Name: Isaac Masters

Title: Predatory bacteria: investigating the role of lipopolysaccharide and outer membrane protein mutants on predation

Running title: Investigating predatory bacteria

Abstract

In recent years, there have been studies that evaluate the potential use of predatory bacteria *Bdellovibrio bacteriovorus* to control the multidrug-resistant (MDR), Gram-negative pathogens associated with human infections. This study was focused on analyzing various Lipopolysaccharide and outer membrane protein mutants of forty *Acinetobacter baumannii* mutants in order to evaluate which factors play a role in predation. Although most of the cycle of the *Bdellovibrio* is known, it is still relatively unknown how these predatory bacteria attach to its prey. By co-culturing *Bdellovibrio bacteriovorus* 109J with a model MDR organism, *Acinetobacter baumannii,* we were able to identify and examine specific mutations that result in decreased predation of the predatory bacteria on the model organism. *Acinetobacter baumannii* with an outer membrane protein (OMP) mutation on OmpA showed a decrease in predation after 24h. Additional experiments were done on one OmpA mutated *Escherichia coli* and three *Pseudomonas aeruginosa* strains but no significant effect was found. We have established that *Bdellovibrio bacteriovorus* reduced the amount of MDR *Acinetobacter baumannii* similarly in all mutants except outer membrane protein A. This research indicates that the attachment mechanism of *Bdellovibrio bacteriovorus* to *Acinetobacter baumannii* might play a vital role in predation. With the help of future studies, the proceeding steps would be to analyze the methodology further in order to solidify if outer membrane proteins play a role in attachment.

Keywords: *Bdellovibrio bacteriovorus, Acinetobacter baumannii,* OmpA mutant, multidrug resistant pathogens

Although the discovery of antibiotics has provided substantial benefits to the field of medicine and have saved countless lives, the misuse of antibiotics over the years has led to the emergence of life-threatening pathogenic multidrug-resistant (MDR) Gram-negative bacteria. In the United States alone, it is estimated that nearly 2 million people are infected and more than 23,000 die annually as a direct result of an MDR infection (Shatzkes et al. 2017). Due to the rising number of MDR infections, the need to investigate new ways and technologies to control and cure infections has become a concern. Furthermore, antibiotics cannot treat Gram-negative pathogens as well as Gram-positives; the antibiotic must penetrate the additional outer membrane in Gram-negative bacteria. Therefore, a significant effort is needed to find antibiotics, or other methods, that can safely treat Gram-negative pathogens.

An alternative approach to combat MDR pathogens is the use of predatory bacteria. Predatory bacteria are small, motile, Gram-negative bacteria that naturally prey on other Gram-negative bacteria in their environment. Among the abundant predator bacteria found in nature, only *Bdellovibrio bacteriovorus* and *Micavibrio bacteriovorus* have been thoroughly studied, and only *Bdellovibrio bacteriovorus* was used in the scope of this particular research. *B. bacteriovorus* is a Deltaproteobacteria that has a single, polar flagellum which causes it to be one of the fastest moving organisms in nature (Shatzkes et al. 2017).

Nutrients for the replication of *Bdellovibrio bacteriovorus* come entirely from the digestion of the single invaded bacterium, now called a bdelloplast, which is bound by the original prey outer membrane (Fig. 1) (Núñez et al. 2003). *B. bacteriovorus* are efficient digesters of prey cells, yielding on average four to six progeny from digestion of

a single prey cell of a genome size similar to that of the *Bdellovibrio bacteriovorus* cell itself (Fenton et al. 2010). The developmental intrabacterial cycle of is largely unknown; however, Fenton et al. (2010) showed that developing *B. bacteriovorus* cells use the inner resources of a prey cell. They showed that *Bdellovibrio bacteriovorus* do not follow the familiar pattern of bacterial cell division by binary fission. Instead, they septate synchronously to produce both odd and even numbers of progeny, even when two separate *B. bacteriovorus* cells have invaded and developed within a single prey bacterium and produce two different amounts of progeny. When replication is complete, they showed that *Bdellovibrio bacteriovorus* cells exit the exhausted prey via discrete pores rather than by breakdown of the entire outer membrane of the prey (Fenton et al. 2010).

Despite adequate knowledge regarding the majority of the life cycle and basic characteristics of *Bdellovibrio bacteriovorus*, the mechanism of how this predator attaches to its prey remains unclear. Previous studies have shown the ability of predatory bacteria to prey on a broad range of Gram-negative bacteria *in vitro*, such as key human pathogens from the genera *Acinetobacter*, *Bordetella*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Salmonella*, *Yersinia*, among others (Shatzkes et al. 2017). Previous studies have also shown the ability of *Bdellovibrio bacteriovorus* to reduce existing multidrug-resistant bacteria by 4-log over a 24-hour period (Kadouri et al. 2013). This research attempts to understand the mechanism in which *Bdellovibrio bacteriovorus* attaches to its prey and to examine various types of mutations that may play a role in predation.

Materials and Methods

A total of 51 MDR clinical mutants were tested, which includes 45 mutants of *Acinetobacter baumannii* (including wild type), 4 strains of *Pseudomonas aeruginosa* (including wild types), and 2 strains of *Escherichia coli* (including wild type). Each mutant used in this study was a Lipopolysaccharide (LPS) or an outer membrane protein (OMP) mutant. The *Acinetobacter baumannii* mutants were obtained from the AB5075 transposon mutant library from the Manoil lab at the University of Washington (Gallagher et al. 2015). Lipopolysaccharides, also known as lipoglycans, are composed of a lipid and a polysaccharide, and are extremely important to the structural integrity of the Gram-negative bacteria. The predatory bacteria used were *B. bacteriovorus* 109J and *B. bacteriovorus* HD100. To initiate a lysate, cocultures of *B. bacteriovorus* and *E. coli* were started and incubated at 30°C on a rotary shaker until the coculture became clear (stock lysate). To prepare an active culture of the predators, cocultures were prepared with host cells and predator lysate (from above). The cocultures were incubated for 24 hrs at 30°C on a rotary shaker to reach a final concentration of about 1 x 10^8 PFU ml^{-1} predator. After 24 hrs, the lysate was passed through a 0.45-μm Millex pore-sized filter to remove residual prey and cell debris (filtered lysate) (Dashiff et al. 2011).

In order to evaluate the ability of *B. bacteriovorus* to prey on the selected MDR pathogens, cocultures were prepared by incubating overnight cultures of prey cells with *B. bacteriovorus*, the predator. Each mutant was cocultured with a mixture of wt *B. bacteriovorus* 109J and a strain of 109J engineered to express a red fluorescent protein (tdTomato). Thus, the population of predator cells could be monitored by measuring

fluorescence at 548 and 586 nm. As a control, a culture containing lysate buffer and prey cells was used. The cultures were incubated for up to 48 hrs, at 30°C in a microplate reader which measured the optical density of the prey and the fluorescence emission of the predator. The absorbance was measured at wavelength of 600nm to determine prey cell populations and the fluorescence at wavelengths of 548 nm and 586 nm for red fluorescence protein (tdTomato) or 485 nm and 528 nm for green fluorescence protein (GFP) to determine predator cell population. GFP can used to monitor the movement or quantity of a live culture of organisms via fluorescence (Chalfie et al. 1994). Each coculture was done in triplicate in a 96-well plate. tdTomato fluorescent protein has been found to be a convenient and adequate tool for monitoring predator growth during predation, monitoring growth in real time, and visualizing different predator growth stages by microscopy. It can be used in prey population to monitor predation, more specifically in the study of predatory bacterial biology and predator-prey interaction (Mukherjee et al. 2016).

The ability of predators to reduce existing colonies of the prey was then confirmed by the reduction in host cell viability, measured by colony forming unit (CFU) enumeration, compared to the control. Each coculture was performed in triplicate for significance (Dashiff et al. 2011). To determine significance ach of the mutant's log reductions were compared to the wild type using a paired t-test and an alpha value of 0.05 with a two-tailed p-value.

The samples were also visualized using a phase contrast microscope. OmpA mutants AB01772, AB01773, AB01774, AB01775 were examined at time points of zero, 30 minutes, 2 hours, and 24 hours after the predator was introduced to the prey.

Cocultures of the *A. baumannii* mutant and *B. bacteriovorus* 109J were made similar to other experiments and incubated at 30°C for a total of 24 hrs.

Results

The ability of *B. bacteriovorus* 109J to attack the prey was first measured by optical density using a microplate reader, then by dilution plating and CFU enumeration of a *A. baumannii* mutants verified in the microplate assay as resistant to *B. bacteriovorus* predation, each compared to the initial host concentration and the predator-free control group. Each mutant was screened using a microplate reader measuring optical density of the prey as well as the fluorescence of the predator (Fig. 2-6). In the initial 38 mutants of *Acinetobacter baumannii*, only six showed a decrease in predation. In order to evaluate further, after initial screening, mutants that presented evidence of delayed predation or decreased fluorescence emission were rescreened to validate the previous findings. Fig. 7 shows the second screen of these potential candidates. In addition to 109J and tdTomato, HD100 and green fluorescent protein *B. bacteriovorus* (GFP) was used in order to determine if predator strain affects predation. These six candidates were mutants AB06446, AB08788, AB01772, AB01774, AB04138, and AB10125. Of these six candidates, only mutants AB01772 and AB01774 showed predation different from the wild type after the second screen. These two mutants show a delay in predation via optical density, as well as lower predator populations via fluorescence emissions (Fig. 7). After the initial screens, two additional OmpA mutants, AB01773 and AB01775, were screened via the microplate reader. Fig. 8 shows the predation of *B. bacteriovorus* 109J, HD100, tdTomato, and GFP on OmpA mutants AB01772, AB01773, AB01774, and

AB01775. Mutant AB01773 produced results similar to the wild type; however, mutants AB01772, AB01773, AB01775 appeared to show a delay in predation. CFU enumeration was done in order to confirm (Table 1). When compared to the wild type, *A. baumannii* OmpA mutant 1772 and *A. baumannii* OmpA mutant 1774 were the only mutants that showed significantly decreased predation. Mutant AB01772 was preyed upon nearly 86% less than the wild type after 24 hrs, and mutant AB01774 was preyed upon nearly 92% less than the wild type after 24 hrs. These mutants were counted again after 48 hrs and still showed a significant decrease in predation (p-value <0.05).

