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Profiling the response of individual gut microbes to free fatty acids (FFAs) found in human milk

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Abstract

Preterm infants have an immature intestinal environment featuring microbial dysbiosis. Human milk can improve the composition of the neonatal gut microbiome by supporting commensal species. Milk free fatty acids (FFAs) provide nutritional energy, participate in endogenous signaling, and exert antimicrobial effects. This study examined the growth of individual commensal and pathobiont microbes in response to unesterified unsaturated FFAs found in milk: oleic, linoleic, arachidonic, and docosahexaenoic acid. Select species of commensal and

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Ethics statement

This project did not use humans or biospecimens and is not considered human subjects research due to the Common rule regulations.

Disclosures

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Megan E. Waller: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Alyssa Gutierrez:** Writing – review & editing. **Taylor D. Ticer:** Writing – review & editing, Validation, Data curation, Conceptualization. **Janiece S. Glover:** Writing – review & editing, Data curation. **John E. Baatz:** Writing – review & editing, Resources. **Carol L. Wagner:** Writing – review & editing, Resources, Conceptualization. **Melinda A. Engevik:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Katherine E. Chetta:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Appendix A. Supplementary data

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pathobiont genera (*Bifidobacterium, Lactobacillus, Streptococcus*, *Staphylococcus, Enterococcus*, *Acinetobacter*, *Pseudomonas*, *Escherichia*, and *Klebsiella)* were cultured with FFAs. The growth of all commensals, except for L. *johnsonii*, was significantly inhibited by the highest concentration (1 %) of all FFAs. *L. johnsonii* was only inhibited by arachidonic acid. In contrast, suppression of pathobionts in response to FFAs was less pronounced. Higher concentrations (0.1 %, 1 %) of docosahexaenoic acid significantly inhibited the growth of five of eight pathobionts. Meanwhile, for oleic, linoleic, and arachidonic acid, only two of eight pathobionts were significantly affected. Intriguingly, the effects for these FFAs were highly complex. For example, *S. agalactiae* growth was enhanced with 1 % oleic acid but suppressed at 0.01 %; however, the effects were directionally opposite for linoleic acid, *i.e.*, suppressed at 1 % but enhanced at 0.01 %. Our genome analyses suggest that pathobiont survival may be related to the number of gene copies for fatty acid transporters. Overall, the effect of FFAs was dose-dependent and species-specific, where commensal growth was broadly inhibited while pathobionts were either unaffected or exhibited complex, bi-directional responses.

Keywords

Human breast milk; Diet; Formula; *Bifidobacterium* ; *Lactobacillus* ; *Enterobacter* ; *Klebsiella* ; *Enterococcus* ; Pathogens; Premature infant; Microbiome; Intestine; Free fatty acid

1. Introduction

Human milk fat contains an array of nutritive, neuroprotective, and immunological properties. The fat portion in human milk spontaneously undergoes lipolysis after expression, leading to increased free fatty acids (FFAs) such as oleic acid, linoleic acid, docosahexaenoic acid, and arachidonic acid. These FFAs are used as fuel, but they also function as highly potent signaling molecules that possess both inflammatory and anti-inflammatory properties (Khor et al., 2020; Ramiro-Cortijo et al., 2020). More recently, a growing body of evidence has highlighted the complex interactions between FFAs and the gut microbiome. Several dietary fatty acids have been found to affect the composition of the gut microbiota in both adult humans and mice (Miyamoto et al., 2019; Mujico et al., 2013; Patterson et al., 2014; Schoeler et al., 2023; Xu et al., 2022). We believe that the FFAs released from milk triglycerides may affect microbial growth and thereby modulate the neonatal microbiome. Importantly, unsaturated fatty acids, as compared to saturated fatty acids, have been shown to carry potent antibacterial properties (Kabara et al., 1972). We suspect that unsaturated fatty acids in human milk may exert antimicrobial effects on pathogenic bacteria in the neonatal gut.

Considering the mounting evidence that the microbiome influences neonatal health, we aimed to investigate the impact of FFAs on the growth of several commensal and pathobiont species that are prevalent in the infant gut. For this study, we selected four highly prevalent fatty acids in human milk: oleic acid, linoleic acid, docosahexaenoic acid, and arachidonic acid. Oleic acid is consistently found to be the most abundant of all fatty acids in breast milk, and linoleic acid is the dominating polyunsaturated fatty acid (PUFA) (Sánchez-Hernández et al., 2019a; Sánchez-Hernández et al., 2019b; Shan et al., 2020; Simon Sarkadi

et al., 2022). Docosahexaenoic acid (DHA) and arachidonic acid (ARA) were additionally chosen as they are clinically relevant long-chain PUFAs and, more importantly, they are frequently added as supplements in products such as infant formulas (Bobiński & Bobińska, 2022; Bousset-Alféres et al., 2022; Calvo-Lerma et al., 2022; Sánchez-Hernández et al., 2019a; Sánchez-Hernández et al., 2019b). We studied the effects of these FFAs in mono-cultures and examined the bacterial genomes for fatty acid transporters using a publicly available web-based library (Adlerberth & Wold, 2009; Arboleya et al., 2012; Aujoulat et al., 2014). We hypothesized that higher concentrations of unsaturated FFAs would broadly promote commensal growth and inhibit or not affect pathobiont growth. Consistent with this concept, this study identified differential responses of these pathobionts and commensals in the presence of free fatty acids.

2. Methods

2.1. Bacterial culture conditions

We selected six commensal and eight pathobiont species. These species were chosen for being a predominant species in either breast milk or infant feces or for being involved in neonatal sepsis or risk for necrotizing enterocolitis (NEC), a severe intestinal morbidity that occurs in the preterm population (Albesharat et al., 2011; Chen et al., 2020; Coleman et al., 2023; Engevik et al., 2022; Greenfield et al., 2024; Gupta, 2002; Haarman & Knol, 2006; Hufnagel et al., 2007; Jiménez et al., 2008; Junick & Blaut, 2012; Lackey et al., 2019; Lee & O'Sullivan, 2010; Luck et al., 2021; Makino, 2018; Moles et al., 2020; Mukherjee et al., 2021; Nagpal et al., 2017; Oh et al., 2018; Patel & Denning, 2015; Paveglio et al., 2020; Pillay et al., 2024; Rinne et al., 2005; Seferovic et al., 2020; Seki et al., 2021; Stafford et al., 2018; Tsunoda et al., 2018; Ward et al., 2016; Yan et al., 2021; You et al., 2020; Zhang et al., 2020). Information for bacterial strain and culture media used in this study are summarized in Table 1. Commensal *Bifidobacterium* and *Lactobacillus* species were grown on De Man-Rogosa-Sharpe (MRS) agar plates, while the pathobionts *Klebsiella, Staphylococcus, Streptococcus, Pseudomonas, Escherichia, Acinetobacter*, and *Enterococcus* species were grown on Brain Heart Infusion (BHI) agar plates. Single colonies of all bacterial species were grown in rich media, MRS broth for commensals and BHI broth for pathobionts, overnight. Commensals were grown at 37 °C in an anaerobic chamber (Anaerobe Systems, AS-150) and pathobionts were grown at 37 °C in an incubator. After 24 h of growth, bacteria were inspected for morphology to ensure absence of contamination using light microscopy at 40× magnification (Motic AE 2000). The optical density was measured at 600 nm (OD_{600nm}) on a Spectronic 200 Spectrophotometer (ThermoFisher). Bacterial cultures were adjusted to an OD_{600nm} of 0.1 in ZMB1, a chemically defined media optimized for gut bacterial strains, supplemented with glucose (Table 1).

