A Positive Correlation between CRISPR-Cas Spacer Abundance and Cell Cycle Duration Across the Bacteria Domain

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Abstract

CRISPR-Cas systems provide adaptive immunity for prokaryotic cells by recognizing and eliminating the recurrent genetic invaders whose sequences had been captured in a prior infection and stored in the CRISPR arrays as spacers. However, the biological factors determining the efficiency of this adaptive immune system have not been fully characterized. Recent studies in cultured bacteria showed that slowing the growth rate of bacterial cells could promote their acquisition of novel spacers. This paper reports a positive correlation between the minimal doubling time and the spacer abundance across the bacteria domain. The correlation is still significant after controlling for the effect of growth temperature. This observation provides evidence for the existence of the trade-off between bacterial growth and defense proposed in the “Killing the Winner” hypothesis.

Keywords

CRISPR-Cas, cell cycle, bacteria, phage, growth rate.

CRISPR-Cas systems provide adaptive immunity for prokaryotic cells against invasive genetic elements. Accumulating evidence indicates that slowing down host cell growth rate could enhance spacer acquisition probably because the slow growth affects virus replication more than the cell's response to the invaders. Using a fluorescent CRISPR adaptation reporter method, Amlinger et al. (1) quantified the adaptation efficiency of Type II CRISPR in the different growth phases of *Escherichia coli*. They found that spacer acquisition occurs predominantly during the late exponential/early stationary phase. In line with this study, McKenzie et al. (2) observed that the *E. coli* cells that adapted and cleared the target DNA earlier grew significantly slower than the population means. The soil-dwelling bacterium *Pseudomonas aeruginosa* can also infect the plant and human bodies. It grows slowly in the cooler temperatures of the soil or during plant infection and rapidly under the higher temperature of human bodies. Høyland-Kroghsbo et al. (3) found that decreasing the growth rate could increase the CRISPR adaptation frequency of the bacterium. Dimitriu et al. (4) recently found that the bacteriostatic agents that slowing down the growth of *P. aeruginosa* could promote the phage-derived acquisition of novel spacers into the CRISPR array. Furthermore, they showed that limited carbon sources associated with slow bacterial growth could also enhance the evolution of CRISPR immunity. The latter result is in line with the previous studies that observed increased CRISPR-Cas immunity in bacteria grown in a nutrient-limited medium (4). Dormancy could be regarded as a prolonged growth state of the prokaryotic cells. In the response of the type VI CRISPR-Cas system to RNA virus infection, the Cas13 enzymes of the bacteria *Listeria ivanovii* were found to destruct both the RNA virus genomes and the host mRNA molecules, which further induce dormancy of the host bacterial cells (5). In the same study, the immunity of the bacterial cells against the unrelated virus was enhanced in the type-VI-CRISPR-Cas-system-induced dormant state.

All these results were obtained from analyzing a few cultured bacteria in laboratories. We

examined whether there is a universal relationship between growth rate and the adaptive immunity across the bacteria domain.

We used the phylogenetic generalized least squares (PGLS) regression analysis to examine the relationship between spacer abundance and growth rate across the bacterial and archaeal domains. A significant positive correlation was observed in the bacterial domain when the growth rates were measured by the predicted minimal doubling times (n = 4143, slope = 1.75, $P = 9 \times 10^{-5}$). When the growth rates were measured by the empirical minimal doubling times, we observed a positive correlation at a marginally significant level (n = 262, slope = 1.37, P = 0.056). To examine whether the empirical minimal doubling times and the predicted minimal doubling times lead to the same conclusion, we examined the 252 bacteria with both empirical minimal doubling times and predicted minimal doubling times. Among these bacteria, the spacer abundance and growth rate are significantly correlated for both cases: slope = 10.6, $P = 3 \times 10^{-8}$ when the growth rate was measured by predicted minimal doubling times, and slope = 1.56, P = 0.026 when the growth rate was measured by empirical minimal doubling times. The empirical minimal doubling times and the predicted minimal doubling times gave the same conclusion. However, the predicted minimal doubling time strongly correlates with the spacer abundance.

