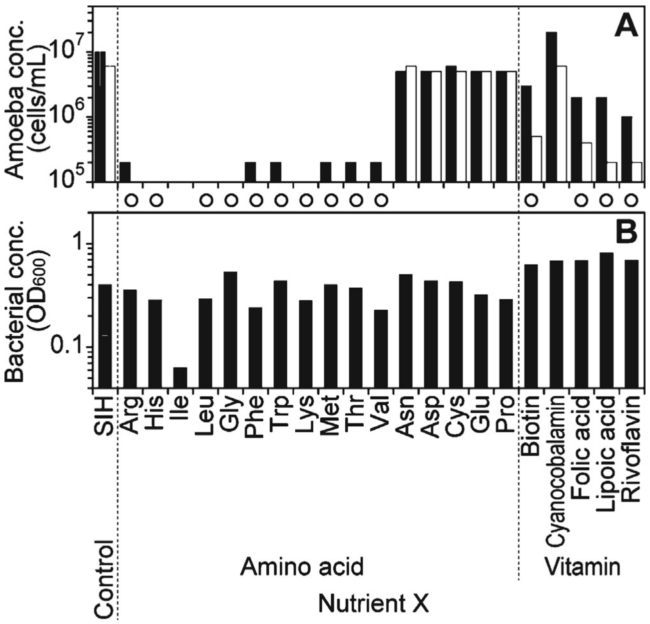

Fig. 2. The growth of the amoeba and the bacterium in monoculture in the absence of each nutrient X. (A) The results of the monoculture of the amoeba in SIH-X. For X to satisfy the requirement, the amoeba must not grow in SIH-X. The omitted nutrient X in each culture is indicated at the bottom of the figure. The amoeba, pre-cultured in SIH, was inoculated in SIH-X at an initial cell concentration of 2×10^5/mL, and the cell concentration was determined after one week (black bar). The amoeba was then transferred to the same medium at the same initial cell concentration, and the cell concentration was determined after 6 or 7 days (white bar). (B) The results of the monoculture of the bacterium in SIH-X + Gln. For X to satisfy the requirement, the bacterium must grow in SIH-X + Gln. The bacterium, pre-cultured in SIH + Gln, was inoculated in SIH-X + Gln at an initial optical density of 0.02 at 600 nm (OD_{600}), and the OD_{600} was determined after 1 day. The symbols ○ between A and B denote the selected candidates. These results are reasonably consistent with existing knowledge (see text), although the experiments have not been replicated.

In nature, bacteria–eukaryote mutualism is commonly observed (Begon et al., 1996; Douglas, 1994; Tarkka et al., 2009), and several studies have reported experimental ecosystems containing bacteria and eukaryotes (Hekstra and Leibler, 2012; Kihara et al., 2011; Matsuyama et al., 2004; Nakajima et al., 2009; Sano et al., 2009; Todoriki et al., 2002a; Tsuchiya et al., 1972; Yamada et al., 2008). However, it remains unclear whether these relationships constituted mutualism, because it is difficult to experimentally determine whether interactions are beneficial if the relationship is not obligate. In contrast, if the relationship is obligate for both species, the interaction must be beneficial to both species. Once synthetic mutualism has been constructed between a bacterium and cells of a mammal (Weber et al., 2007). However, the mammalian cell is no longer an individual mammal, and this situation may have reduced ecological relevance. Accordingly, the construction of synthetic obligate bacteria–eukaryote mutualism (SOBEM) is valuable for investigating the possibility of establishing novel bacteria–eukaryote mutualism in nature.

In this study, we constructed a SOBEM by mixing two model organisms and testing 14 interaction patterns. More precisely, we mixed a bacterium, *E. coli*, and an amoeba, *Dictyostelium discoideum*, under 14 conditions in which both species could not grow in monoculture but potentially could grow in coculture. When *E. coli* and *D. discoideum* mutually compensated for the lack of required nutrients, lipoic acid and glutamine (Gln), respectively, both species grew continuously through several subcultures, essentially establishing mutualism. Our results shed light on the establishment of bacteria–eukaryote mutualism and indicate that a bacterium and eukaryote pair in nature has a non-negligible possibility of establishing mutualism through their encounter if the pair is potentially mutualistic.

2. Materials and methods

2.1. Strains and culture conditions

For the bacterium, we used a green-labeled glutamine auxotrophic *E. coli* strain OSU6 (ΔglnA::(Kan^r)P_{tetA}-*gfpuv5* F$^-$ *endA1 gyrA96 thi-1 hsdR17*(r_K^-, m_K^+) *supE44 relA1*) (Yamada et al., 2008), which is a derivative of *E. coli* DH1 (obtained from the National BioResource Project, National Institute of Genetics, Shizuoka, Japan). For the amoeba, we used an aggregation-defective cellular slime mold *D. discoideum* strain HS175 (erkB−) (Segall et al., 1995), which was a kind gift from Dr. Mineko Maeda of Osaka University. This mutant lacks lipopolysaccharide-dependent enhanced bactericidal activity, which has been shown to be dependent on the MAPK ErkB (Walk et al., 2011), although the predation of the bacterium by this mutant was actually detected (Fig. S1). All of the cultures were grown at 22 °C in a synthetic medium, SIH (Han et al., 2004), or its derivatives, as described in the corresponding text for each experiment. The Gln concentration was 2 mM in all of the Gln-containing media. The antibiotic kanamycin was added at 25 μg/mL to each culture; ampicillin was also added at 50 μg/mL for the amoeba monocultures. The cultures were static (for the amino acids as nutrient X in Figs. 2 and 3) or agitated (for the vitamins as nutrient X in Figs. 2 and 3; 180 rpm rotation in a 300 mL conical flask with 50 mL culture liquid). Before culturing, we washed the bacterium and the amoeba using centrifugation (twice at 4820 × *g* for 5 min and at 890 × *g* for 5 min, respectively) with phosphate buffer (8.9 mM KH_2PO_4 and 2.5 mM NaH_2PO_4; pH 6.5) to exclude the carry-over of supplements from the preculture.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.biosystems.2013.05.006.