To assess bacterial growth in response to oleic acid, linoleic acid, docosahexaenoic acid, and arachidonic acid, we generated ZMB1 with 0.01 %, 0.1 %, and 1 % FFAs. All solutions were adjusted to pH 7 prior to the experiment. These concentrations are in the range of freshly expressed milk samples up to 48 h in storage (<1 % to 1–4 % FFA per g fat at 48 h) (Lavine & Clark, 1987). These concentrations were secondarily chosen because they supported the growth of the bacteria while not affecting the optical density of samples.

Growth was recorded after aerobic (pathobionts) and anaerobic (commensal) incubation as described above for 20 h, and then they were measured using OD_{600nm} on a Biotek Synery H1 plate reader. Anaerobic conditions were selected for the growth of commensal bacteria to mimic the oxygen-deprived environment of the intestinal lumen. Aerobic conditions were selected to optimize the growth of the pathobionts and simulate pathological states where oxygen levels are elevated, such as during inflammation or epithelial disruption. The experiments were each performed two separate times in triplicate.

2.2. Genome analysis

Bacterial transporters involved in free fatty acid uptake were examined in the selected bacteria as previously described (Engevik et al., 2022). The bacterial transporters were identified in the KEGG database (KEGG: Kyoto Encyclopedia of Genes and Genomes, https://www.genome.jp). The KEGG IDs for fadL (K06076) and atoE (K02106) were then searched in the IMG database (Integrated Microbial Genomes Database, https://img.jgi.doe.gov) for the following bacterial species: *B. bifidum* (77 genomes), *B. longum* (291 genomes), *B. infantis* (48 genomes), *B. breve* (98 genomes), *L. johnsonii* (24 genomes), *L. rhamnosus* (153 genomes), *S. epidermidis* (178 genomes), *S. agalactiae* (709 genomes), *E. coli* (4410 genomes), *P. aeruginosa* (973 genomes), *A. baumannii* (1363 genomes), *E. faecalis* (423 genomes), *K. aerogenes* (140 genomes), and *K. pneumoniae* (1224 genomes). Genomes that harbored at least one gene copy of a KEGG ID were considered to possess free fatty acid transporters. To determine how many bacterial strains from a given species possessed free fatty acid transporters, we used the following equation:

$$Bacteral\ genomes\ harboring\ a\ KEGG\ ID(\%) = \frac{(Bacterial\ \ genomes_{+KEGG\ ID})}{(Bacterial\ \ genomes_{total})} * 100 \quad (1)$$

2.3. Statistics

The data are shown as mean ± standard deviation (stdev). One-way Analysis of Variance (ANOVA) was used to determine the significance between pairwise comparisons (Fig. 1–8). To calculate statistics for our ANOVA and to generate the graphs seen below, we used GraphPad Prism (GraphPad Software, Inc. La Jolla, CA). On each graph, an "*" indicates a $p < 0.05$.

3. Results

We systematically examined bacterial growth in the presence of various FFAs of both beneficial commensal and detrimental pathobiont microbes (Table 1). Commensal species included *Bifidobacterium* and *Lactobacillus* species, which help establish a healthy gut microbiome in early life. We examined the growth of four species of *Bifidobacterium* (*B. bifidum* ATCC 11863, *B. longum* ATCC 55813, *B. infantis* ATCC 15697, and *B. breve* ATCC 15698) and two species of *Lactobacillus* (*L. johnsonii* ATCC 33200 and L. *rhamnosus* ATCC 53163) in response to the unsaturated fatty acids oleic acid, linoleic acid, arachidonic acid, and docosahexaenoic acid at 0.01 %, 0.1 %, and 1 % concentrations. Oleic acid at concentrations of 0.1 % and 1 % significantly inhibited the growth of all species

except for L. *johnsonii* (Fig. 1A–F). Interestingly, oleic acid at the lower concentrations of 0.01 % and 0.1 % enhanced the growth of L. *johnsonii* (Fig. 1E). The effects of linoleic acid were very similar to that of oleic acid, where bacterial growth was inhibited at concentrations of 0.1 % and 1 % for all species except for *L. johnsonii* (Fig. 2A–F). Arachidonic acid inhibited the growth of all six species at the highest concentration of 1 %, but the growth of *B. longum* and *B. bifidum* were promoted at the lowest concentration of 0.01 % (Fig. 3A–F). The results for docosahexaenoic acid mirror those of oleic and linoleic acid, where the growth of all species except L. *johnsonii* was inhibited at 0.1 % and 1 % (Fig. 4A–F). Similar to oleic acid, docosahexaenoic acid at the intermediate concentration of 0.1 % enhanced the growth of *L. johnsonii* (Fig. 4E). These data suggest that higher concentrations of FFAs tested at 0.1 % or above generally suppressed the growth of commensal species.

To address how pathobionts grow in the presence of FFAs, we studied the growth of *S. epidermidis* ATCC 51025, *S. agalactiae* ATCC 13813*, E. coli* K12*, P. aeruginosa* CB1, *A. baumannii* ATCC 747*, E. faecalis* ATCC 29212, *K. aerogenes* NCMB 10102, and *K. pneumoniae* CB1. First, we examined the response of these pathobionts to oleic acid (Fig. 5A–H), and interestingly only two out of the eight species were affected by oleic acid. *S. agalactiae* demonstrated significantly elevated growth at 1 % but significantly decreased growth at 0.01 % (Fig. 5B). *E. faecalis* had inhibited growth at 0.1 % and 1 % (Fig. 5H). The results for linoleic acid (Fig. 6A–H) were remarkably similar to oleic acid, where the growth of only two species, *S. agalactiae* and *E. faecalis*, was altered. *S. agalactiae* had significant elevated growth at 0.1 % linoleic acid but was inhibited at 1 % (Fig. 6B). Similar to oleic acid, linoleic acid inhibited *E. faecalis* at 1 % (Fig. 6H). The results for *S. agalactiae* (group B strep) are particularly of interest because it is a well-established causative agent for newborn sepsis and meningitis (Lohrmann et al., 2023). Arachidonic acid generally had no effect on the growth of the pathobionts (Fig. 7A–H) except for *S. agalactiae* which showed promoted growth at 1 % but diminished growth at 0.1 % (Fig. 7B) and *A. baumanii* which was inhibited at the lower concentrations of 0.01 % and 0.1 % (Fig. 7E). The results for docosahexaenoic acid were strain-dependent (Fig. 8A–H). *P. aeruginosa, K. pneumoniae*, and *K. aerogenes* were unaffected, whereas growth of the other five species was inhibited in the presence of 0.1 % and 1 % docosahexaenoic acid (Fig. 8A–H). These findings indicate that higher concentrations of FFAs at 0.1 % or higher did not affect the growth of most pathobiont species, with a few species showing improved growth in the presence of FFAs.

