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Evidence for the priming effect in single strain and simplified communities of estuarine bacteria

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Evidence For The Priming Effect In Single Strain And Simplified Communities Of Estuarine Bacteria

by

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Abstract

The flow of terrestrially derived organic between rivers and coastal environments reveals a substantial utilization of this recalcitrant carbon in estuaries. The priming effect (PE), a greater mineralization of recalcitrant carbon in the presence of labile organic matter (LOM), has been proposed to explain the increased degradation. Despite being widely studied in soils, the mechanism for PE is not well understood especially in regard to aquatic bacteria. To better understand the mechanism, biomass production in single strain and intentional communities of Roseobacters was observed to study the effect of chemical complexity and concentration of LOM on PE. Acetate, coumarate, casamino acids, and tryptone served as sources of LOM at concentrations of 1, 4, 40, and 400 μm-C. PE was observed in both *Citriecella* sp. SE45 and *Sagitulla stallata* E37 single strain experiments along with the intentional community. Higher LOM concentrations yielded PEs with the greatest amount of biomass production, while acetate conditions yielded PEs with the greatest longevity. Community priming with coumarate suggests a synergistic relationship between SE45 and E37 with increased abundance of both strains accompanied by PE not seen with single strain growth. The evidence for Roseobacter PE in this study can used to gather a better understanding of mechanisms of aquatic priming and global carbon cycling.

1. Introduction

Organic matter (OM) is an essential cabon source for riverine microorganisms comprised of both terrigenous (t-DOM) and autochthonous organic matter. These carbon sources vary in regards to bioavailability from labile leachate from primary producers to recalcitrant vascular plant matter (Hotchkiss et al. 2014). Lignin in vascular plant matter is a chemically stable polymer that makes it difficult to degrade for carbon utilization. Despite its resistance to decay, t-DOM decreases noticeably between riverine and coastal environments (Bianchi 2011). The combination of labile and recalcitrant carbon has shown to increase carbon use efficiency in riverine bacteria (Fonte et al. 2013). For this reason, the priming effect (PE) has been proposed as a mechanism to explain the disappearance of OM. Widely studied in soil bacteria, PE shows an increased mineralization rate of recalcitrant OM in the presence of LOM (Kuzyakov et al. 2000). Recently PE has been observed when a natural estuarine community was primed with labile protein to remineralize degraded phytoplankton, indicating that it may be a mechanism of recalcitrant OM degradation in aquatic systems (Steen et al. 2016).

Members of the Roseobacter clade, with their high metabolic activity, hold potential to undergo a PE. Their ability to catabolize aromatic carbon is essential for utilizing recalcitrant OM in their environement. *Citreicella* sp. SE45 and *Sagittula stallata* E37 are lignolytic, hence why they were used in this study (Buchan et al. 2005). The high abundance and metabolic activity of Roseobacters in estuarine environments could lead to a substantial decrease in OM degradation if they are capable of being primed.

In this study, we tested the influence of LOM chemical structure and concentration on PE in Roseobacters. Four types of LOM were chosen based on their range in chemical complexity: acetate, the simplest molecule; coumarate, an aromatic monomer; casamino acids, all necessary free amino acids; and tryptone, an oligopeptide. Previous findings in soil systems indicate that PE is concentration dependent so we tested four concentrations (1, 4, 40, and 400

uM-C) of all of the sources of LOM. By examining a concentration gradient of LOM, we hope to gain a better understanding of necessary conditions of priming.

We conducted experiments to observe priming capabilities in both single strains and a simplified community of bacteria. Single strain experiments were used to elucidate priming capabilities in E37 and SE45 within a controlled lab setting. The simplified community was used to better understand the roles of interspecies interactions in PE. This information can be used to formulate a mechanism for aquatic priming and its effect on the global carbon cycle.

