npj Science of Food

https://doi.org/10.1038/s41538-026-00759-x

Article in Press

Human milk and infant formula influence lactobacilli metabolism in a species-specific manner

Received: 14 May 2025

Accepted: 5 February 2026

Cite this article as: Gutierrez, A., Chetta, K.E., Puckett, B. *et al.* Human milk and infant formula influence lactobacilli metabolism in a species-specific manner. *npj Sci Food* (2026). https://doi.org/10.1038/s41538-026-00759-x

Alyssa Gutierrez, Katherine E. Chetta, Brenton Puckett, Sigmund J. Haidacher, Thomas D. Horvath & Melinda A. Engevik

Human milk and infant formula influence lactobacilli metabolism in a species-specific manner

Alyssa Gutierrez[1], Katherine E. Chetta[2,3], Brenton Puckett[1], Sigmund J. Haidacher[4,5], Thomas D. Horvath[4,5,6], Melinda A. Engevik[1,7] *****

1. Department of Regenerative Medicine & Cell Biology, Medical University of South Carolina (MUSC)
2. Department of Pediatrics, C.P. Darby Children's Research Institute, MUSC
3. Department of Pediatrics, Division of Neonatal-Perinatal Medicine, MUSC, Shawn Jenkins Children's Hospital
4. Department of Pathology and Immunology, Baylor College of Medicine (BCM)
5. Texas Children's Research Institute (TCRI) – Microbiome Center, Texas Children's Hospital
6. Department of Pharmacy Practice & Translational Research, University of Houston (UH)
7. Department of Microbiology & Immunology, MUSC

***Corresponding Author:** Melinda A. Engevik, engevik@musc.edu. Address: 173 Ashley Ave, Charleston, SC 29425

Funding: This study was supported by T32DK124191-01A1 (AG), NATS NIH KL2TR001452 (KEC), Darby Pediatrics pilot grant (KEC), UL1TR001450 (KEC), 3P20 P20 GM130457 (COBRE in Digestive & Liver Disease, MUSC; GM130457-04S1 supplement), P30 DK123704 (Digestive Disease Research Center, MUSC). The Virginia and L.E. Simmons Family Foundation Mass Spectrometry Laboratory housed within the Texas Children's Research Institute - Microbiome Center is supported by an NIH S10 Shared Instrumentation Grant 1S10ODO36416-01 (TDH), and by a NIH P30DK056338 (TDH).

Keywords: *Lactobacillus*, milk, formula, short-chain fatty acids, LC-MS/MS, metabolites

Abstract:

The neonatal gut is colonized by commensal bacteria, including lactobacilli, that play a critical role in promoting host health. While lactobacilli are known for producing bioactive metabolites, species-specific responses to neonatal diets remain unclear. We hypothesized that human milk and infant formula would differentially influence lactobacilli metabolite production. Six species (*Lactobacillus acidophilus, Levilactobacillus brevis, Lactobacillus johnsonii, Lacticaseibacillus paracasei, Limosilactobacillus reuteri, Lacticaseibacillus rhamnosus*) were cultured in defined media with human milk, formula, or water, and supernatants were analyzed via untargeted LC-MS/MS metabolomics. Principal coordinates analyses showed distinct metabolomic profiles among lactobacilli species and across dietary treatments. Human milk enhanced the production of several metabolites, including hydroxyphenyllactic acid, tyrosine, indoles, vaccenic acid, and di-peptides. Interesting, several unique metabolites were upregulated in the presence of infant formula, including isonicotinic acid. These results highlight the differential effects of human milk and infant formula on the metabolomic profiles of lactobacilli species, emphasizing significant species-specific variation and pronounced production of potentially beneficial compounds in response to human milk. This work underscores the importance of understanding diet-microbe interactions to optimize neonatal gut health.

Introduction

Immediately after birth, the neonatal gut is quickly colonized by microbes, which influence intestinal, immune, and neurological development, and may have lasting effects on health outcomes[1-12]. Among the earliest colonizers are lactobacilli species, which are transferred from mother to child through human milk and form a substantial component of the infant gut microbiota[13-19]. Given that lactobacilli species are widely recognized for their health benefits in both early and adult life, they are frequently utilized as probiotics in clinical trials. In human infants, lactobacilli-based probiotics have demonstrated safety and efficacy in improving growth, feeding tolerance, and infantile colic[20-24].

Lactobacilli exert many of their beneficial effects by metabolizing dietary nutrients to produce an array of bioactive compounds such as indoles, vitamins, histamine, phenolic derivatives, bacteriocins, and short-chain fatty acids[25-33]. These metabolites can regulate immune processes, act as antioxidants, and contribute to gut homeostasis[34-43]. However, the composition and abundance of these microbial products are highly dependent on both the bacterial strain and the surrounding nutrient environment[44-48]. For example*, Lactobacillus acidophilus, L. gasseri, L. crispatus*, and *Lacticaseibacillus rhamnosus* generate diverse metabolites, with certain compounds being unique to specific species, but these metabolites are dependent on dietary availability[29].