Finally, we performed a genome analysis and correlation analysis of our pathobiont species to evaluate which of our species genomes had at least one copy of bacterial transporters involved in the uptake of FAs (supplemental file). We found that of the pathobionts, *K. pneumoniae* and *K. aerogenes* possess the transporter fadL, while *E. coli, P. aeruginosa*, and *A. baumannii* possess both fadL and atoE. Only one commensal species, *B. infantis*, possessed the atoE transporter. There were positive correlations between the percentage of genomes containing the fadL transporter and bacterial growth with arachidonic acid and linoleic at 1 %. There was also a correlation between the growth in the presence of 1 % ARA and atoE. However, there were no correlations between oleic acid or docosahexaenoic acid and the percentage of the genome containing the transporters atoE or fadL.

4. Discussion

The goal of this study was to examine how individual microbes respond to major unsaturated FFAs found in human milk. The results of this study could have important implications for both the milk and preterm infant gut microbiome. Human milk has been shown to have a unique microbiome profile which helps to seed the newborn intestine and is a major driver of the neonatal gut microbiome (Parra-Llorca et al., 2018; Sugino et al., 2021). Microbial sharing has numerous neuro-developmental benefits for the offspring (Parra-Llorca et al., 2018). Milk that is pumped, and not directly breastfed, is also known to change in storage and contains increased free fatty acids due to the presence of milk lipases (Berkow et al., 1984). FFAs are antimicrobial, and the bacterial colony content of expressed milk has been shown to decrease after 5 days in the refrigerator (4 °C) (Desbois & Smith, 2010; Sosa & Barness, 1987). Unsaturated fatty acids have more potent bactericidal effects than saturated FFAs, especially against gram-negative species (Das, 2018). We selected four unsaturated free fatty acids based on their prevalence in human milk, and we hypothesized that they would promote commensal bacteria but inhibit pathobiont growth. However, we found highly species-specific and concentration-dependent effects of various FFAs. In general, most commensals were suppressed by FFAs at the highest concentration of 1 %. However, suppression was not seen at the lowest concentration of 0.01 %, and some commensal species showed improved growth such as L. *johnsonii, B. longum*, and *B. bifidum*. Interestingly, pathobionts exhibited more diverse responses to the FFA supplementation. Most pathobionts were unaffected when exposed to any concentration of oleic acid, linoleic acid, and arachidonic acid, whereas many pathobionts were affected by docosahexaenoic acid. For the few pathobionts significantly affected by at least one FFA, such as *S. agalactiae*, growth was altered in both directions depending on concentration and FFA type. Altogether, our data confirm that individual FFAs can differentially modulate microbial populations. However, these interactions are incredibly complex due to species-specific and concentration-dependent effects.

Recent clinical studies have observed microbiome changes with pumped *versus* fresh milk. A recent study by Fehr et al. demonstrated that breastfeeding term infants harbored more of their mother's milk microbiome as measured in their stool than infants who received pumped milk (Fehr et al., 2020). Interestingly, the study revealed that *Bifidobacterium* species were less prevalent in infants whose mothers pumped their milk, although no difference in relative abundances was detected. We speculate that the extended storage of human milk, through the accumulation of released FFAs, may impair the ability of commensal bacteria in the milk microbiome to seed the preterm infant gut properly.

Certain commensal species, specifically the *Bifidobacterium* and *Lactobacillus* species, have significant immunological and digestive benefits for infants (Adlerberth & Wold, 2009; Arboleya et al., 2012; Aujoulat et al., 2014). Bifidobacteria and Lactobacilli are beneficial to preterm infants, too, as they are associated with lower rates of necrotizing enterocolitis (NEC) (Hagen & Skelley, 2019; Seghesio et al., 2021). Pathobionts such as *Klebsiella* and *E. coli*, as well as other proteobacteria, have been shown to bloom before NEC, indicating that a shift to pathobionts and a reduction in commensals may be a critical preceding risk factor for NEC (Pammi et al., 2017).

In contrast to our initial hypothesis that the unsaturated FFAs would enhance commensal growth, our results demonstrate that these FFAs broadly inhibit the growth of the commensals at high concentrations. This aligns with the notion that the administration of fresh human milk, which contains low concentrations of FFAs, would best support the viability of commensal milk microbes that influence the infant microbiome. Our results indicate that FFAs, even at seemingly minimal concentrations of 1 %, can drastically affect bacterial growth. We selected specific FFA concentrations based on the amount of FFAs in freshly expressed and stored milk (Lavine & Clark, 1987). Milk contains lipases that continuously hydrolyze triglycerides and generate FFAs, even in freezer storage. FFAs progressively accumulate with storage time, from less than 1 % to up to 8 % in refrigerated conditions at 24 h (Lavine & Clark, 1987). Our higher levels of FFAs mirror the amount of FFAs found in stored milk. Although the majority of the FFAs are absorbed in the small intestine (Lönnerdal, 2003), FFAs can reach the colon and are found in the stool at low levels (Shen et al., 2021). In this study, our lower concentrations of FFAs are roughly correlated with levels observed in stool. We chose to examine bacterial growth over 20 h due to the rapid response of the gut microbiome to the diet. Animal and human studies have demonstrated that the gut microbiome can dramatically shift its composition in a 24-h period in response to diet (David et al., 2014; Turnbaugh et al., 2009; Wu et al., 2011). Therefore, we believe that the concentrations of FFAs and the timeline used in our *in vitro* studies are applicable to changes that may be seen in mammalian hosts. Our data indicates that high levels of FFAs are inhibitory to commensal bacteria over 20 h of growth. These data suggest that fresh milk, which contains lower levels of FFAs, may help promote commensal microbes in the preterm gut (Berkow et al., 1984; Hamosh et al., 1996).

FFAs can inhibit bacteria through various mechanisms, but it is generally thought that the dominant antimicrobial function of FFAs is through membrane disruption. FFAs are amphipathic and can accumulate in bacterial membranes (Desbois & Smith, 2010; Yoon et al., 2018). High doses of FFAs destabilize the bacterial membrane, leading to increased cell permeability and cell lysis (Carson & Daneo-Moore, 1980; Casillas-Vargas et al., 2021). In addition to cell lysis, some FFAs exert antimicrobial effects by inhibiting key pathways in bacteria. For example, oleic acid, linoleic acid, and arachidonic acid inhibit bacterial glucosyltransferases that mediate glucan production in *Streptococcus* species. Another study found that linoleic acid inhibited bacterial enoylacyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, in *Staphylococcus aureus* (Zheng et al., 2005). FFAs have also been shown to disarm bacterial pathogens of their virulence factors, including biofilm production, swarming motility, and virulence expression (Borreby et al., 2023; Kumar et al., 2020; Yoon et al., 2018). We speculate that the FFAs in breast milk likely perform similar effects on bacteria in the infant gut.