2. Methods

2.1 Bacteria

We used six strains of Roseobacter to study PE in the lab: *Citreicella* sp. SE45, *Sagittula stallata* E37, *Sulfitobacter* sp. NAS-14.1, *Sulfitobacter* sp. EE-36, *Phaeobacter* sp. Y4I, and *Roseovarius nubinhibens* ISM. The strains contain varying capabilities for aromatic carbon catabolism (**Table 1**). SE45 and E37 were chosen for the single strain experiments based on their potential to degrade OM.

Table 1. The aromatic carbon catabolism pathways for the six roseobacter strains are shown. The ability to breakdown stable aromatic structures increases priming potential and utilization of recalcitrant carbon.

Ring-cleaving pathways	SE45	Y4I	ISM	E-37	EE-36	NAS-14.1
β-ketoadipate	Yes	Yes	Yes	Yes	Yes	Yes
Gentisate	Yes	X	X	Yes	X	X
Benzoate	X	X	X	Yes	X	X
Phenylacetic acid	Yes	Yes	X	Yes	Yes	Yes
Homoprotocatechuate	X	Yes	X	Yes	X	X
Homogentisate	Yes	Yes	X	Yes	X	X

2.2 Treatments

Treatments were labeled LOM, NOM, PRI, and ABM corresponding to the carbon source used. LOM treatments contained solely the labile carbon source: acetate, coumarate, casamino acids, or tryptone. These were tested at 1 µM-C, 4 µM-C, 40 µM-C, and 400 µM-C. NOM contained a 2 mM solution of Suwannee River natural organic matter (NOM). PRI treatments contained both NOM and one of the four labile carbon sources at the varying concentrations. ABM treatments were used as a control, containing no carbon source. There were five replicates of each treatment..

2.3 Inoculation

The strains were cultured individually in 2mM p-hydroxybenzoate (POB) broth in a shaking incubator at 30°C. Optical density (OD) measured by spectrophotometer (GENESYS) was used to determine cell density by comparing OD to predetermined growth curves for the individual strains. For single strain experiments, the cultures in POB were diluted with Aromatic Basal Media (ABM) to 10^6 cells mL^{-1} and 100 µL was added to each treatment to establish a seeding density of 10^4 cells mL^{-1} in 10 mL of media. For the intentional community containing all six strains of Roseobacters, the POB cultures were diluted to 10^6 cells mL^{-1} and 1 mL of each strain was combined to create a 6 x 10^6 cell mL^{-1} community master mix. The treatments were inoculated with 16.7 µL of the master mix to create a starting concentration of 10^4 cells mL^{-1} in 10 mL of media.

2.4 Data Collection and Analysis

The cultures were incubated in a shaking incubator at 30°C in the dark for two weeks. Samples containing 200 µL of the cultures were taken from the culture on days 0, 1, 2, 4, 7, 10, and 14 to measure viable counts. The samples were serially diluted with ABM to plate 30-300 cells with 100µL plated onto YTSS (2.50 g yeast extract, 4.00 g tryptone, 15.00 g sea salts, 15.00 g agar, 1 L distilled water). Colonies were counted after a 48 h incubation at 30°C in the dark.

The viable counts for the replicates were averaged. A composite score was created by summing LOM and NOM viable counts to show the baseline of Roseobacter growth without priming. The data was analyzed with a two-way ANOVA and Tukey Test to show statistical differences between the PRI and composite growth curves and the growth at differing LOM concentrations.

3. Results

3.1 Single Strain Experiments

E37. We see the most evidence for priming in the 400 µM-C treatments (**Fig 1A**) with significant differences ($p<0.05$) between the composite and PRI growth in acetate, coumarate, and casamino acids. The priming in acetate treatments has the greatest longevity of the four LOM with significant priming at five of the six time points tested in the 400 µM-C. The magnitude of priming decreases with the lower LOM concentration treatments. Significant priming can be seen with 40 µM-C casamino acids at two time points, and only at one time point in 4 µM-C casamino acids. The 1 µM-C treatments showed the least priming with similar composite and PRI growth curves. Tryptone had the weakest effect on priming with a composite score higher than priming treatment in most cases.