Mutants AB01772, AB01773, AB01774, and AB01775 were also observed under a phase contrast microscope to examine predator attachment and bdelloplast formation. Visual images were taken at time points of zero, 30 minutes, 2 hrs, and 24 hrs after *B. bacteriovorus* was introduced to the prey (Fig. 8-12). Attachment in the mutants at t0 appeared to be consistent with the wild type and no significant difference was noted. However, at t30 min, mutants AB01772 and AB01774 showed roughly 50% fewer predator attachments, while AB01773 and AB01775 were consistent with the wild type. Additionally, at t2 hrs, mutants AB01772, AB01774, and AB01775 showed fewer bdelloblasts and more attachments that the wild type, indicating that predation is delayed.

To understand the effect of OmpA mutations on predation of bacteria other than *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Escherichia coli* were examined. *P. aeruginosa* strain 14 was used as the wild type along with *P. aeruginosa* B2, and OmpA mutants *P. aeruginosa* A7 and *P. aeruginosa* F2. Each mutant was tested in a microplate reader cocultured with *B. bacteriovorus* 109J and HD100 to test optical density of the prey, as well as GFP to measure the fluorescence of the predator (Fig. 13).

In order to confirm microplate results, CFU enumeration was performed on these mutants (Table 2). These mutants were not done in triplicate, thus, standard deviation could not be calculated. However, *B. bacteriovorus* did not appear to show decrease predation on *P. aeruginosa* OmpA mutants.

Furthermore, *Escherichia coli* OmpA mutant was also examined. *E. coli* 7637 was used as the wild type and *E. coli* 8942 as the OmpA mutant. Both strains were cocultured with *B. bacteriovorus* 109J, HD100, tdTomato, and GFP and examined in the microplate reader for 48 hrs (Figure 14). In order to confirm, CFU enumeration was done (Table 3). *B. bacteriovorus* did not significantly reduce *E. coli* OmpA mutants.

Discussion

Due the emergence of multidrug-resistant (MDR) Gram-negative bacteria, new therapeutic alternatives to antibiotics have become a necessity. The lack of viable antibiotics to treat these infections has urged researchers to think of new approaches to pathogen control, such as predatory bacteria (Shatzkes et al. 2017). Predatory bacteria, *Bdellovibrio bacteriovorus* for example, prey on MDR bacteria regardless of their antimicrobial resistance; thus, might be used as therapeutic agents where other antimicrobial drugs fail (Kadouri et al. 2013). Although his research did not attempt to assess the predation of various multidrug-resistant bacteria, it did attempt to examine various outer membrane structures of *Acinetobacter baumannii* in order to determine which structures play a role in predation.

The ability of *B. bacteriovorus* 109J to attack the prey was first measured by optical density using a microplate reader (Fig. 2-6). Each mutant was screened using a

microplate reader measuring optical density of the prey as well as the fluorescence of the predator. By using a control that lacked the predator, we determined that the bacterial culture was not dying on its own (data not shown). The initial screens of the 38 mutants show the ability of *B. bacteriovorus* to attack *A. baumannii*. *B. bacteriovorus* was able to prey upon every mutant of multi-drug resistant *A. baumannii*, reducing the optical density to 0.1 for nearly every mutant. Additionally, tdTomato, a red fluorescent protein tagged 109J (predatory bacteria), was used to show that the fluorescence intensity of the predator increases as the predator preys, indicating that the number of predators are increasing by feeding on the prey (Mukherjee et al. 2016).

Out of the 38 LPS or OMP mutants tested, only six showed predation different from the wild type. These six mutants were screened an addition time in order to verify the initial results (Fig. 7). In addition to 109J and tdTomato, HD100 and green fluorescent protein *B. bacteriovorus* (GFP) was used in order to determine if predator strain affected predation. These six candidates were mutants AB06446, AB08788, AB01772, AB01774, AB04138, and AB10125. Of these six candidates, only mutants AB01772 and AB01774 showed predation different from the wild type after the second screen. These two mutants show a delay in predation via optical density, as well as lower fluorescence emissions. Mutants AB01772 and AB01774 are outer membrane protein (OmpA) mutants. Unlike LPS mutants, outer membrane protein mutants have mutations found on the membrane, which is superficial to the peptidoglycan. OmpA is a β-barrel porin that is highly conserved among bacterial species, and has been associated with a variety of interesting biological properties in *in vitro* model systems (Smith et al. 2007). OmpA has been shown to bind host eukaryote epithelia, target mitochondria, translocate

to the nucleus, and induce cell death and can also bind factor H, which may allow *A. baumannii* to develop serum resistance (Choi et al. 2008). Factor H is a complement control protein that circulates in human plasma (Sofat et al. 2013). Furthermore, OmpA has also been associated with antimicrobial resistance in related pathogens, including *A. baumannii* (Viveiros et al. 2007). Although the exact mechanism of action is not clear, it is possible that OmpA participates in the extrusion of compounds from the periplasmic space through the outer membrane and couples with inner membrane efflux systems, such as major facilitator superfamily (MFS) efflux pumps lacking the outer membrane protein component (Smani et al. 2014).

In order to create certain mutations, the genome of the strain was altered at specific locations by using a transposon. In *Acinetobacter baumannii* 5070, the wild type, OmpA mutations are made via transposon between the genome coordinates of 654,225 and 655,325 (Gallagher et al. 2015). After the initial screens, two other OmpA mutants were screened via the microplate reader. Fig 7 shows the predation of *B. bacteriovorus* 109J, HD100, tdTomato, and GFP on OmpA mutants AB01772, AB01773, AB01774, and AB01775. Mutant AB01773 produced results similar to the wild type; however, mutants AB01772, AB01773, AB01775 appeared to show a delay in predation, indicating that OmpA could potentially play a role in predation. In order to confirm, CFU enumeration was done on mutants AB01772, AB01773, AB01774, and AB01775 (Table 1). Of the four OmpA mutants, only AB01772 and AB01774 showed significant differences in log reduction of prey cells. Additionally, only AB01772 and AB01774 have been confirmed as OmpA mutants in the transposon mutant library in the Manoil Lab at the University of Washington. Therefore, only AB01772 and AB01774 are

unequivocally true OmpA mutants. As a result, the genomic location of mutations in AB01773 and AB01775 could be contributed to a mutation outside of the *ompA* locus.

Mutants AB01772, AB01773, AB01774, and AB01775 were also observed under a phase contrast microscope to examine predator attachment and bdelloplast formation (Fig. 9-13). This information could indicate which types of mutations play a factor in the predation of the predator. In the experiment, each mutant was compared to the wild type and was monitored for number of predator attachment, observed timing of attachment, amount and timing of bdelloplasts formed, and how effective the predator was at escaping from inside the bdelloplast. For all 4 mutants, attachment at t0 was consistant with the wild type and no significant difference was noted. At t30 min, mutants AB01772 and AB01774 had fewer predator attachments, while AB01773 and AB01775 were consistent with the wild type. At t2 hrs, mutants AB01772, AB01774, and AB01775 showed fewer bdelloblasts and more attachments than the wild type, indicating that predation is delayed. Mutant AB01773 was similar to the wild type. At 24 hrs, the wild type coculture was entirely *Bdellovibrio bacteriovorus*, indicating that the predator had consumed all the prey and escaped the bdelloplasts. However, all the mutants contained bdelloplasts, with mutants AB01772 and AB1774 containing primarily bdelloplasts and few free-swimming predators, possibly indicating that the predator was "trapped" in the bdelloplast and unable to escape due to the mutation in the outer membrane protein. Fenton et al. (2010) found that when replication is complete, *Bdellovibrio* cells exit the exhausted prey via discrete pores rather than by breakdown of the entire outer membrane of the prey. This is consistent with the hypothesis that *Bdellovibrio* can remain trapped inside prey if OmpA is mutated. Thus, in *Acinetobacter baumannii,* OmpA mutations

show a decreased ability for *Bdellovibrio bacteriovorus* to escape the bdelloplast and seek other prey, ultimately decreasing predation.

In order to test whether OmpA mutations have an effect on predation of bacteria other than *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Escherichia coli* were examined. *P. aeruginosa* strain 14 was used as the wild type along with *P. aeruginosa* B2, and OmpA mutants *P. aeruginosa* A7 and *P. aeruginosa* F2. Each mutant was tested in a microplate reader cocultured with *B. bacteriovorus* 109J and HD100 to test optical density of the prey, as well as GFP to measure the fluorescence of the predator (Fig. 14). The optical densities of PaA7 and PaF2, the OmpA mutants, with 109J and HD100 provide evidence that outer membrane protein mutations do not play a role in predation of *P. aeruginosa;* all of the mutants are similar to the wild type, Pa14. One explanation to this finding is that in *P. aeruginosa,* there could be other outer membrane proteins other than OmpA that allow *B. bacteriovorus* to adequately attach and enter the cell. In order to confirm, CFU enumeration was performed on these mutants (Table 2). These experiments were not done in triplicate, thus, standard deviation could not be calculated. However, the OmpA mutants did not produce a decrease in predation as was found in *A. baumannii*.

