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Interplay between microbial trait dynamics and population dynamics revealed by the combination of laboratory experiment and computational approaches

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ABSTRACT

Filament formation is a common bacterial defense mechanism and possibly has a broad impact on microbial community dynamics. In order to examine the impact of filament formation on population dynamics, we developed an experimental system with a filamentous bacterium *Flectobacillus* sp. MWH38 and a ciliate predator *Tetrahymena pyriformis*. In this system, the effective defense of *Flectobacillus* resulted in the extinction of *Tetrahymena* by allowing almost no population growth. The result of a kairomone experiment suggested the existence of chemical signals for filament formation. To examine the mechanism further, we developed a quantitative mechanistic model and optimized the model for the experimental result using the simulated annealing method. We also performed a global parameter sensitivity analysis using an approximated Bayesian computation based on the sequential Monte Carlo method to reveal parameters to which the model behavior is sensitive to. Our model reproduced the population dynamics, as well as the cell size dynamics of *Flectobacillus*. The model behavior is sensitive to the nutrient uptake of *Flectobacillus* and the propensity of filament formation. It robustly predicts the extinction of *Tetrahymena* at the condition used in the experiment and predicts the transition from equilibrium to population cycle at higher nutrient conditions. Contrary to the previous study that disproved the presence of chemical signals for filament formation, our result suggested the importance of chemical signals at low predator density, suggesting the variety in bacterial resistance mechanisms that act at different stages of predator-prey interactions.

1. Introduction

In recent years, there has been a growing interest in the inducible defense of prey species against their predators (Van Donk et al., 2011; Matz and Kjelleberg, 2005), especially in the context of the effects of phenotypic plasticity on ecological processes, such as predator-prey dynamics (Yamamichi et al., 2011; Kishida et al., 2010; Miner et al., 2005). Inducible defense is a defense mechanism that is activated only when predation risk is sensed (Agrawal, 1998). Recent studies have revealed that this kind of phenotypic plasticity is common in various biological taxa including lichens (Blom et al., 2010; Corno and Jürgens, 2006; Hahn et al., 1999; Hahn and Höfle, 1998), alga (Van der Stap I et al., 2008; Verschoor et al., 2004), zooplankton (Boeing and Ramcharan, 2010; Kratina et al., 2010), plants (Nicotra et al., 2010; Koorneef et al., 2008; Sultan, 2000), insects (Moczek, 2010) and amphibian (Kishida et al., 2007).

Filamentous bacteria are frequently observed in freshwater lakes, temporarily in high concentrations (Schauer and Hahn, 2005; Pernthaler et al., 2004; Jürgens and Stolpe, 1995; Sommaruga and Psenner, 1995), and also appear in brackish (Engström-Öst et al., 2002) and marine (Caron et al., 1988) systems. The enhanced development of filamentous morphotypes has been often linked to an increase in bacterivores, which was demonstrated by food web manipulation experiments (Šimek et al., 2001, 1999; Jürgens et al. 1999; Šimek et al. 1999; Jürgens et al., 1994, 1999). The interplay between size-selective grazing and differences in the cell size distribution of different bacterial strains results in an altered bacterial community composition in chemostat experiments with mixed bacterial assemblages and bacterivorous nanoflagellates (Hahn et al., 1999; Pernthaler et al., 1997; Šimek et al., 1997). These results suggest the potential importance of filamentous bacteria on the microbial community dynamics.

Flectobacillus is a common rod form gram-negative bacteria including some strains that defend against predators by increasing cell size (filament formation; Corno and Jürgens, 2006; Hahn et al., 1999). Filament formation is effective to avoid grazing by the gape-limited

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bacterivores (Güde, 1979), and at the same time clearly indicates the defense level that we can measure based on the cell size distribution. Hahn et al. (1999) developed an experimental predator-prey system with *Flectobacillus* sp. MWH38 as a prey and *Ochromonas* sp. as a predator. They showed that *Ochromonas* decreased along with the increase of large cells in the *Flectobacillus* population. Corno and Jürgens (2006) also reported the similar effect of filament formation in an experimental system with *Flectobacillus* GC-5 and *Ochromonas* sp.

Although the two preceding studies presented similar results, Corno and Jürgens (2006) suggested the existence of chemical signals for filament formation, while Hahn et al. (1999) explained that filament formation was only due to the effect of predation and growth conditions. Hahn et al. (1999) argued that the grazing of *Ochromonas* on *Flectobacillus* reduces small cells, and enhances further growth among ungrazed (large) cells by releasing them from intraspecific resource competition. They regarded that the defense was "growth rate controlled." However, they did not perform any additional experiments to test the presence of chemical signals. Corno and Jürgens (2006) showed that, in *Flectobacillus* GC-5, it is not only grazing itself, but also chemical signals that trigger filament formation.