SE45. The different types of LOM produced varying time patterns in regard to onset of priming in 400 µM-C treatments (**Fig 1B**). There is initial priming in tryptone whereas casamino acids shows priming toward the end of the incubation. The 400 µM-C acetate treatments shows initial priming that disappears and then returns on day 4, lasting nearly the rest of the experiment. The effect of changing LOM concentration is indiscernible compared to that of the E37 single strain experiment.

E37 vs. SE45. The two strains exhibited noticeably different growth patterns throughout the two weeks. E37 showed a rapid increase, exceeding SE45 growth by day 2 in 1, 4, and 40 µM-C treatments (**Fig 1C**). The differences in initial growth disappear in the 400 µM-C treatments with the exception of casamino acids. E37 also displays a rapid decline in viable counts around day 7, exemplified by 4 µM-C and 40 µM-C casamino acids. In contrast SE45 has a steady growth after day 1 and shows an increase in the 1 µM-C treatments.

3.2 Intentional Community

Overall Priming. Similar to the E37 single strain experiment, we see the greatest evidence for priming in the 400 µM-C LOM treatments (**Fig 2A**). There's delayed priming in the 400 µM-C acetate and 400 µM-C tryptone, beginning around day 4 with continual priming for the rest of the experiment. In the 400 µM-C coumarate treatment, we can see significant differences between the PRI and composite curves early on. As the difference between the growth curves decreases, PRI viable counts remain higher than composite until day 14. There's minimal priming at the 4 µM-C and 40 µM-