Furthermore, *E. coli* 8942 (an OmpA mutant) did not show a significant difference in predation from the wild type (Fig. 15). CFU enumeration of predation was performed on this mutant in order to confirm the result from the microplate reader experiment and then compared to the wild type, in triplicate. The OmpA mutant 8942 did not show a significant change in predation from the wild type (Table 3). Because the *E. coli* OmpA mutant did not show a decrease in predation, OmpA mutations do not

appear to play a role in predation in *E. coli.* However, OmpA plays a vital structural role in *E. coli,* and previous research suggests that a perfect β-barrel structure of OmpA is important for outer membrane stability (Wang 2002). One possibility is that *E. coli* has other outer membrane proteins that allow *B. bacteriovorus* to enter and exit the cell where *A. baumannii* does not. Thus, even though OmpA is important for the structural integrity of *E. coli,* it may not be as important for the entry and exiting of the *Bdellovibrio.* As a result, OmpA mutations seem to only play a factor in *A. baumannii*.

Thus, due to the increased occurrence of Gram-negative multidrug-resistant pathogens, most of which can no longer be effectively treated with conventional antibiotics, predatory bacteria could potentially be used at the clinical level to treat these infections. To fully understand these predators, studies such as this one must be done in order to examine the methodology of predation. This study's data suggests that OmpA could potentially play a role in predation in *Acinetobacter baumannii.*

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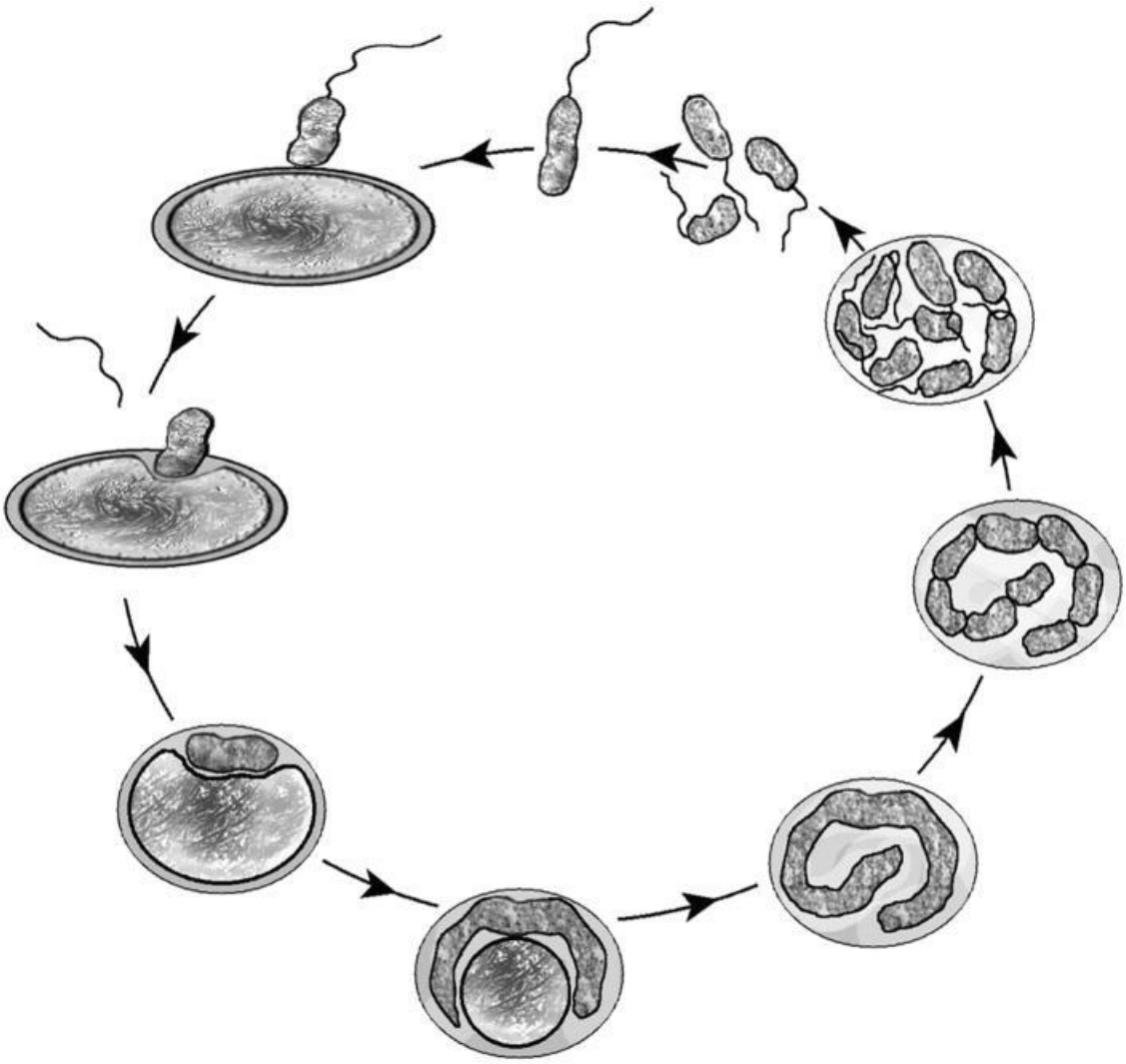

Figure 1. Diagram of the life cycle of the bacterial predator *Bdellovibrio bacteriovorus*, which consists of a free-swimming stage, and a growth stage spent inside its prey bacterium. A *Bdellovibrio* predator and the killed prey cell in which it is growing are together termed a bdelloplast (Núñez et al. 2003).

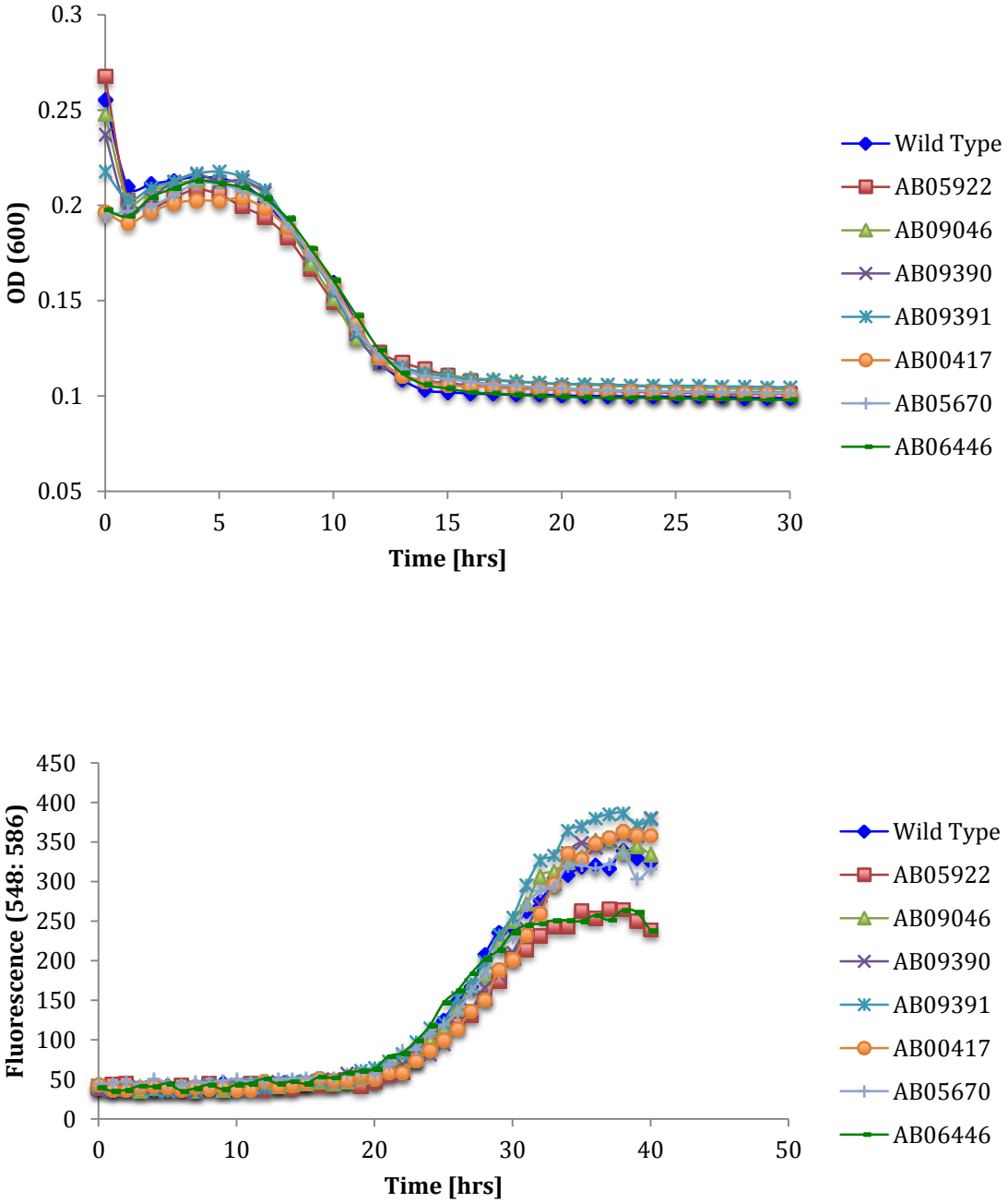

Figure 2. Effect of *B. bacteriovorus* on *Acinetobacter baumannii* mutants. Top panel shows culture density (OD_{600}) of wt and mutant *Acinetobacter baumannii* over time. Bottom panel shows fluorescence of *Bdellovibrio* population ($F_{548:586}$) over time. Each coculture was made in a 96-well microplate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing wt 109J and fluorescence 109J at every hour for those wells containing the red fluorescent protein, tdTomato.

