WT and *ΔompA E.coli* compete in the same niche for colonization in the mouse intestine

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Abstract

Escherichia coli is known to contain several outer membrane porin proteins that potentially play a role in its ability to colonize its host. One of the most abundant is the Beta barrel outer membrane protein A, or ompA. To study its role in colonization, an ompA-deletion mutant of commensal *E.coli* MG1655 was constructed. The Δ*ompA* strain was administered to mice in a series of experiments to determine how the absence of *ompA* would affect the colonization of *E.coli* in the mouse intestine. The ΔompA mutant alone colonized in numbers equal to the wild type, supporting the idea that ompA is not vital to colonization. However, when WT and Δ*ompA E.coli* strains were administered simultaneously, WT outcompeted the Δ*ompA* by almost 4 log fold within 15 days of competition. To determine if the *ompA* mutant competes in the same niche as the WT, mice were associated with WT and then challenged with the ompA mutant. A second experiment was also done in reverse order. When WT was administered first followed by ompA, ompA was unable to colonize. Likewise, when *ompA* was administered first followed by WT, WT was unable to colonize. The results demonstrated that both strains compete in the same niche and once the niche is fully occupied by one strain, the other cannot colonize.

Introduction

Escherichia Coli is a ubiquitous gram-negative bacterium found in both the environment and in every living mammal (1). Despite *E.coli* being among the most studied microorganisms, its mechanisms for colonization remain unknown. To explore components of this bacterium that could be potential factors in its ability to colonize, we look at the basis of membrane functionality. The permeability of the outer membrane in microorganisms is greatly attributed to the presence of porin proteins. These beta-barrel proteins form large channels that cross the membrane, allowing passive diffusion of molecules in and out of the cell (2). This transport often leads to signaling that then enables a variety of functions in bacteria. From this, it could be estimated that porin proteins could have the potential to contribute to the colonization efficiency of *E.coli*.

Each outer membrane protein, or Omp, is responsible for unique functions within the cell and *E.coli* is known to contain many. OmpA, a vital protein for outer membrane stability and structure, is involved in many interactions between *E.coli* and host cells (3). This makes OmpA a protein of interest in studying its role in colonization. From this, we posed two questions: Is OmpA required for colonization? and, does mutant *ompA* compete in the same niche as the wild type? This information would contribute a small piece of the greater idea to mechanisms by which *E.coli* uses to colonize the intestine.

The competitive exclusion principle states that two of the same species cannot coexist in the same niche, because they are competing for the same resources (2). Following this theory, two strains of MG1655 *E.coli* would both compete in the same niche, therefore, could not stably coexist. However, would deletion of *ompA* cause the *E.coli* to become genetically distinct enough to compete in a different niche than the wild type? We predict that the theory of

competitive exclusion will apply to both strains of MG1655 *E.coli*, in that regardless of the mutation making the strains distinct, they are similar enough to compete in the same ecological niche.

To test this, we isolated a sample of commensal wild type *E.coli* MG1655 and constructed an ompA-deletion mutant. In a series of four experiments, the Δ*ompA* strain was administered to a different set of 6 mice independently, simultaneously with WT, and twice non-simultaneously with WT. Mice were treated with streptomycin water and fecal sample collections were diluted and plated on regulated intervals. These sets of experiments should determine if OmpA is vital for colonization and if the removal of *ompA* still allows the strain to compete in the same niche as the WT.

Methods

For the use of this study, the ΔompA mutant was constructed by a graduate student using the allelic replacement method described by Datsenko and Wanner and administered to mice on the appropriate day of each experiment (4).

To prepare the mice for experimentation, they were treated with streptomycin via water intake to clear out facultative anaerobes in the cecum and open a niche for *E. coli* to colonize. They were then fasted for 18 hours before association to WT or ΔompA *E. coli* MG1655. Approximately one gram of feces were collected from each mouse at 5 hours, 24 hours, and every following 48 hours for the duration of the experiments. Fecal samples were serially diluted in 1% tryptone and plated on MacConkey agar with appropriate antibiotics. After incubation at 37°C for 24-48 hours, the colony forming units were counted to determine the concentration of WT or ΔompA *E.coli* present.

Results

As seen in Figure 1, the deletion of *ompA* in commensal *E.coli* MG1655, Δ*ompA*, was successful in the intestinal monocolonization of 6 mice for the duration of the 7-day experiment. When a set of six mice were administered both Δ*ompA* and WT *E.coli* MG1655 simultaneously, as seen in Figure 2, both strains were able to colonize with WT showing a higher affinity for colonization. As seen in Figure 3, WT was administered to a set of 6 mice on Day 0, followed by Δ*ompA* administered on Day 9 of the 21-day experiment. WT successfully colonized for the entirety of the experiment, while Δ*ompA* colonized initially then was outcompeted by WT. As seen in Figure 4, the *E.coli* strains were administered in opposite order, resulting in WT being outcompeted by Δ*ompA*.