Previous studies observed that bacterial CRISPR abundance is positively correlated with growth temperatures (6-9), with an abrupt jump at around 45°C (10). Here, using spacer abundance as the dependent variable, the minimal doubling time and the growth temperature as the independent variables, we performed multiple PGLS regression analyses. The results show that the minimal doubling time and the growth temperature are independently correlated with spacer abundance (Table 1).

In archaea, we did not observe a significant correlation between spacer abundance and growth rate (P > 0.100), no matter when the growth rate was measured by the empirical

minimal doubling times (n =104) or the predicted minimal doubling times (n =256). Multiple PGLS regression analyses showed that archaeal spacer abundance is not significantly correlated with either minimal doubling time or growth temperature ($P > 0.10$ for both cases).

The authors reporting the enhancing effects of slow growth on spacer acquisition in cultured bacterial species generally explain their observation by the mechanisms of spacer acquisition (1, 3, 11). The mechanistic explanation could be applied to the correlation between spacer acquisition and growth rate across the bacteria domain. Furthermore, we could put the spacer acquisition into an ecological context. Studies on natural bacterial communities support the existence of trade-offs between competition ability (faster growth) and invulnerability to predation (including virus resistance) (12-14). With the limited resources available in natural environments, the bacteria that allocate more resources to defense have to grow slowly, and the bacteria with high competition ability allocate less resource to virus resistance. In addition, there is evidence that prophages were more frequent in bacteria with small minimal doubling times (15). Targeting prophages might lead to auto-immunity and produce a selective force driving the loss of CRISPR–Cas systems or against spacer acquisition (16, 17).

MATERIALS AND METHODS

The datasets of prokaryotic empirical minimal doubling times and predicted minimal doubling times were retrieved from reference (18) and the EGGO database (accessed Nov 26, 2021) (19), respectively. To cover more prokaryotes, we used the optimal growth temperatures predicted by the program Tome (version 1.0.0) (20). The phylogenetic trees and taxonomic information of bacteria and archaea were retrieved from the Genome Taxonomy Database (GTDB, accessed Apr 8, 2022). The prokaryotic genome assembly files were downloaded from the GenBank database (accessed Apr 26, 2021) (21). The CRISPR-Cas

systems of the prokaryotic genomes were annotated using CRISPRCasFinder (version 4.2.20) (22). The avoid the outlier effects on the statistical results, we only retained the minimal doubling times shorter than 60h. To avoid underestimation of the CRISPR-Cas systems, partially assembled genomes have been discarded, and only those with assembly levels of "complete genome" or "chromosome" were retained in our analyses. Because there is no phylogenetic divergence information of different strains of the same prokaryotic species in GTDB (23), we used the representative genome of each species in this study. In addition, all the suppressed genomes of the GenBank database were discarded.

We estimated the phylogenetic signals (λ) of the analyzed characters using the R (Version 4.0.2) package phytools (Version 0.7-47) (24). All the analyzed characters exhibit significant phylogenetic signals, indicating that phylogenetic comparative methods are required in the analyses to control the effect of common ancestry. The correlations between different characters were estimated by the PGLS regression using the R (Version 4.0.2) package phylolm (version 2.6.2) (25). Pagel's λ model has been applied in this study.

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Table 1. Correlations of bacterial CRISPR spacer abundance with the minimal doubling time and the growth temperature.

		MDT		Growth temperature	
	N	Slope	*P*	Slope	*P*
Spacer abundance[1]	262	1.84	0.011	2.06	0.001
Spacer abundance[2]	4142	1.66	2×10^{-4}	1.43	4×10^{-8}

The correlations were examined using multiple PGLS regression analyses, with the positive sign of the regression slope to indicate the positive correlation. [1] Correlation of spacer abundance with empirical MDT and optimal growth temperature. [2] Correlation of spacer abundance with predicted MDT and optimal growth temperature. MDT, minimal doubling time.