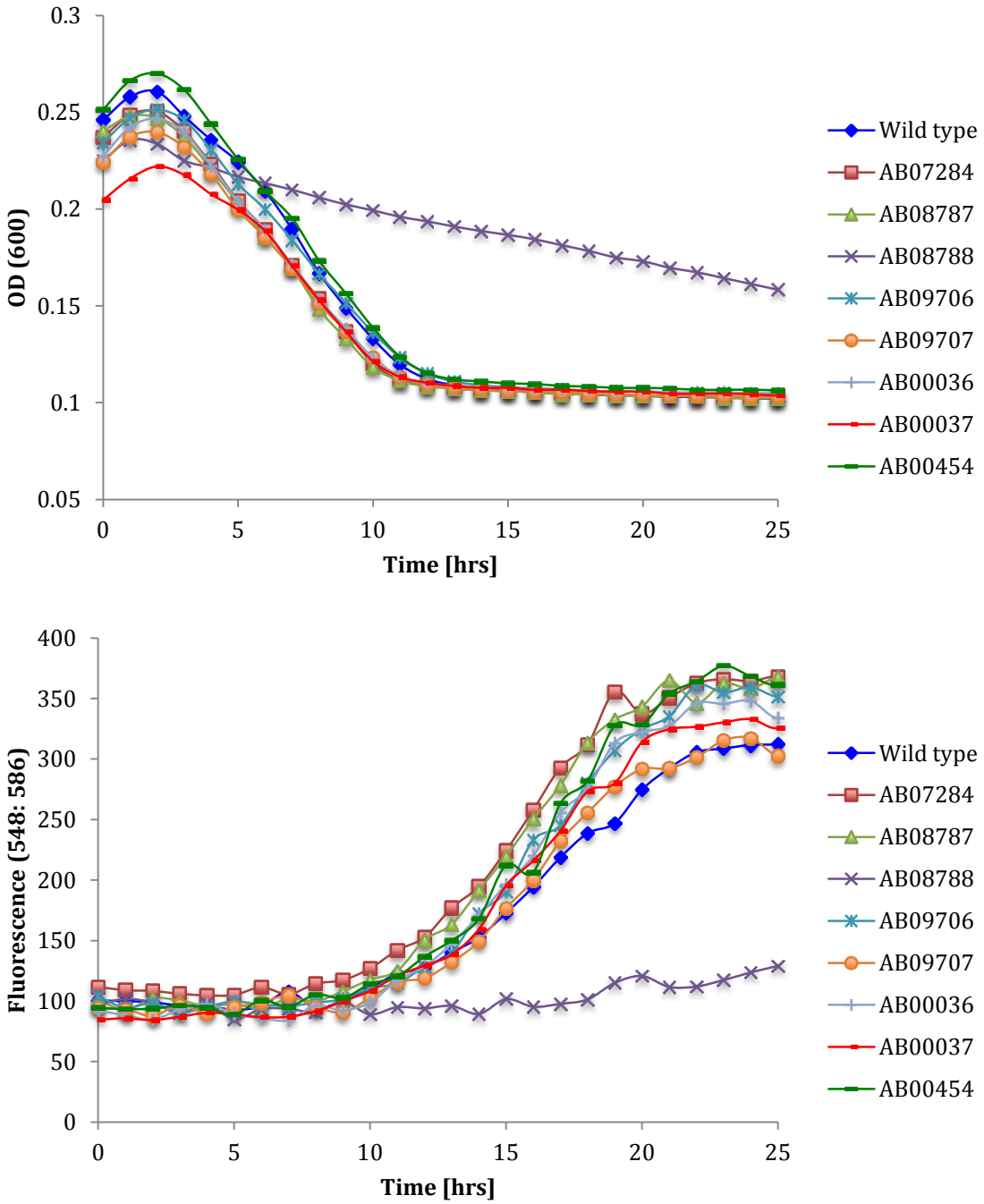

Figure 3. Effect of *B. bacteriovorus* on *Acinetobacter baumannii* mutants. Top panel shows culture density (OD_{600}) of wt and mutant *Acinetobacter baumannii* over time. Bottom panel shows fluorescence of *Bdellovibrio* population ($F_{548:586}$) over time. Each coculture was made in a 96-well microplate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato.

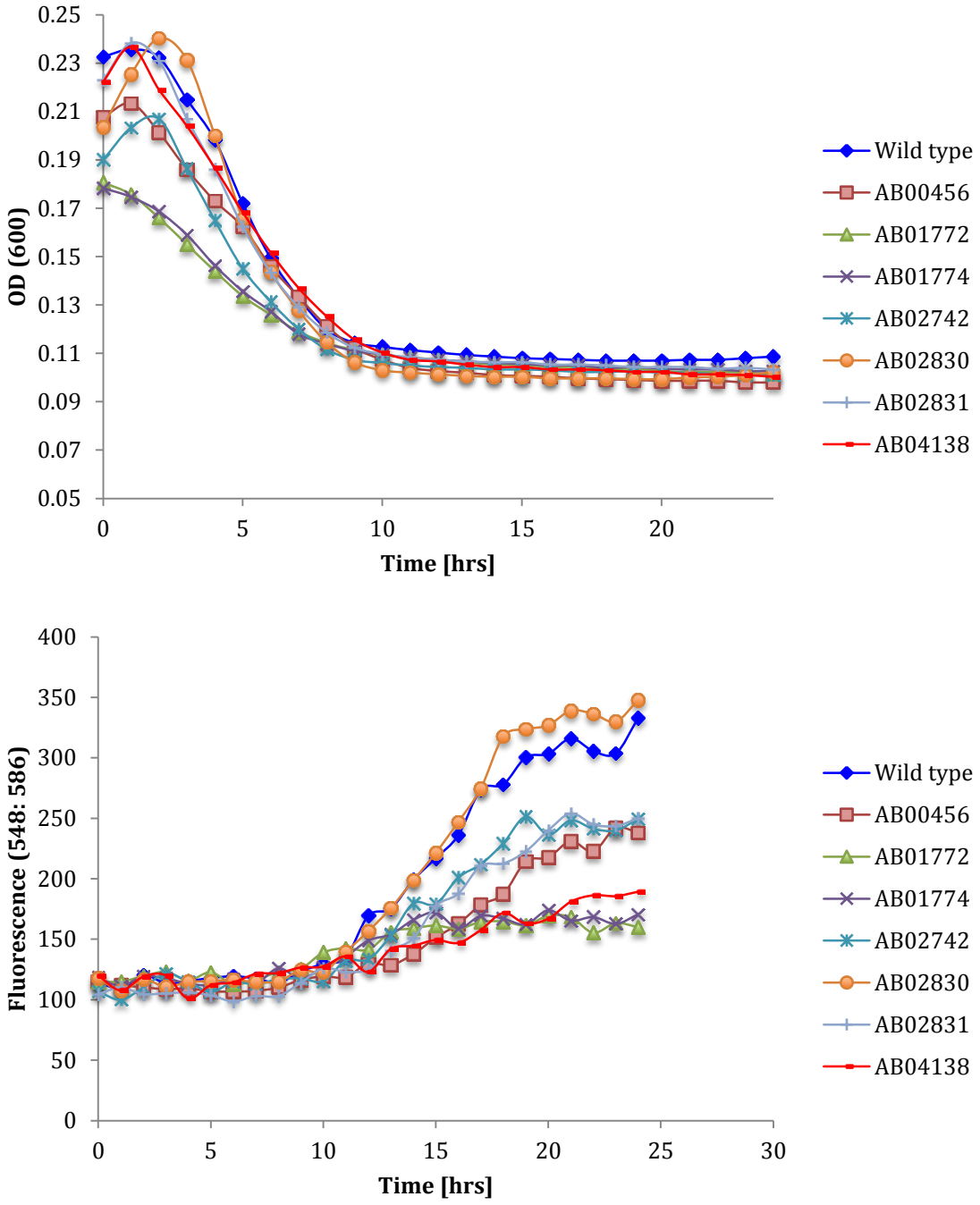

Figure 4. Effect of *B. bacteriovorus* on *Acinetobacter baumannii* mutants. Top panel shows culture density (OD_{600}) of wt and mutant *Acinetobacter baumannii* over time. Bottom panel shows fluorescence of *Bdellovibrio* population ($F_{548:586}$) over time. Each coculture was made in a 96-well microplate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato.