When performing the genome analysis, the commensal species lacked gene copies for bacterial transporters involved in the uptake of FAs except for *B. infantis*, which possessed the transporter fadL. This suggests that while *B. infantis* may utilize FFAs, this is uncommon among other commensal bacteria. Another study found that unsaturated FFA concentration in milk was negatively correlated with *Bifidobacterium* but positively correlated with *Streptococcus* species, which mirrors the data presented herein (Quin & Gibson, 2020).

The genome analysis of our eight tested pathobionts revealed that five had at least one copy of the bacterial transporter fadL and three had both fadL and atoE. There was also a positive correlation associated with the bacteria harboring fadL and growth with 1 % oleic acid or 1 % arachidonic acid. These results suggest that the presence of FA transporters may equip the pathobiont species with the ability to grow efficiently in the setting of high FFAs. Interestingly, fatty acid transporters may also impart antibacterial resistance for some bacteria (Casillas-Vargas et al., 2021). Our data further support the notion that pathobionts whose genomes lack fatty acid transporters, such as *S. agalactiae* and *E. faecalis*, are more likely to be suppressed in high concentrations of FFAs. In this study, we did not directly test the expression of these transporters in response to FFAs. Similar to other nutrient transporters, we speculate that certain FFAs can modulate the expression of bacterial fatty acid transporters. This might explain the complex, bi-directional results we see for *S. agalactiae*, where 1 % oleic acid enhanced growth but 1 % linoleic acid suppressed growth. In the future, we suspect a close examination of bacterial transporters in response to milk components would be informative.

Overall, these data suggest that unsaturated FFAs in human milk can shape the microbiome's species-level constituents in human milk or the neonatal intestine. Our findings additionally support the idea that fresh human milk, which contains lower FFA contents compared to milk that has been previously stored, may be best for promoting infant intestinal health as it relates to the gut microbiota. We recognize that our approach is limited by strictly utilizing *in vitro* methods, and we anticipate that future mouse studies will provide more insightful findings on the complex relationship between milk FFAs and early-life gut microbiota. Animal models will also shed light on the physiological consequences of FFA-microbiota interactions. We hope that these findings will set the stage for further research that will optimize feeding guidelines for preterm infants, who are highly susceptible to detrimental intestinal diseases such as necrotizing enterocolitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data will be made available on request.

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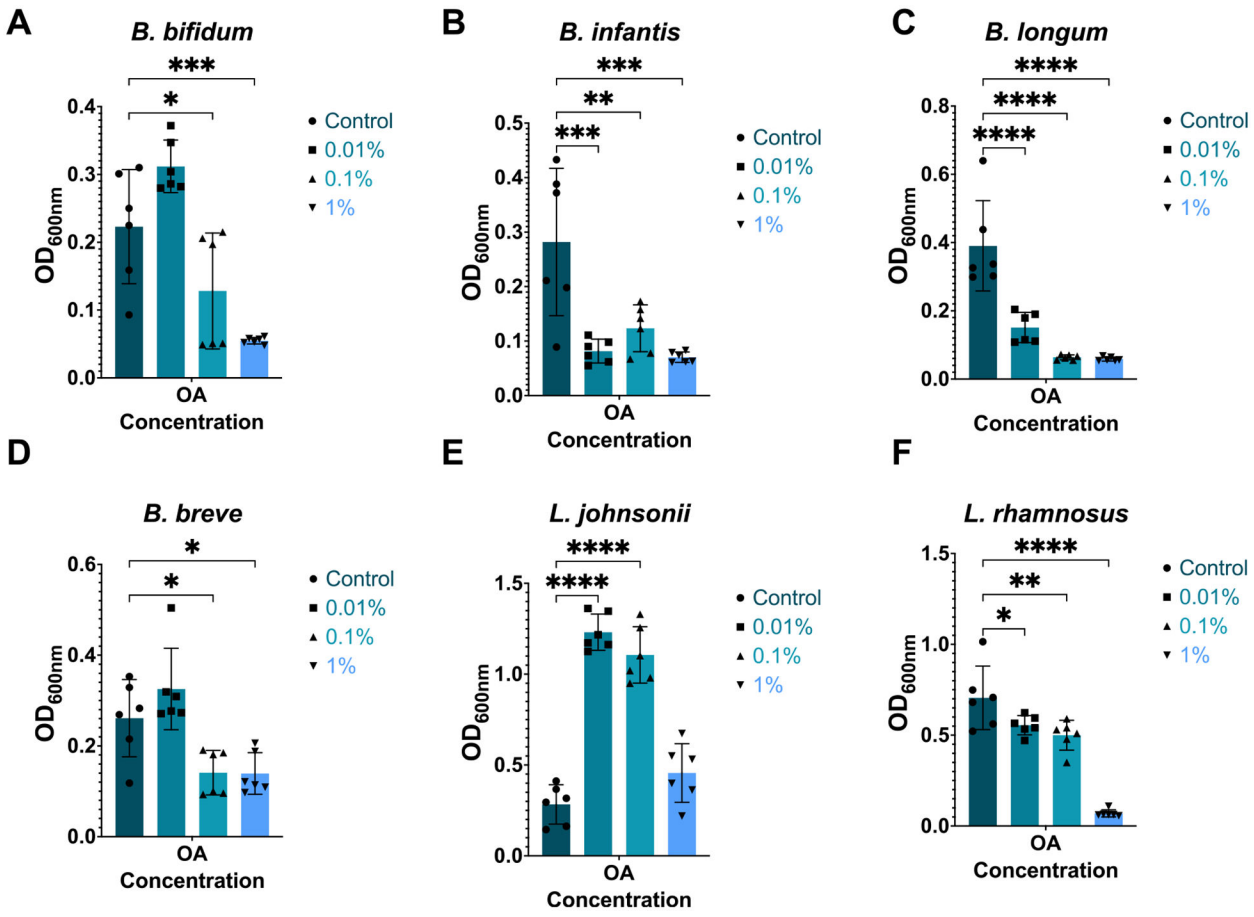

Fig. 1.

