Degradation of the generalist phage in minimal media cannot be clearly attributed to any particular media component

We investigated the cause of phage degradation in our minimal media by incubating 103 EH7 particles per well at 37°C with shaking at 432 rotations per minute in glucose minimal media for 48 hours. We found that EH7 degraded below the limit of detection within 24 hours, half the time period of our standard phage competition assays (Supplemental Figure 3A). However, phage recovery was possible following the addition of cells in some cases (Figure 5B), suggesting that infectious phage particles remained. P22*vir* titer was unchanged over the course of 48 hours (Supplemental Figure 3A, two-way ANOVA, Tukey’s HSD, p = 1.0).

Given previous observations that environmental factors such as salinity [63, 64, 65, 66], pH [61, 62, 63], and metal presence can have significant impacts on phage titer [67, 68, 69], we attempted to identify the cause of degradation in our minimal media by removing one component at a time. We created minimal media without metals, without sulfur, without phosphorus, or some combination thereof. Again, we incubated 103 EH7 particles per well at 37°C with shaking at 432 rotations per minute in each minimal media type for 48 hours. However, phage degraded below the limit of detection regardless of which media component was absent, suggesting that metals and osmolarity were not driving the reduction in titer (Supplemental Figure 3C; Supplemental Table 10). Additionally, because phage titer can be reduced as the result of adsorption of viral particles to plastic surfaces, we tested our phage by incubating it in LB [70, 71, 72]. We determined that adsorption of phage particles to the plastic was likely not driving the decrease in phage titer, given that EH7 density was unchanged following 48 hours of incubation in LB in the same 96-well plate where degradation in minimal media was observed (Supplemental Figure 3B, Welch’s t-test, p = 0.00035). This was true despite the low starting phage density, which has been shown to increase the likelihood of rapid phage degradation due to adsorption to plastic [61, 70].

Finally, we investigated which component of LB was responsible for the preservation of phage titer by removing one component at a time. We repeated our previous experiments by incubating 103 EH7 particles per well at 37°C with shaking at 432 rotations per minute in each LB type for 48 hours. The titer of phage incubated in solutions containing tryptone were unchanged over the window of the 48 hour experiment (Supplementary Figure 3D; Supplemental Table 10g). When incubated in a solution of yeast and salt, the density of EH7 particles was also not significantly changed (Supplementary Figure 3D; Supplemental Table 10g). However, titer did decrease significantly relative to other conditions when phage were incubated in only yeast or only salt (Supplementary Figure 3D, yeast: two-way ANOVA, Tukey’s HSD, p < 0.001 for all multiple comparisons, salt: two-way ANOVA, Tukey’s HSD, p < 0.001 for all multiple comparisons). These results suggest that tryptone may play a role in the stability of EH7 titer in LB.