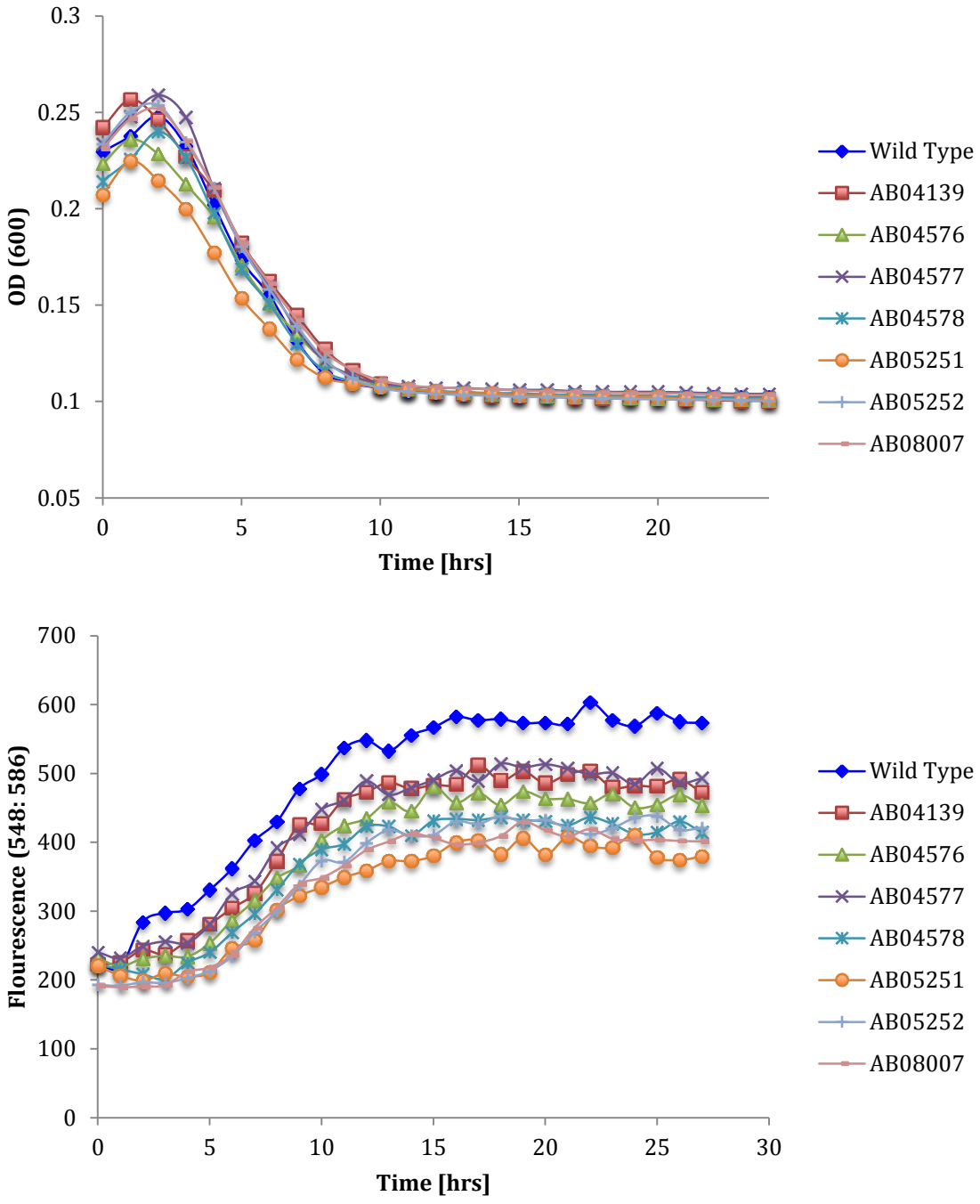

Figure 5. Effect of *B. bacteriovorus* on *Acinetobacter baumannii* mutants. Top panel shows culture density (OD_{600}) of wt and mutant *Acinetobacter baumannii* over time. Bottom panel shows fluorescence of *Bdellovibrio* population ($F_{548:586}$) over time. Each coculture was made in a 96-well microplate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato.

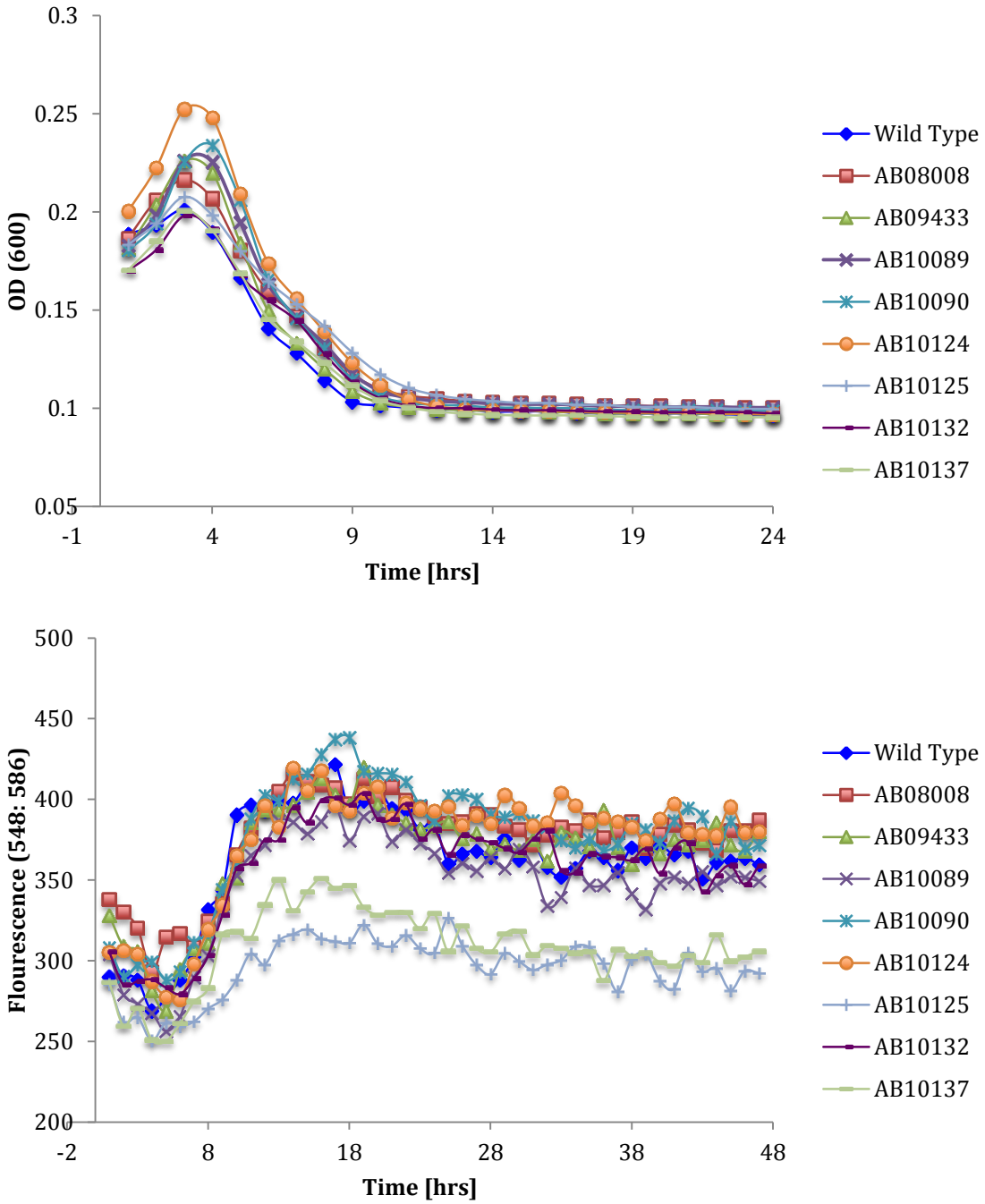

Figure 6. Effect of *B. bacteriovorus* predation on *Acinetobacter baumannii* mutants. Top panel shows culture density (OD_{600}) of wt and mutant *Acinetobacter baumannii* over time. Bottom panel shows fluorescence of *Bdellovibrio* population ($F_{548:586}$) over time. Each coculture was made in a 96-well microplate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato.

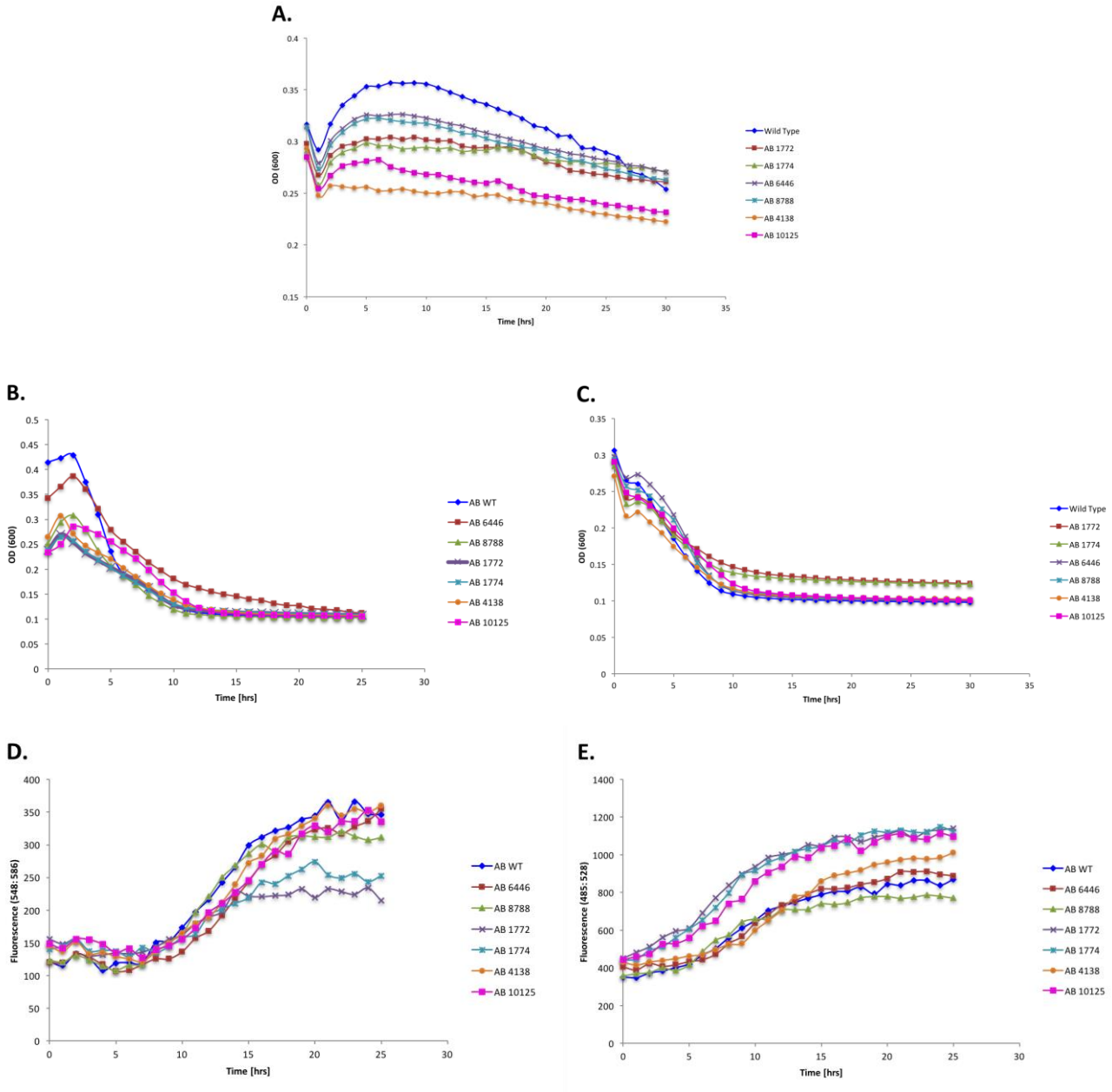

Figure 7. Second screen of *A. baumannii* predation resistant candidates. (**A.**) Each candidate cocultured with *B. bacteriovorus* without predator as a control (**B.**) Candidates cocultured with *B. bacteriovorus* 109J (**C.**) Candidates cocultured with *B. bacteriovorus* HD100 (**D.**) Candidates cocutured with *B. bacteriovorus* 109J expressing tdTomato and (**E.**) Candidates cocultured with *B. bacteriovorus* HD100 expressing GFP. Each coculture was made in a 96-well static plate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and HD100, and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato and green fluorescent protein, GFP.