Figure 1. Monocolonization of ΔompA

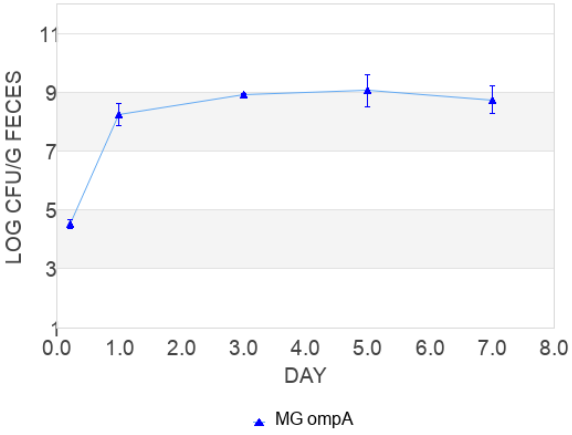


Figure 2. Competition of ΔompA vs. WT

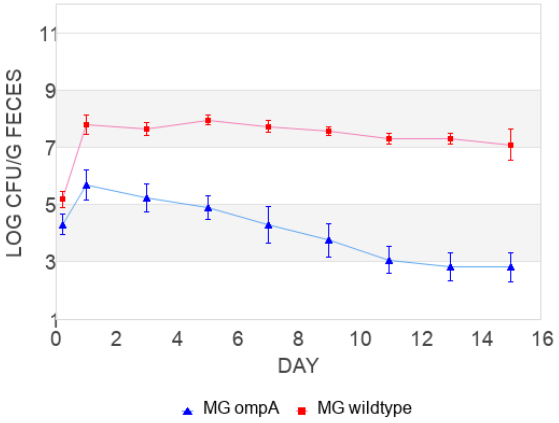


Figure 3. Challenge WT vs. ΔompA

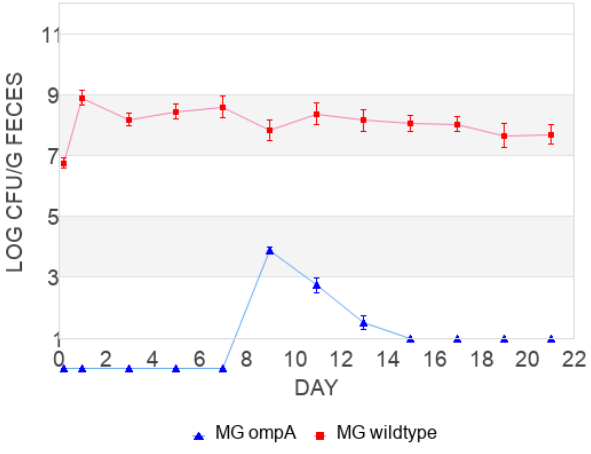
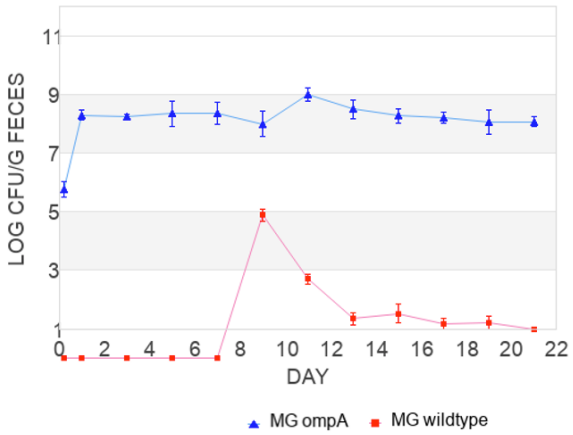


Figure 4. Challenge ΔompA vs. WT



Discussion

The success of Δ*ompA E.coli* MG1655 in the monocolonization experiment indicates that OmpA is not a vital outer membrane protein for *E.coli* colonizing the mouse intestine. This opened the possibility for further experimentation to explain the use and importance of OmpA. Knowing that Δ*ompA* can colonize independently and WT can colonize independently, the two were then put against each other to compete. While Δ*ompA* did colonize, it could not compete to the standard of which WT did. Meaning, OmpA is not crucial to colonization, but it is helpful for its efficiency. Knowing WT can outcompete Δ*ompA*, we then look at if they colonize the same niche or are independent of one another.

Although WT can outcompete Δ*ompA* in the competition, it is not so genetically advantaged that it can replace Δ*ompA* if Δ*ompA* has already colonized the niche in the challenge. Regardless of which strain was administered to the mice first in the challenge experiments, once the niche was fully occupied, the opposite strain could not effectively colonize thereafter. Both Δ*ompA* and WT could not colonize at the same rate when administered separately, because although they are genetically distinct, they remain within the same species and therefore cannot stably coexist. This supports the hypothesis that the competitive exclusion principle does apply, and both Δ*ompA* and WT *E.coli* MG1655 compete in the same niche.

These findings contribute to the larger picture of the outer membrane proteins in E. coli in its entirety. Further directions could be made to answer why Δ*ompA* has a disadvantage in competition even though it acts similar to WT in the challenge experiments. Could this be because OmpA provides greater resilience against bile salts or toxic substances in the intestine? Additionally, competition between pathogens and the microbiome supports the idea that probiotics can be used as a preventative measure (5). Applying this concept, could deletion of

ompA prevent E. coli from interacting with other microbial communities? And, if so, what could this mean for what we know about the microbiome and how it benefits its hosts? Answering these questions could not only contribute to the knowledge of E. coli, but also to the normal flora in its entirety.

References

(1) Conway, T., & Cohen, P. S. (2015). Commensal and pathogenic *escherichia coli* metabolism in the gut. *Microbiology Spectrum*, *3*(3). https://doi.org/10.1128/microbiolspec.mbp-0006-2014

(2) Willey, J. M., Sandman, K. M., Wood, D. H., & Prescott, L. M. (2022). *Prescott's microbiology*. McGraw Hill.

(3) Wang, Y. (2002). The function of ompa in escherichia coli. *Biochemical and Biophysical Research Communications*, *292*(2), 396–401.

(4) Datsenko, K. A., & Wanner, B. L. (2000). One-step inactivation of chromosomal genes in *escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences*, *97*(12), 6640–6645. https://doi.org/10.1073/pnas.120163297

(5) Meador, J. P., Caldwell, M. E., Cohen, P. S., & Conway, T. (2014). Escherichia coli pathotypes occupy distinct niches in the mouse intestine. *Infection and Immunity*, *82*(5), 1931–1938. https://doi.org/10.1128/iai.01435-13