In this paper, we developed an experimental system with *Flectobacillus* sp. MWH38 and *Tetrahymena pyriformis,* and studied the population dynamics and the filament formation of the bacterium. In a continuous flow-through system, we showed that the effective defense of *Flectobacillus* resulted in the extinction of *Tetrahymena* by allowing almost no population growth. Further, we performed a kairomone experiment to reveal the effect of chemical signals on *Flectobacillus* filament formation. Unlike the Hahn et al. (1999)'s argument, the kairomone experiment suggested the existence of chemical signals for filament formation. We then developed a quantitative mechanistic model and optimized the model to reproduce the experimental result using the simulated annealing method (Mendes and Kell, 1998; Kirkpatrick and Vecchi, 1983). This allowed us to closely examine the mechanism of trait dynamics, to verify the validity of parameter values that the past experimental studies evaluated, and to see if the model without the chemical signals' effect explains the experimental data. The results suggested that the existence of chemical signals is convincing. In addition to this, we performed a global parameter sensitivity analysis using the approximate Bayesian computation based on the sequential Monte Carlo (ABC SMC) method (Hartig et al., 2011; Toni et al., 2009; Sisson et al., 2007), in order to reveal parameters to which the model behavior is sensitive. Here, we use the term "global" because our analysis does not consider each free parameter separately, but considers a set of parameter vectors (combination of all free parameters) sampled with respect to the proximity of simulation result to the experimental data. Moreover, we investigated the dynamical behavior of the *Flectobacillus-Tetrahymena* system by analyzing the model sensitivity to the nutrient concentration of in-flow media and the maximum defense level. Finally, we summarize the result and the advantages of our approach, discuss the implications of our result in comparison with previous studies, and discuss the role of filamentous bacteria in natural microbial communities.

2. Materials and methods

2.1. Chemostat experiment

2.1.1. Organisms

For the prey species of our predator-prey system, we used *Flectobacillus* sp. MWH38 (*Flectobacillus*, hereafter) that was originally isolated from the German lake Heidbergsee, and provided by courtesy of Dr. Martin W. Hahn at the University of Innsbruck. We isolated a single clone from the provided strain using agar plates with LB medium and freeze-preserved until the experiment. For the predator species, we used a ciliate species *Tetrahymena pyriformis* ATCC 3005 (*Tetrahymena*, hereafter) that had been cultured by Dr. Toshiyuki Nakajima at Ehime University by PYG medium and provided by courtesy of Dr. Nakajima. When we cultured *Flectobacillus* and *Tetrahymena* together in the later experiments, we used the COMBO medium (Kilham et al., 1998) supplemented with glucose (100 mg/L) (the modified COMBO medium, hereafter).

2.1.2. Kairomone experiment

For the preliminary examination of the filament formation of *Flectobacillus* in response to the kairomone released by *Tetrahymena*, we conducted a kairomone experiment in which *Flectobacillus* was exposed to the filtrate of a separate *Flectobacillus-Tetrahymena* coculture. The kairomone solution was made by filtering with a membrane filter (0.2-μm pore size) the culture medium that *Flectobacillus* and *Tetrahymena* had cocultured in the modified COMBO medium for nine days. Then, the kairomone solution was mixed with the fresh modified COMBO medium by 50:50 to make a treatment with kairomone. The control was made with the fresh medium without the kairomone solution. *Flectobacillus* was inoculated in the treatment and control medium and cultured for about 11 days. The size distribution of *Flectobacillus* was examined every two days during the culture as described below. It should be mentioned that the kairomone solution here contained the chemicals released by *Tetrahymena* as well as the ingredients of grazed *Flectobacillus* cells, both of which can be a signal for filament formation.

2.1.3. Continuous culture experiment

In order to observe the population dynamics of the *Tetrahymena-Flectobacillus* predator-prey system and the filament formation of *Flectobacillus*, we developed the continuous culture using chemostats. The organisms were incubated in the chemostats with 200 ml of the modified COMBO medium at 25 °C at a continuous dark condition. The culture was gently stirred by aeration and the dilution rate was 0.1 per day. *Flectobacillus* was inoculated at the start of the experiment with the initial density of 43.3×10^5 cells/ml, and after *Flectobacillus* had grown enough, *Tetrahymena* was inoculated at the day six with the initial density of 77 cells/ml. The continuous culture experiment was conducted in triplicate and the abundance of organisms and the size distribution of *Flectobacillus* were monitored at 1–2 days intervals by sampling a 5 ml aliquot from the chemostats.

2.1.4. Measurement of abundance and cell size distribution

The sample water was fixed by glutaraldehyde at the final concentration of 1.5%. The number of *Tetrahymena* was counted under an inverted microscope. *Flectobacillus* cells were examined using an epifluorescence microscope after DAPI staining. The bacterial cells were counted and the biovolume of cells was estimated by analyzing photos (two to seven photos per sample) taken under the microscope with an image processing software (Image J 1.42q). The volume of each cell was calculated using the long and short diameters assuming a cylinder shape of cell. The total biomass of *Flectobacillus* was calculated using the number and the volume of cells.

2.2. Simulation

2.2.1. Model description

In our continuous culture experiment, we observed the filament formation of *Flectobacillus* as indicated by the change in the size distribution, in which we categorized cells into seven size classes. We used these size classes in our model in order to compare the simulation results of the model with the experimental results.

Fig. 1 illustrates our assumption of how each *Flectobacillus* cell grows its size or divide into daughter cells. We assumed that cell division occurs as the division of a whole cell into cells in the smallest size class. We assumed that cells in $i = 1$ to $n - 1$ size classes respond to predator kairomone and change the allocation between cell growth and cell division as a function of predator density, while the cells in $i = n$