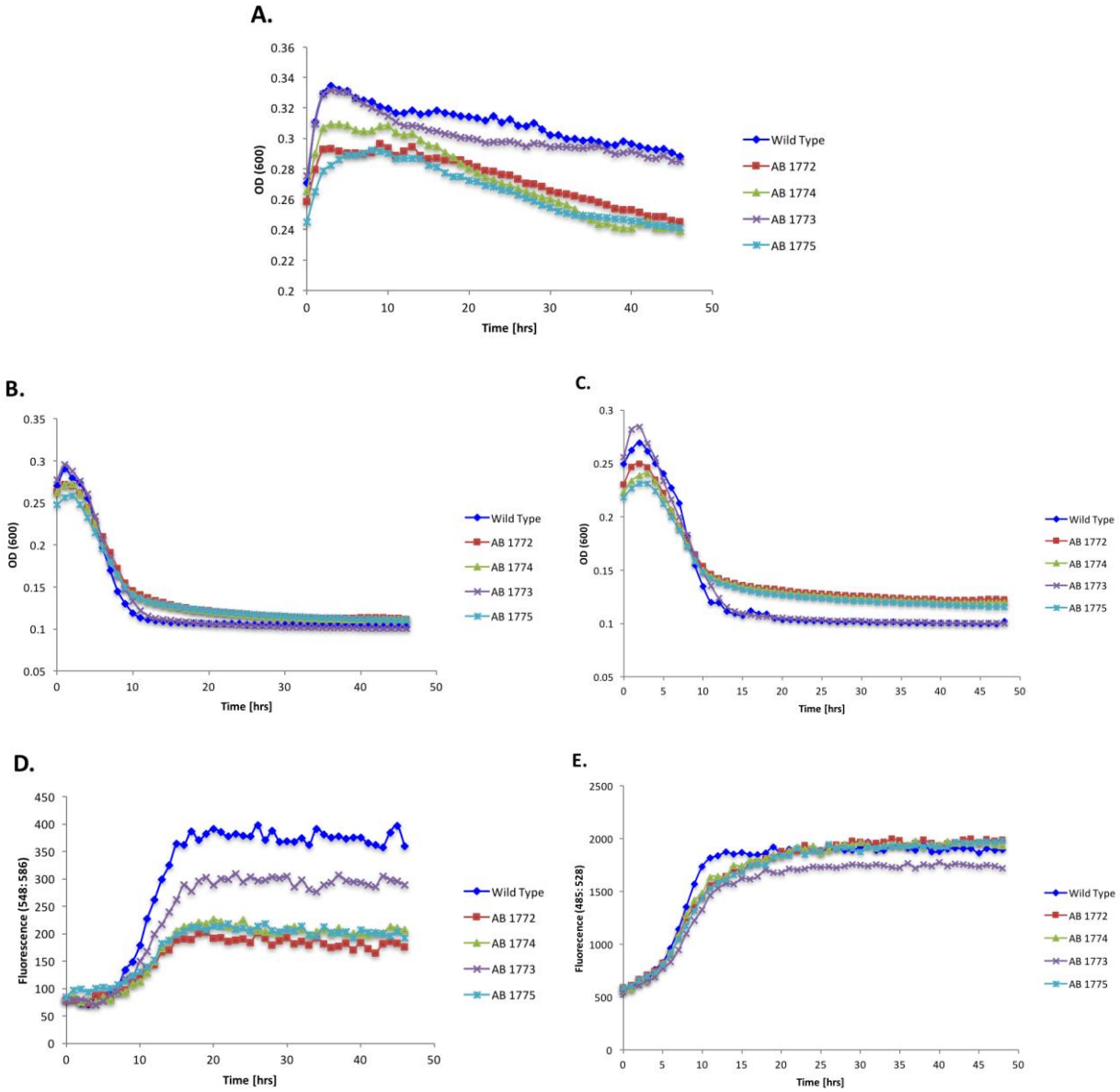

Figure 8. Predation of four different *A. baumannii* OmpA mutants. (**A.**) Each candidate cocultured with *B. bacteriovorus* without predator as a control (**B.**) Candidates cocultured with *B. bacteriovorus* 109J (**C.**) Candidates cocultured with *B. bacteriovorus* HD100 (**D.**) Candidates cocutured with *B. bacteriovorus* 109J expressing tdTomato and (**E.**) Candidates cocultured with *B. bacteriovorus* HD100 expressing GFP. Each coculture was made in a 96-well static plate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and HD100, and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato and green fluorescent protein, GFP.

Table 1. Average CFU enumeration for *Acinetobacter baumannii* mutants. 109J was used for both species. The values shown are averages for a triplicate experiment.

Bacteria tested	CFU log reduction following predation (24 hrs)	CFU log reduction following predation (48 hrs)	p-value
Acinetobacter			
A. baumannii WT 5075	3.37 ± 0.83	n.a.	n.a.
A. baumannii OmpA mutant 1772	0.47 ± 0.50	0.67 ± 0.09	0.01
A. baumannii OmpA mutant 1773	3.44 ± 0.42	n.a.	0.98
A. baumannii OmpA mutant 1774	0.27 ± 0.19	0.83 ± 0.08	0.02
A. baumannii OmpA mutant 1775	2.85 ± 0.11	n.a.	0.47

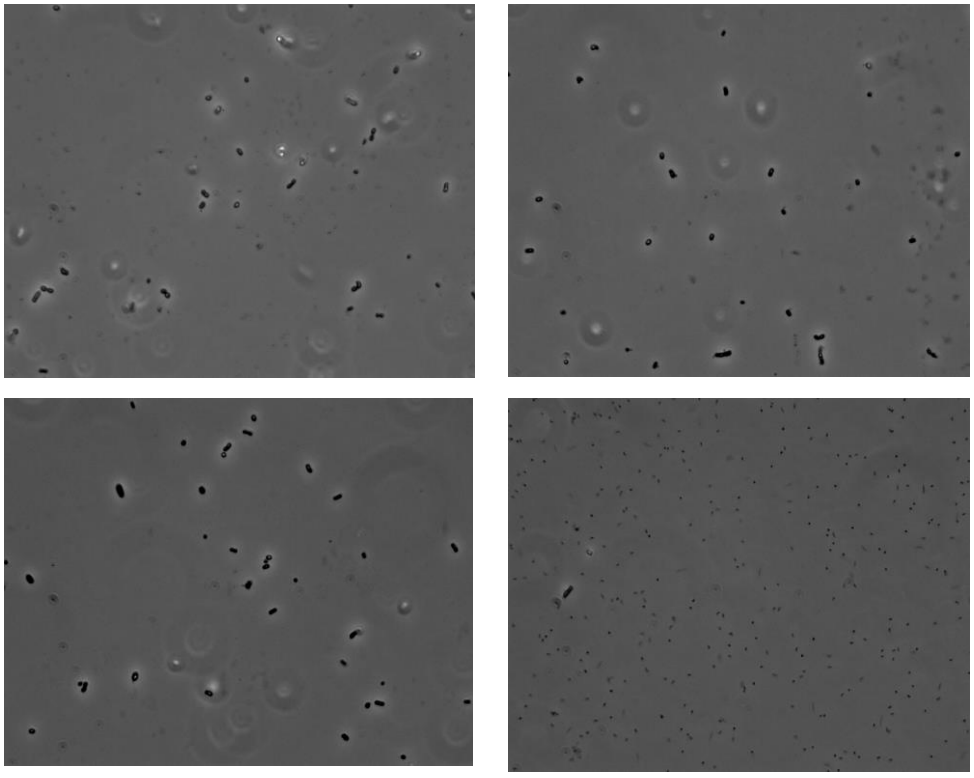

Figure 9. Microscopy images of AB05070 (wild type) cocultured with *B. bacteriovorus* at t0 (top left), t30 mins, (top right) t2 hrs (bottom left), and t24 hrs. (bottom right).

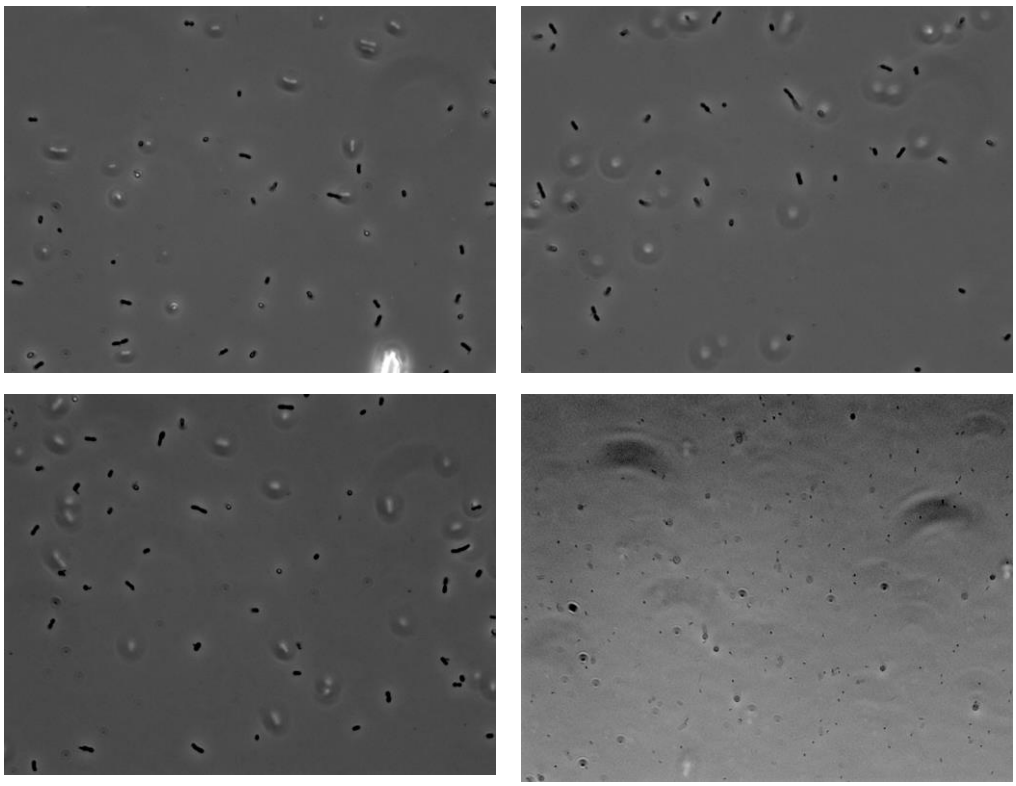

Figure 10. Microscopy images of AB01773 (wild type) cocultured with *B. bacteriovorus* at t0 (top left), t30 mins, (top right) t2 hrs (bottom left), and t24 hrs. (bottom right).