Bifidobacterium species A. *B. bifidum* ATCC 11863, B. *B. longum* subspecies *infantis* ATCC 15697C. *B. longum* ATCC 55813 D. *B. breve* ATCC 15698 and *Lactobacillus* species E. *L. johnsonii* ATCC 33200, and F. *L. rhamnosus* ATCC 53163 were grown in a chemically defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Oleic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

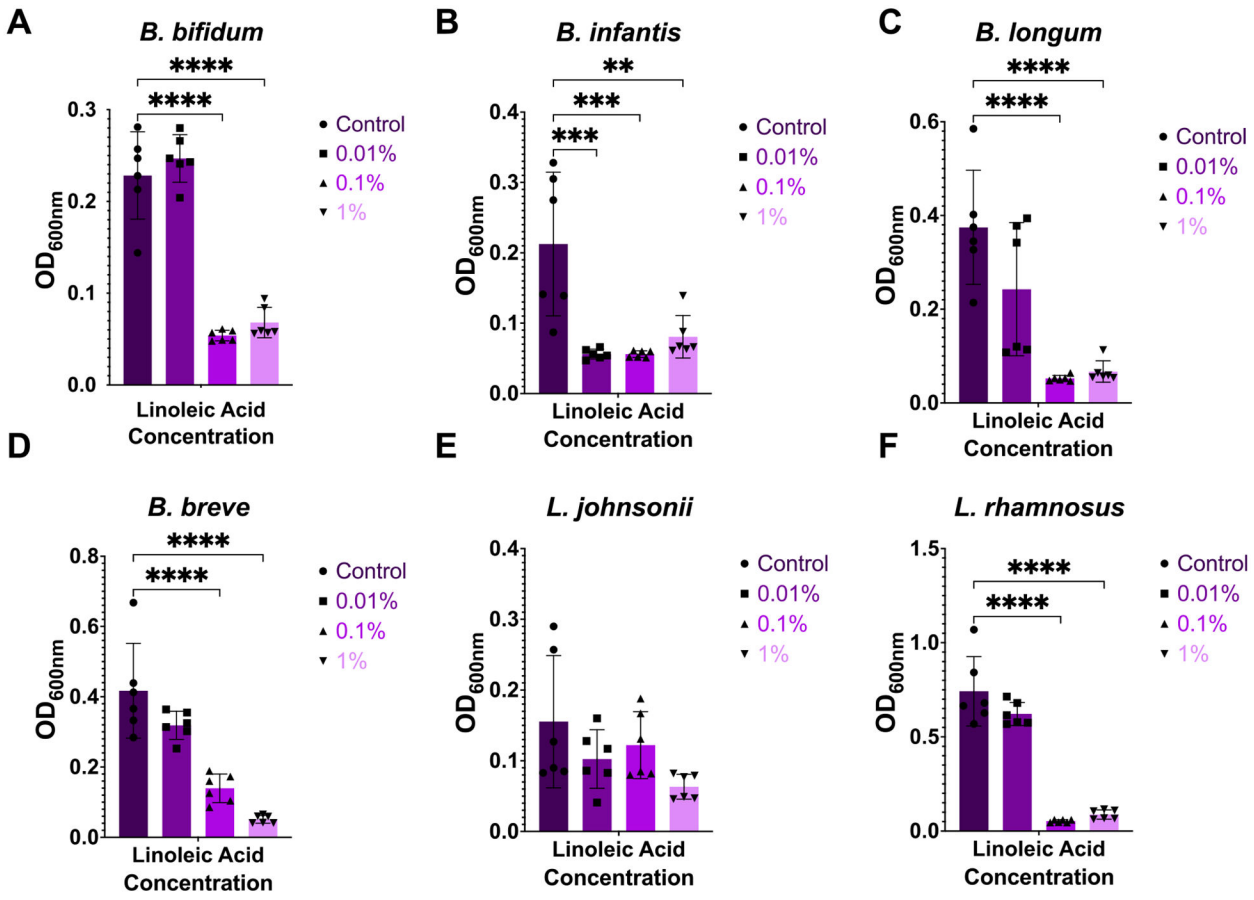

Fig. 2.

Bifidobacterium species A. *B. bifidum* ATCC 11863, B. *B. longum* subspecies *infantis* ATCC 15697C. *B. longum* ATCC 55813 D. *B. breve* ATCC 15698 and *Lactobacillus* species E. L. *johnsonii* ATCC 33200, and F. L. *rhamnosus* ATCC 53163 were grown in a chemically defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Linoleic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

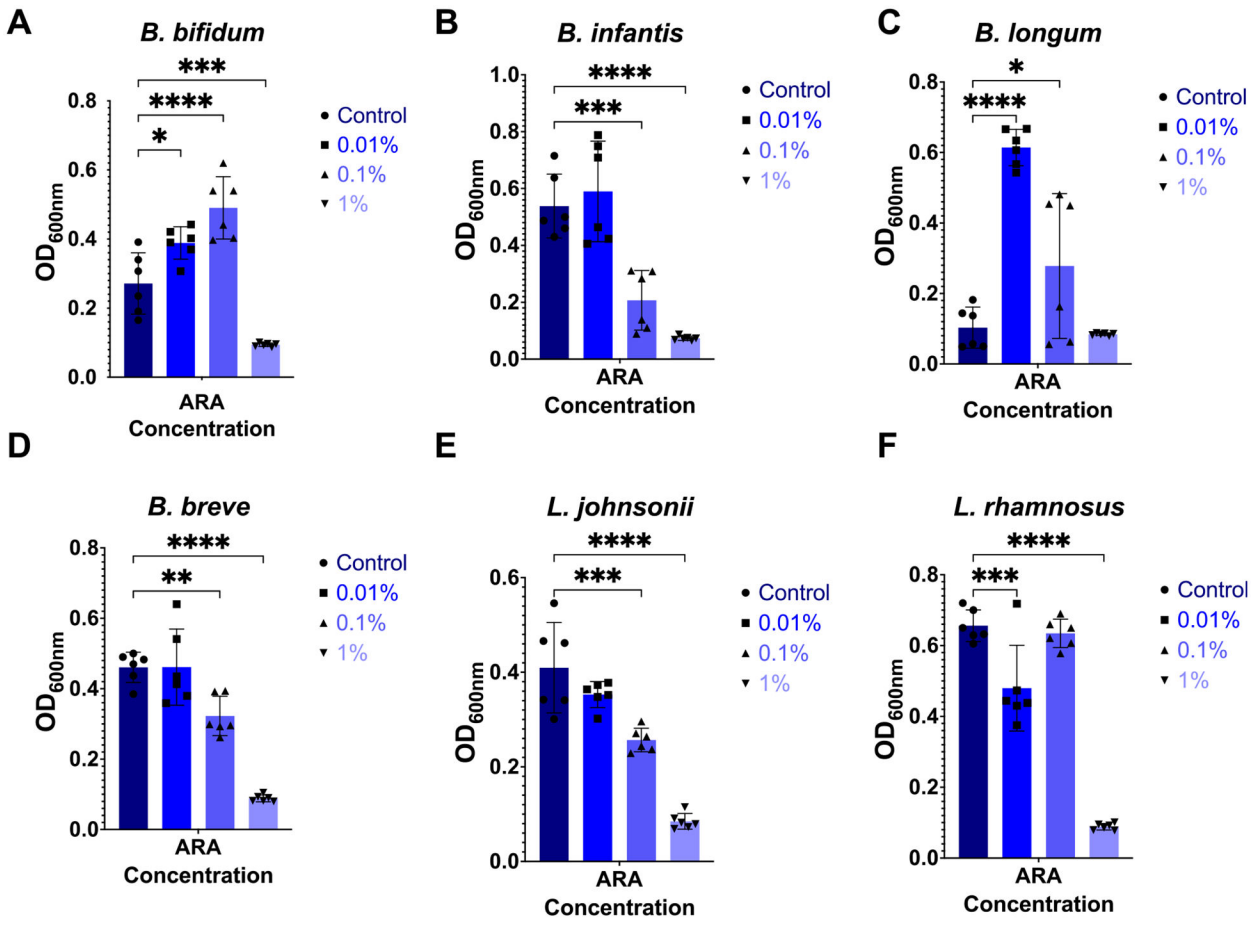

Fig. 3.