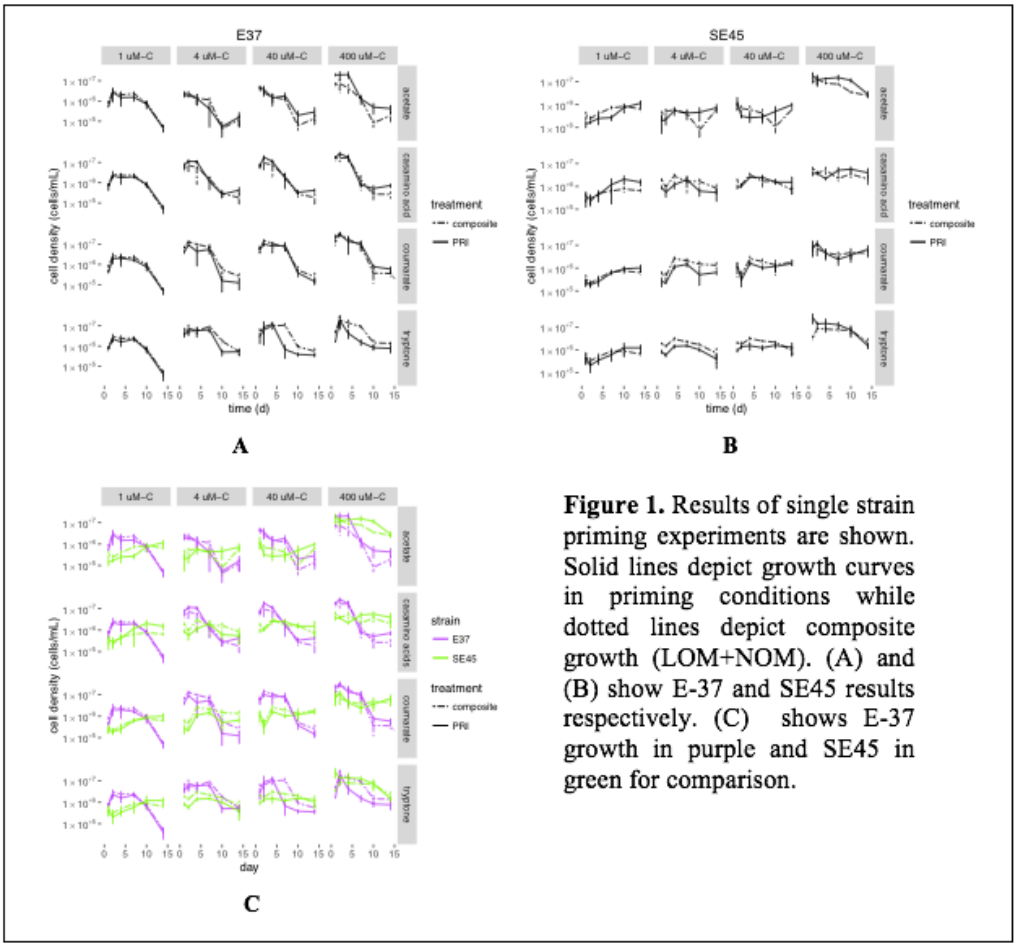

Figure 1. Results of single strain priming experiments are shown. Solid lines depict growth curves in priming conditions while dotted lines depict composite growth (LOM+NOM). (A) and (B) show E-37 and SE45 results respectively. (C) shows E-37 growth in purple and SE45 in green for comparison.

C concentrations and no instances of priming with 1 µM-C.

Community composition. There is a general decrease in community diversity with increasing concentration of LOM. At 1 µM-C, all six strains are represented until at least day 7 (**Fig 3**). With increasing LOM concentration, we see community members disappear more quickly. By day 2 in 400 µM-C acetate, casamino acids, and tryptone, treatments the community is dominated by Y4I.

In coumarate treatments (**Fig 3C**), we see a larger percentage of SE45 in the community with increasing LOM concentration. At 400 µM-C coumarate, SE45 is the dominating species in the community alongside a higher percentage of E37 than seen with other LOM sources.

In comparison to the controls (**Fig 3E**), the diversity seen in PRI treatments at 1 µM-C more closely resembles the NOM control while the 400 µM-C diversity resembles the corresponding LOM treatments. In those low LOM concentrations, we also see in increased abundance specifically of SE45 and E37, the strains most capable of consuming NOM.

The differentiating factor between the LOM and PRI community consumption is the survival and growth of E37 and SE45. There are higher percentages of E37 and SE45 in PRI treatments throughout the experiment compared to the LOM treatments with the exception of 400 µM-C tryptone.

3.3 Single Strain Vs. The Community

E37. At lower LOM concentrations, the growth curve for E37 (**Fig 2B**) in the community mimics E37 single strain growth. At the 4 µM-C and 40 µM-C treatments, we can see the E37 growth in the community begins to resemble the total

community growth curve, specifically 4 µM-C acetate and µM-C coumarate.

Instances over the two weeks where the single strain growth curve is higher than the total community growth shows the restrictions placed on E37 growth in the presence of other organisms. This is seen clearly in the 4 µM-C and 40 µM-C treatments of casamino acids, coumarate, and tryptone.

SE45. We can see instances in the coumarate treatments where SE45 in the community growth exceeds that of single strain growth (**Fig 3C**). This is seen specifically at 400 µM-C coumarate on day 7 and 1 µM-C coumarate day 4. The SE45 in the community growth curves observed at 1 µM-C very closely resembles the total community growth curve. The growth at higher LOM concentrations show no apparent pattern.

E37 vs. SE45. Instances of significant community priming in 400 µM-C acetate and 400 µM-C casamino acids resemble those shown in both E37 and SE45 single strain experiments. The community priming observed initially in 400 µM-C coumarate and later on in 400 µM-C tryptone are not accounted for by the single strain experiments (**Fig 3D**). In comparison to E37, there are fewer instance of SE45 single strain growth exceeding that of total community growth.