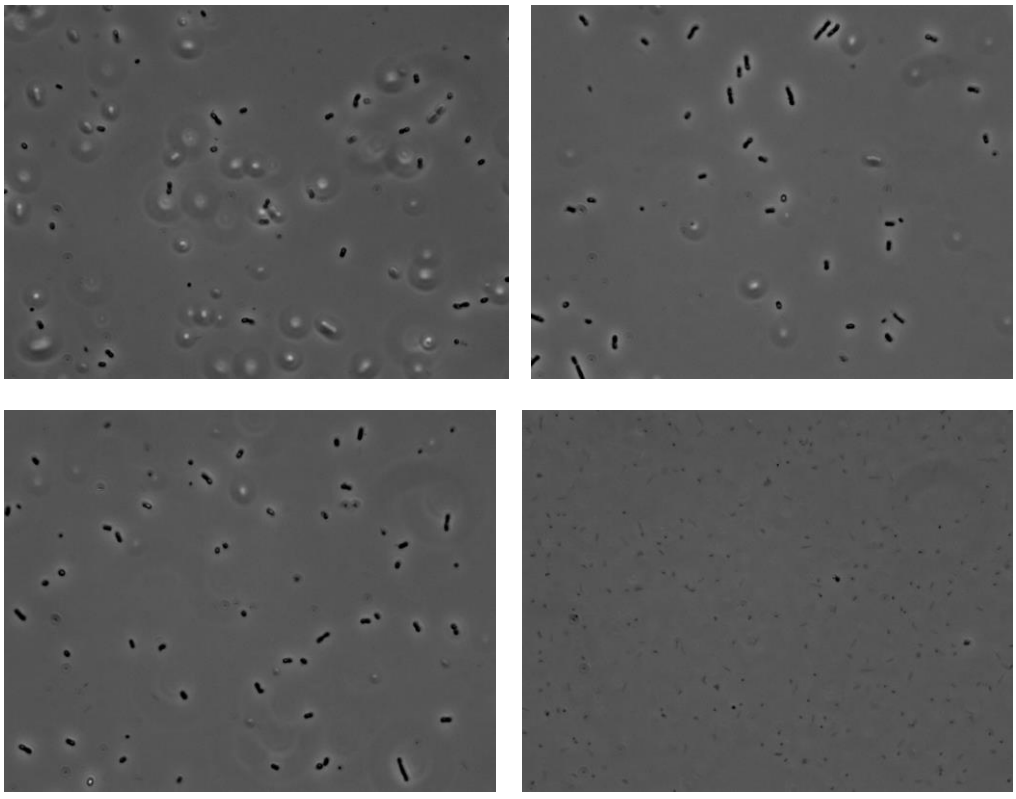

Figure 11. Microscopy images of AB01773 (wild type) cocultured with *B. bacteriovorus* at t0 (top left), t30 mins, (top right) t2 hrs (bottom left), and t24 hrs. (bottom right).

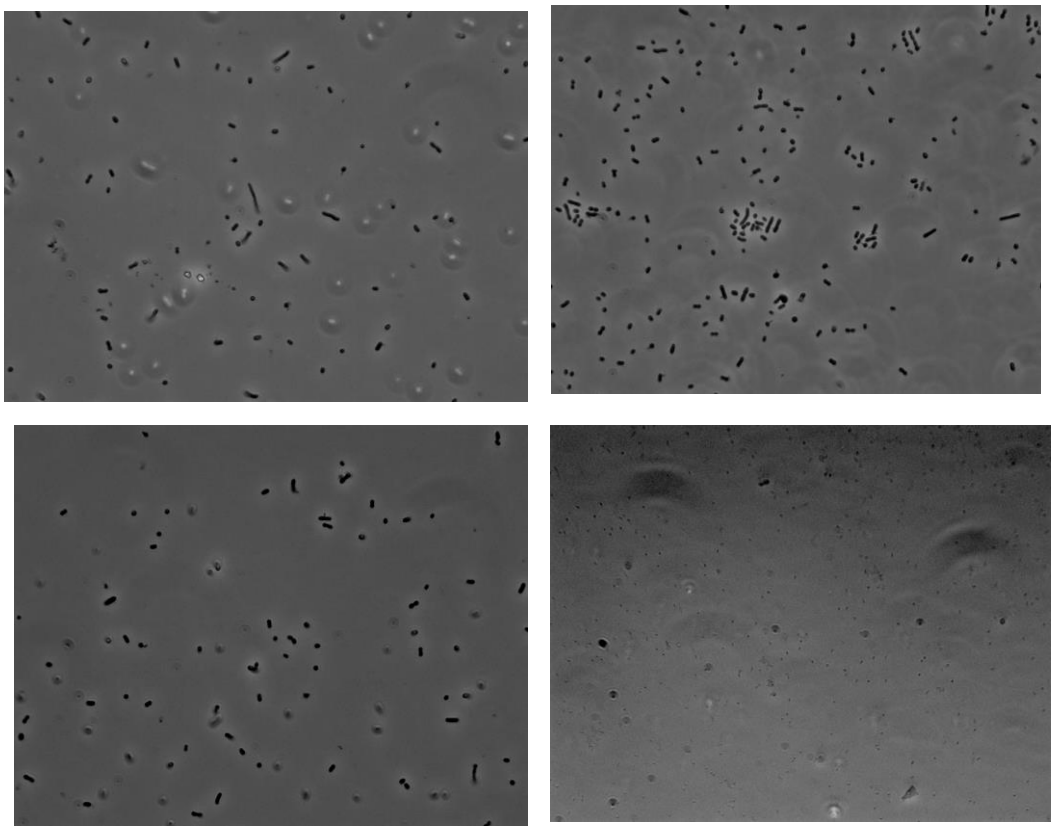

Figure 12. Microscopy images of AB01774 (wild type) cocultured with *B. bacteriovorus* at t0 (top left), t30 mins, (top right) t2 hrs (bottom left), and t24 hrs. (bottom right).

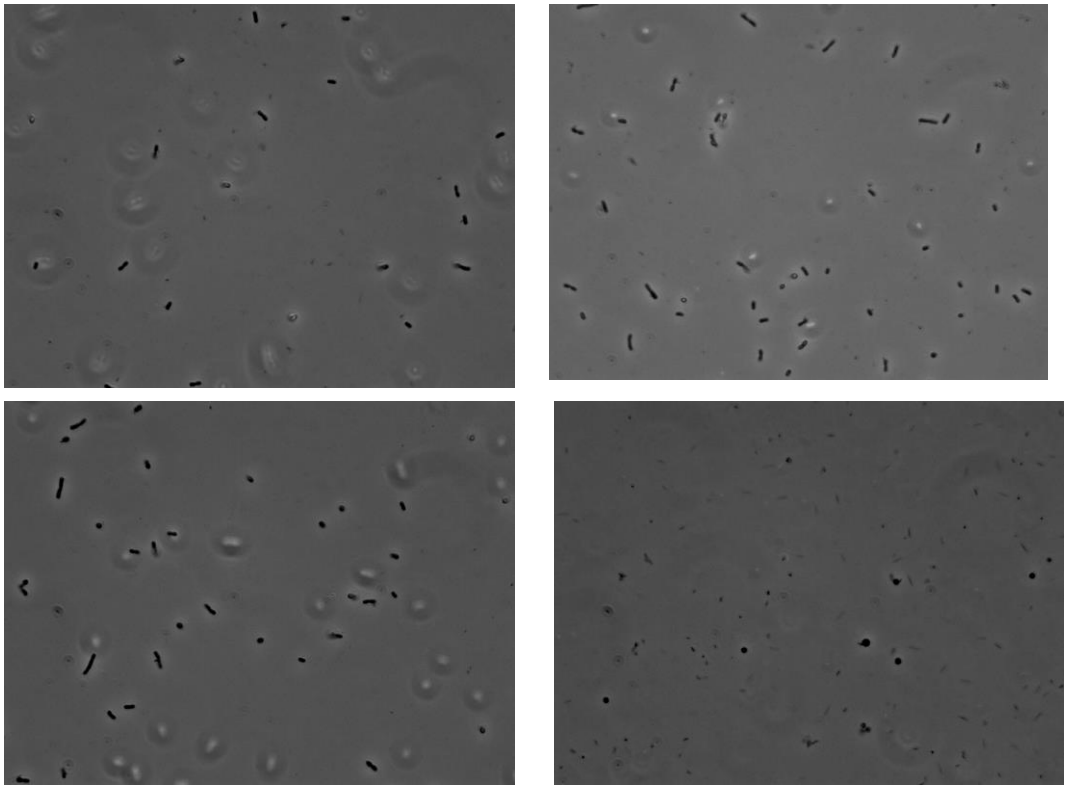

Figure 13. Microscopy images of AB01775 (wild type) cocultured with *B. bacteriovorus* at t0 (top left), t30 mins, (top right) t2 hrs (bottom left), and t24 hrs. (bottom right).