Bifidobacterium species A. *B. bifidum* ATCC 11863, B. *B. longum* subspecies *infantis* ATCC 15697C. *B. longum* ATCC 55813 D. *B. breve* ATCC 15698 and *Lactobacillus* species E. *L. johnsonii* ATCC 33200, and F. *L. rhamnosus* ATCC 53163 were grown in a chemically defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Arachidonic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

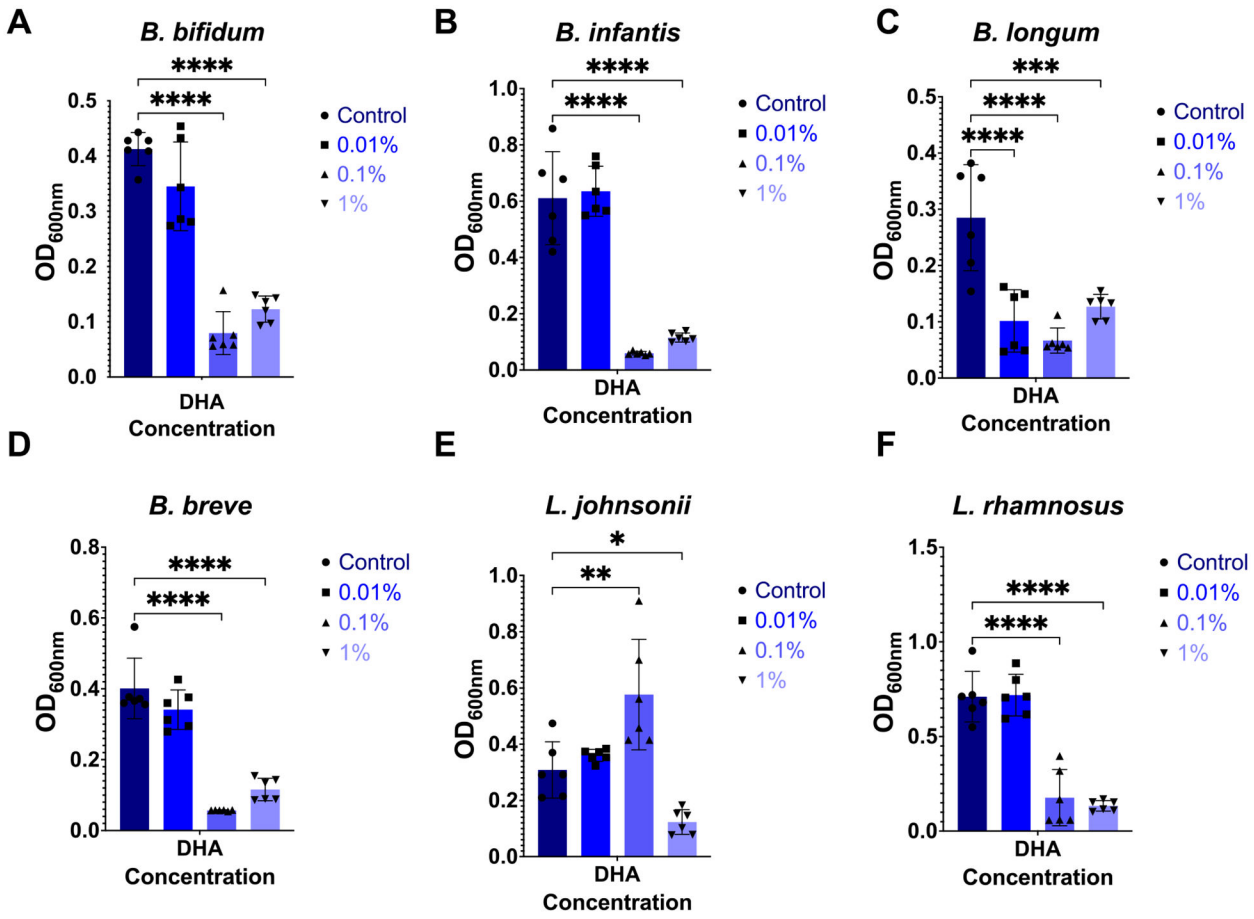

Fig. 4.

Bifidobacterium species A. *B. bifidum* ATCC 11863, B. *B. longum* subspecies *infantis* ATCC 15697C. *B. longum* ATCC 55813 D. *B. breve* ATCC 15698 and *Lactobacillus* species E. *L. johnsonii* ATCC 33200, and F. *L. rhamnosus* ATCC 53163 were grown in a chemically defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Docosahexaenoic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