4. Discussion

4.1 Single Strain Priming

Despite the close relation between SE45 and E37 and their abilities to catabolize aromatic carbon, they each exhibited unique

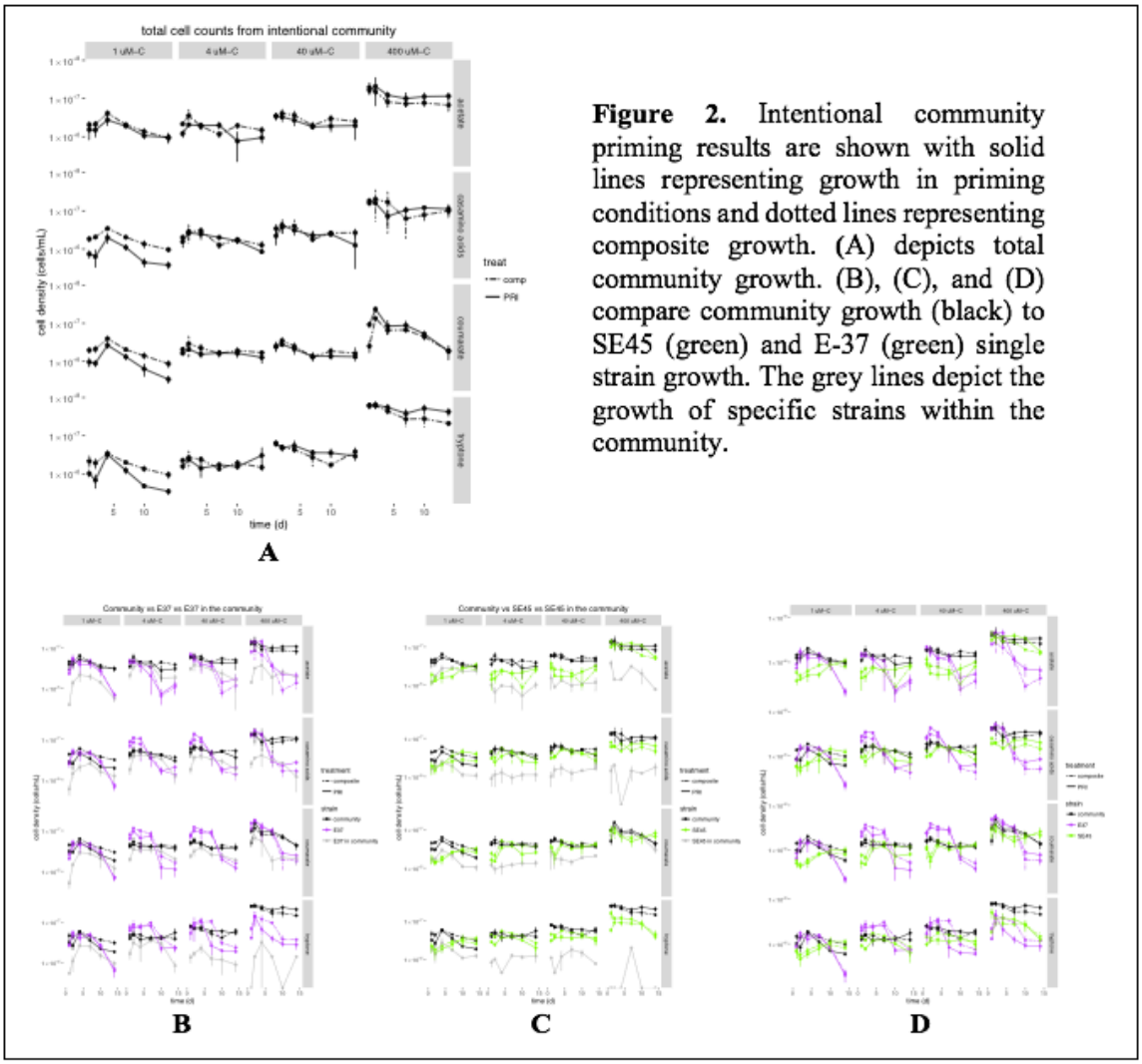

Figure 2. Intentional community priming results are shown with solid lines representing growth in priming conditions and dotted lines representing composite growth. (A) depicts total community growth. (B), (C), and (D) compare community growth (black) to SE45 (green) and E-37 (green) single strain growth. The grey lines depict the growth of specific strains within the community.