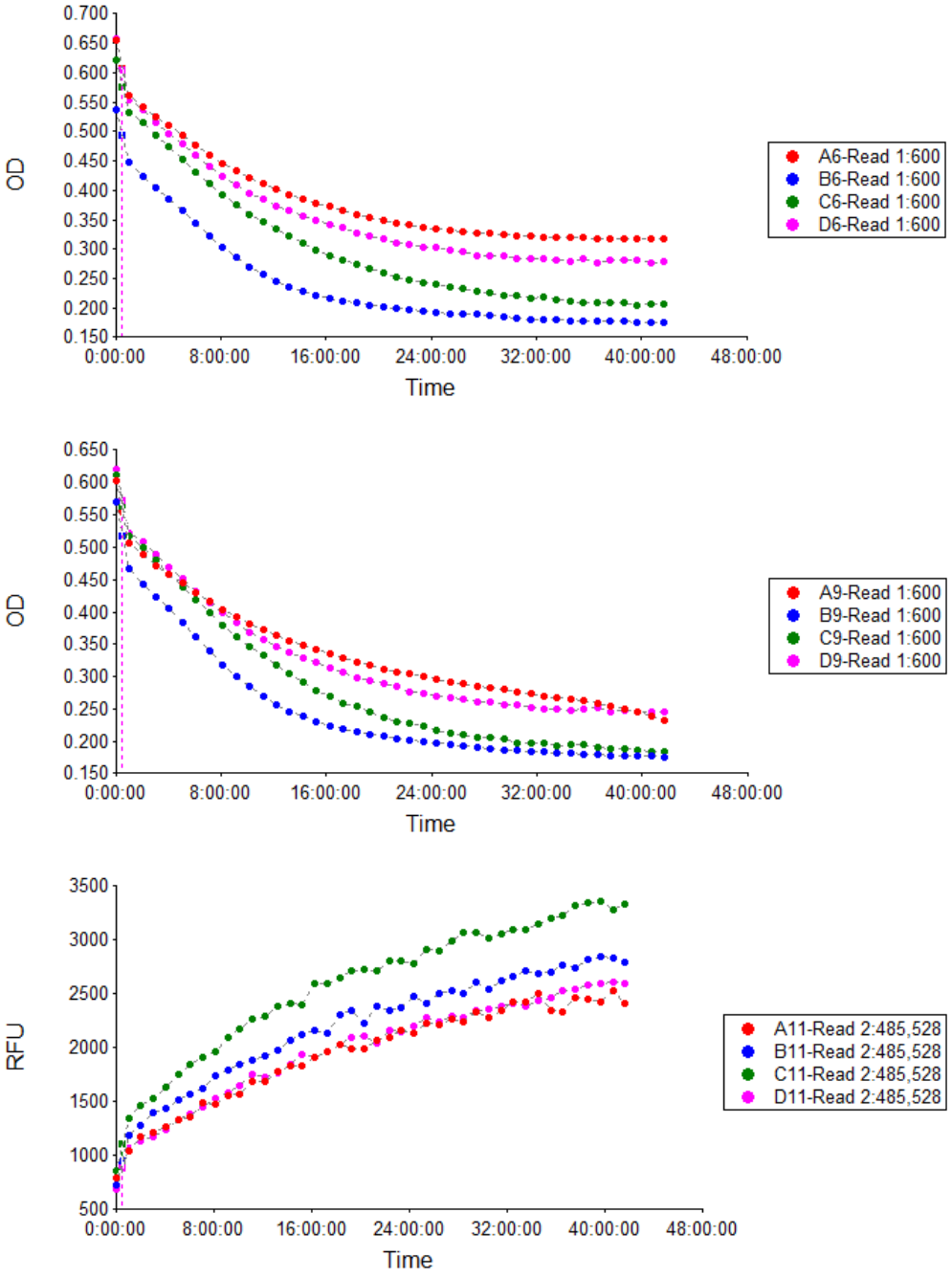

Figure 14. Predation of Pa14 (red), PaB2 (pink), PaA7 (green), and PaF2 (blue) with *B. bacteriovorus* 109J (top), and HD100 (middle), and GFP (bottom).

Table 2. Log reduction of *Pseudomonas aeruginosa* mutants cocultured with 109J and incubated at 30°C for 24 hrs.

Bacteria tested	CFU log reduction following predation (24 hrs)
Pseudomonas	
Ps. aeruginosa PA14	3.52
Ps. aeruginosa B2	3.61
Ps aeruginosa OmpA mutant A7	3.57
Ps. aeruginosa OmpA mutant F2	4.02

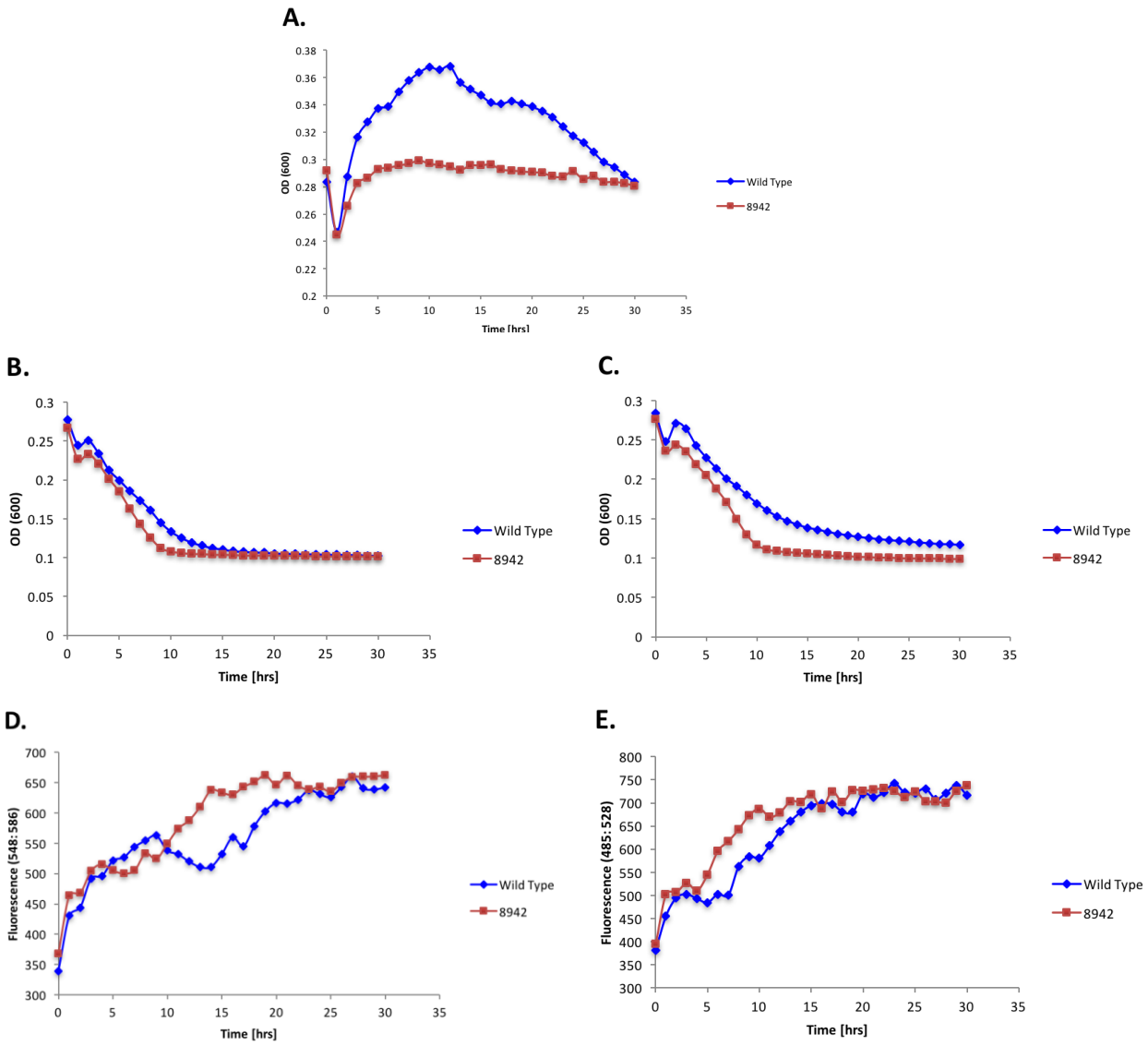

Figure 15. Predation by *B. bacteriovorus* on *Escherichia coli* OmpA mutant 8942. (**A.**) OmpA mutant cocultured with *B. bacteriovorus* without predator as a control (**B.**) Cocultured with *B. bacteriovorus* 109J (**C.**) Coocultured with *B. bacteriovorus* HD100 (**D.**) Cocutured with *B. bacteriovorus* 109J expressing tdTomato and (**E.**) Cocultured with *B. bacteriovorus* HD100 expressing GFP. Each coculture was made in a 96-well static plate and incubated at 30°C for 48 hrs in a microplate reader. Optical density was measured every hr for those wells containing 109J and HD100, and fluorescence at every hour for those wells containing the red fluorescent protein, tdTomato and green fluorescent protein, GFP.

Table 3. Log reduction of *Escherichia coli* mutants cocultured with 109J and HD100, separately, and incubated at 30°C for 24 hrs.

Bacteria tested	CFU log reduction following predation (109J)	CFU log reduction following predation (HD100)
Escherichia		
E. coli WT 7636	3.08 ± 0.34	3.58 ± 0.42
E. coli OmpA mutant 8942	3.86 ± 0.44	3.71 ± 0.15