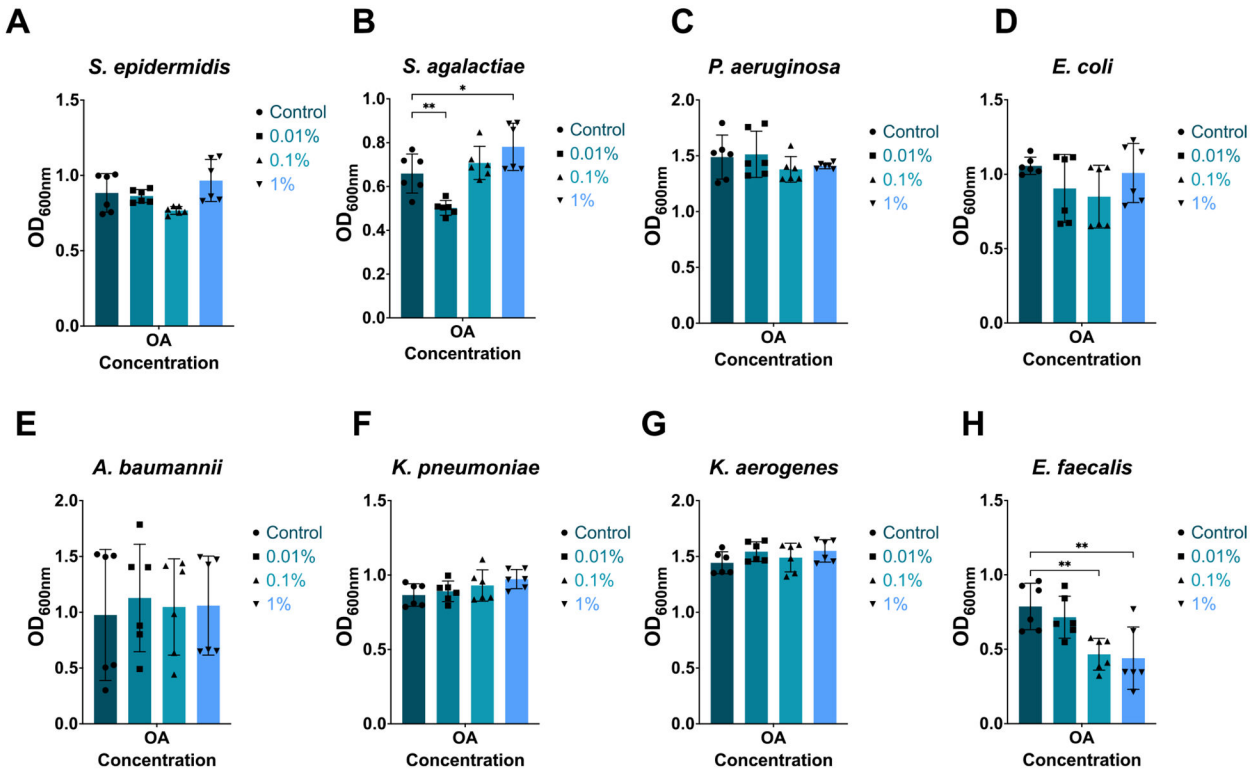

Fig. 5.

Pathobiont species A. *S. epidermidis* ATCC 51025, B. *S.* agalactiae ATCC 13813, C. *P. aeruginosa* ATCC CB1, D. *E. coli* K12, E. *A. baumanii* ATCC 747, F. *K. pneumoniae* CB1, G. *K. aerogenes* NCMB 10102 and H. *E. faecalis* ATCC 29212 were grown in a fully defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Oleic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

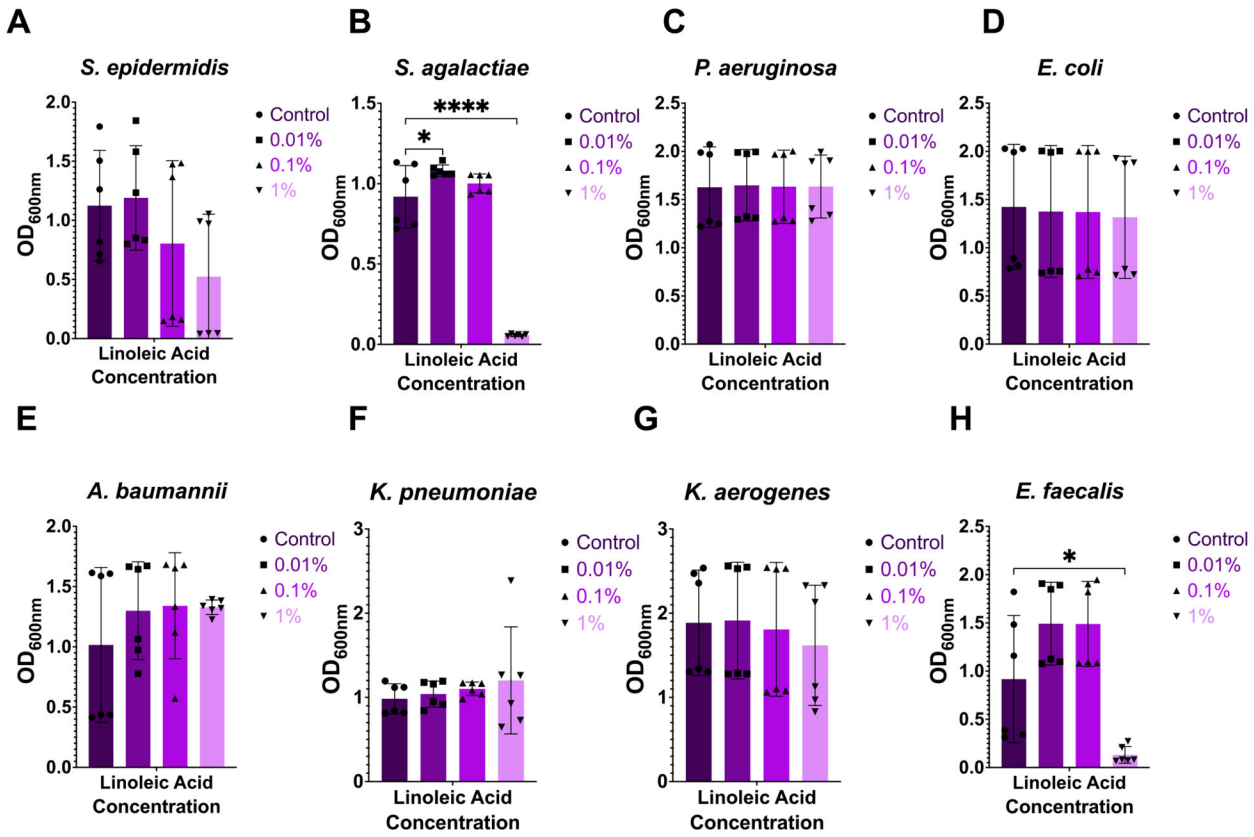

Fig. 6.

Pathobiont species A. *S. epidermidis* ATCC 51025, B. *S.* agalactiae ATCC 13813, C. *P. aeruginosa* ATCC CB1, D. *E. coli* K12, E. *A. baumanii* ATCC 747, F. *K. pneumoniae* CB1, G. *K. aerogenes* NCMB 10102 and H. *E. faecalis* ATCC 29212 were grown in a fully defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Linoleic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