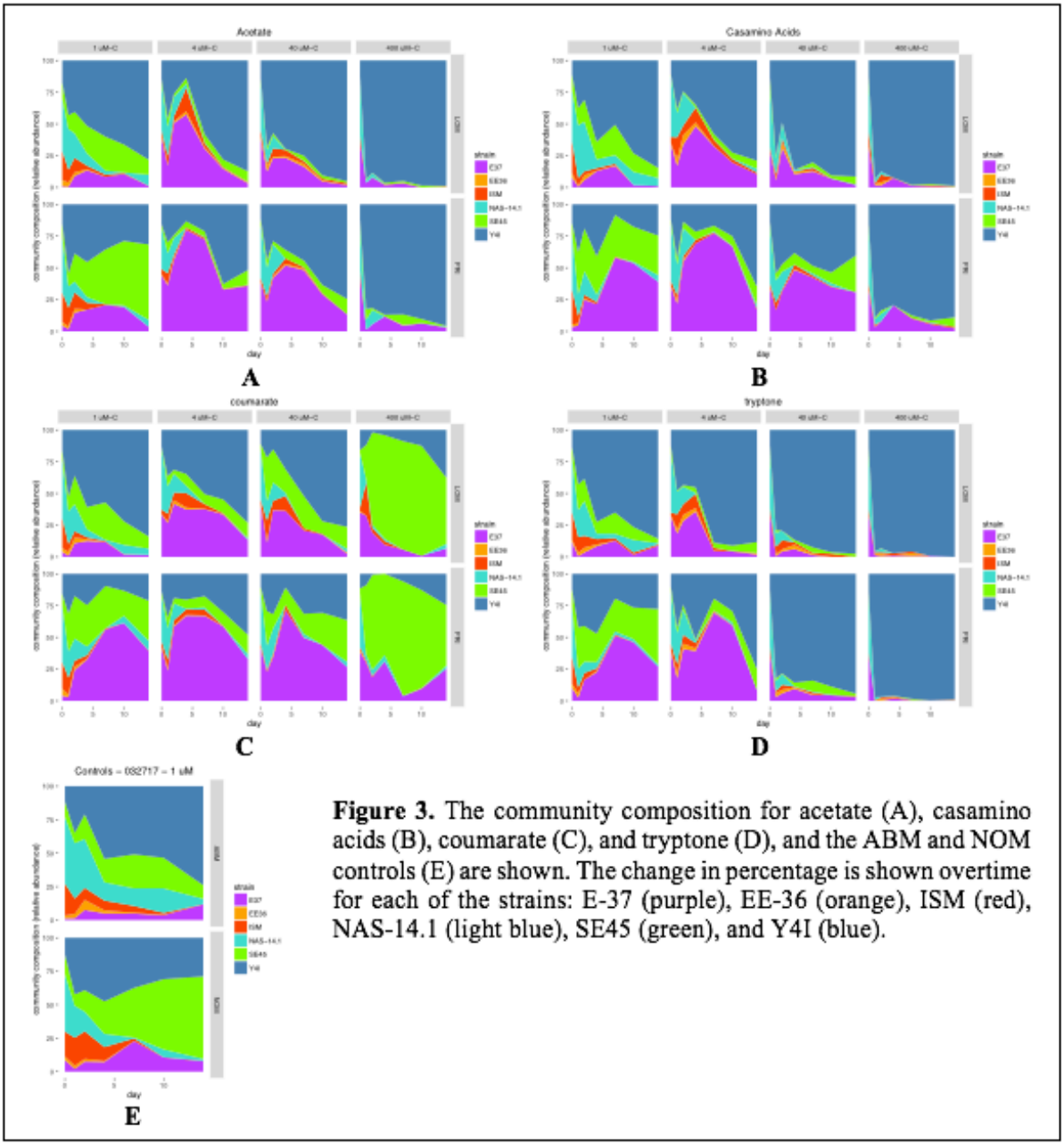

Figure 3. The community composition for acetate (A), casamino acids (B), coumarate (C), and tryptone (D), and the ABM and NOM controls (E) are shown. The change in percentage is shown overtime for each of the strains: E-37 (purple), EE-36 (orange), ISM (red), NAS-14.1 (light blue), SE45 (green), and Y4I (blue).

growth patterns in the priming conditions. The single strain experiments provided significant evidence for priming in acetate, casamino acids, and coumarate in both SE45 and E37. Priming was observed with tryptone only in SE45. Tryptone, an oligopeptide, requires the use of extracellular enzymes to break it down. E37 has the ability to grow with tryptone as the sole carbon source, thus verifying the presence of the necessary extracellular proteases. However, the lack of tryptone priming in E37 points to the fact that these enzymes may not be involved in priming with tryptone treatments. Acetate, on the other hand, has a much simpler chemical structure and the ability to feed directly into the citric acid cycle. This could explain why priming with acetate produced PE with the greatest longevity in both SE45, E37, and the simplified community.

The strains were also affected differently by the concentration of LOM. E37 showed a consistent gradient with increasing magnitude and duration of PE with increasing LOM concentration from 1 µM-C

to 400 μM-C. Although SE45 also demonstrated the greatest PE in 400 μM-C LOM treatments, the lower LOM concentration did not display a similar gradient to that of E37 growth. These data show that the organisms utilize LOM in different ways and could have differing thresholds that affect PE.

4.2 Intentional Community Priming

Significant PE for all four types of LOM were demonstrated in the simplified community experiment. The change in community diversity shows the shift between NOM-driven to LOM-driven community composition with increasing LOM concentration. The 400 μM-C acetate, casamino acids, and tryptone treatments contain primarily Y4I