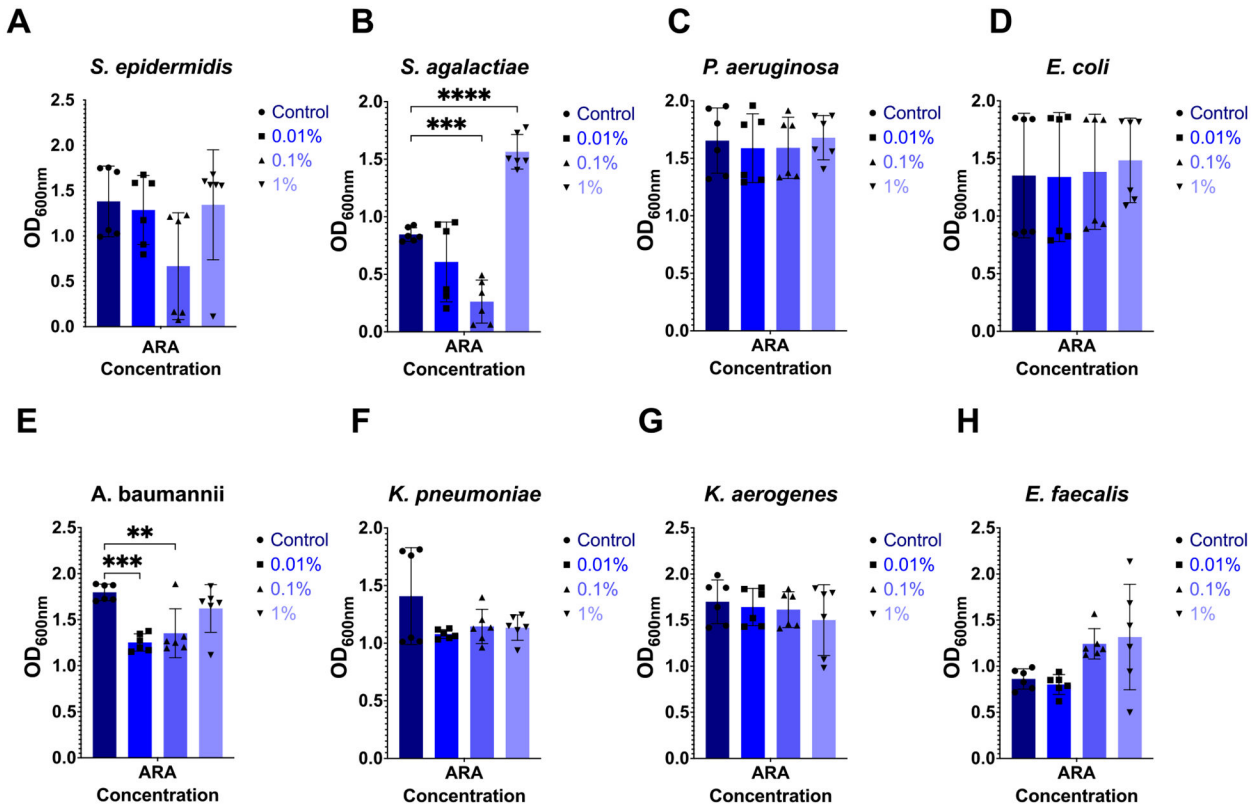

Fig. 7.

Pathobiont species A. *S. epidermidis* ATCC 51025, B. *S.* agalactiae ATCC 13813, C. *P. aeruginosa* ATCC CB1, D. *E. coli* K12, E. *A. baumanii* ATCC 747, F. *K. pneumoniae* CB1, G. *K. aerogenes* NCMB 10102 and H. *E. faecalis* ATCC 29212 were grown in a fully defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Arachidonic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

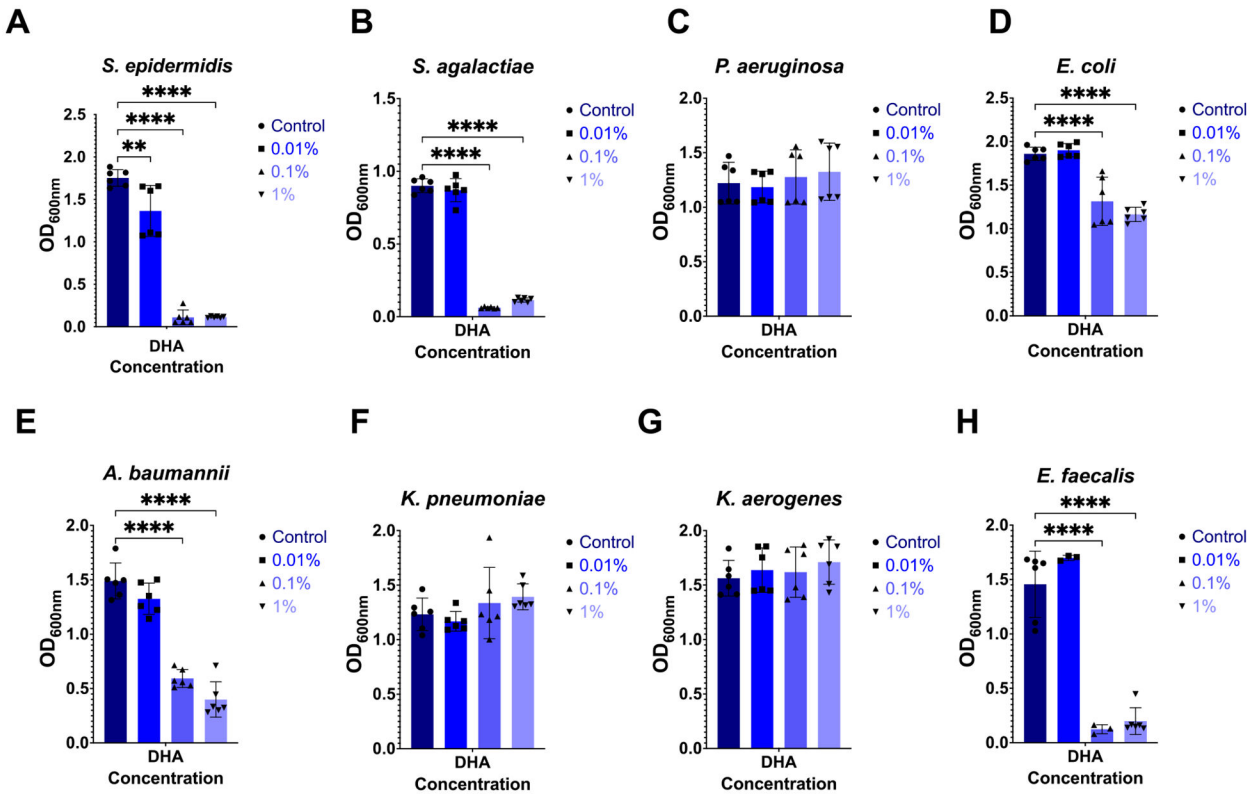

Fig. 8.

Pathobiont species A. *S. epidermidis* ATCC 51025, B. *S.* agalactiae ATCC 13813, C. *P. aeruginosa* ATCC CB1, D. *E. coli* K12, E. *A. baumanii* ATCC 747, F. *K. pneumoniae* CB1, G. *K. aerogenes* NCMB 10102 and H. *E. faecalis* ATCC 29212 were grown in a fully defined media, ZMB1, with 0.01 %, 0.1 % and 1 % Docosahexaenoic Acid. Growth was examined at OD_{600nm} after 20 h. All data are presented as mean ± stdev. One-way ANOVA; * $p < 0.05$.

Table 1

Bacterial strains and growth conditions used in this study.

Bacteria	Strain	Rich Media	Defined Media
B. bifidum	ATCC 11863	MRS	ZMB1
B. longum	ATCC 55813	MRS	ZMB1
B. infantis	ATCC 15697	MRS	ZMB1
B. breve	ATCC 15698	MRS	ZMB1
L. johnsonii	ATCC 33200	MRS	ZMB1
L. rhamnosus	ATCC 53163	MRS	ZMB1
S. epidermidis	ATCC 51025	BHI	ZMB1
S. agalactiae	ATCC 13813	BHI	ZMB1
E. coli	K12	BHI	ZMB1
P. aeruginosa	CB1	BHI	ZMB1
A. baumannii	ATCC 747	BHI	ZMB1
E. faecalis	ATCC 29212	BHI	ZMB1
K. aerogenes	NCMB 10102	BHI	ZMB1
K. pneumoniae	CB1	BHI	ZMB1