after day 2. The increased community abundance of SE45 and E37 observed in priming treatments could indicate the importance of increased ability to catabolize aromatic carbon in PE. The community priming may, therefore, be an additive priming from the two strains. However, this does not account for the significant priming in 400 μM-C tryptone, where the two strains maintained low abundance even in priming treatments. These data suggest priming capabilities in a strain not studied in the single strain experiments, Y4I.

4.3 Synergistic Coumarate Priming

Priming with 400 μM-C coumarate produced an increase in growth unmatched by the single strain experiments. Community composition data reveal a higher percentage of E37 than shown in all other priming treatments and SE45 nearly dominating the culture. The PRI growth curve remains above that of the composite throughout the two-weeks diminishing in a correlating way to the decreased abundance of E37 around day 5. We can also see the SE45 in the community growth curve surpass single strain SE45, indicating a benefit to living in the community in 400 μM-C coumarate priming conditions. With the distinct increase in E37 abundance, the benefit may be a synergistic relationship between the two.

4.4 Conclusion

Roseobacter strains SE45 and E37 demonstrate PE with varying complexity and concentrations of LOM through increased biomass in priming conditions. These strains can therefore be used to gain insight on the mechanism for priming in aquatic bacteria. Studying cellular respiration and enzyme activity can show how the LOM and NOM interact and are incorporated into the cell to create the increase in biomass. To account for the unexpected priming in 400 μM-C tryptone, studies should focus on single strain priming in other strains, such as Y4I. Understanding the mechanism of priming in Roseobacters, as highly abundant and metabolically active members of the estuarine community, can help create a more holistic view of carbon cycling.

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