The interplay between diet and microbes is particularly relevant during infancy. Breast-fed infants are known to harbor higher levels of lactobacilli compared to formula-fed infants[49,50], and some species, such as *Limosilactobacillus reuteri* produce markedly different metabolites when exposed to human milk versus formula[51]. These observations suggest that the nutritional environment can shape not only microbial composition but

also microbial function. We speculate that the ability of commensal microbes, including lactobacilli species, to exert health-promoting effects is highly dependent on dietary context. Supporting this notion, a recent study by Repa *et al*. found that a probiotic mix containing *Lactobacillus* and *Bifidobacterium* reduced the incidence of necrotizing enterocolitis (NEC), however, this benefit only occurred in breastfed infants, with no improvements in those who were formula-fed[52]. Accordingly, the well-established benefits of breastfeeding, including reduced risks of NEC, sepsis, and asthma, may be partially mediated by diet-enhanced microbial metabolism[53-58]. These findings underscore the hypothesis that probiotics may only be effective when their metabolic outputs are supported by specific dietary substrates, such as those found in human milk.

Currently, the metabolite profiles produced by various lactobacilli species in the context of infant diets remain poorly characterized. To address this gap in knowledge, we performed untargeted metabolomics on six lactobacilli species, *L. acidophilus, L. brevis, L. johnsonii, L. paracasei, L. reuteri,* and *L. rhamnosus,* cultured in a chemically-defined medium supplemented with either human milk or infant formula. These species were selected based on their prevalence in infant gut and human milk microbiota, as well as their documented probiotic potential in pediatric populations[15,20-22,59-64]. Our results reveal that while certain metabolites were produced in all conditions, numerous metabolites were uniquely produced in a species- or diet-specific manner. Notably, supplementation with human milk resulted in more diverse metabolite profiles and significant enrichment of antioxidants and compounds known to suppress inflammation, as compared to formula. These findings indicate that dietary substrates can influence microbial metabolic output in ways potentially meaningful to host health. By elucidating these species- and diet-

specific metabolic profiles, this study contributes to a deeper understanding of how probiotics and nutrition intersect to influence infant health. Ultimately, these insights may inform the design of tailored probiotic strategies that align with infant feeding practices to support optimal microbiota and neonatal development.

Results

Species-specificity of lactobacilli metabolites

To investigate the impact of human milk versus infant formula on lactobacilli metabolic output, we cultured six lactobacilli species in a chemically-defined medium supplemented with human milk, infant formula, or water as a control, followed by untargeted metabolomics analysis on culture supernatants using LC-MS/MS. Principal component analysis (PCA) for each media condition demonstrated species-specific clustering, with *L. acidophilus* ordinating the farthest away from the other clusters, indicating the most unique overall metabolomic signature of the six species (**Figure 1A, C, E**). Interestingly, the cluster for *L. paracasei* can be observed overlapping that of other species', indicating significant similarities with *L. johnsonii* and *L. rhamnosus* depending on media conditions.

We next sought to further characterize these inter-species differences and similarities by examining the number of metabolites that were exclusively produced by one species, shared by all six species, or a smaller combination of species. Our metabolite intersection analyses revealed that while several metabolites were shared among species, the majority were uniquely produced by individual species or a combination of two species, highlighting large diversity across species' metabolic outputs (**Figure 1B, D, F**). For example, in the water-supplemented cultures, 3 metabolites were produced by all six species, whereas 14 metabolites were uniquely produced by a single species (**Figure 1B**). We found that for all media conditions, *L. acidophilus* produced the greatest number of unique metabolites, which is consistent with our findings through PCA that *L. acidophilus* had the most unique overall metabolomic signature. *L. acidophilus*

strikingly yielded 20 unique metabolites that were not produced by the other species when cultured with human milk, compared to only 6 unique metabolites in the context of infant formula (**Figure 1D, E**). Notably, the total number of metabolites produced by all six species was greatest in the presence of human milk, compared to water and infant formula. This evidence suggests that human milk offers a wider variety of substrates for lactobacilli to metabolize and generate diverse compounds.

Diet-specificity of lactobacilli metabolites

We next examined diet-specific responses of each individual lactobacilli species, using PCA to assess overall alterations to metabolomic signatures depending on supplementation with water, human milk, or infant formula (**Figure 2**). All six species demonstrated distinct clustering by media condition. Infant formula had the most distinctly separated cluster for *L. acidophilus* and *L. rhamnosus*, while human milk resulted in highly distinct clustering for *L. brevis, L. johnsonii,* and *L. paracasei*. PCA for *L. reuteri* indicated highly distinct metabolomic profiles for all three culture conditions.

The number and diversity of produced metabolites per culture condition varied across the six lactobacilli species (**Figure 3**). We consistently observed that numerous metabolites were produced in all three culture conditions, indicating conserved metabolism by each lactobacilli species that seem to persist regardless of additional environmental dietary substrates. As expected, few metabolites were uniquely produced in water-supplemented cultures, consistent with limited substrate availability and confirming that observed metabolic changes in human milk and formula conditions were nutrient-driven. The highest number of metabolites tended to be produced exclusively in the presence of human milk. In particular, *L. acidophilus* uniquely produced 22

metabolites with human milk, compared to 0 and 3 unique metabolites with water and formula, respectively. This pattern of low numbers of unique to water and formula was seen for all six species.

Assessment of individual metabolites with speculated relevance to host health

As previously indicated by our intersection analyses (**Figure 1B, D, F**), several metabolites were shared across the six species. In **Figure 3**, we highlighted select metabolites of interest that have documented relevance to host health. For example, the compound hydroxyphenyllactic acid was exclusively produced in the presence of human milk for all six species. Tyrosine displayed the same pattern of exclusivity to human milk. Contrarily, no metabolites were produced exclusively in formula cultures that were shared by all six species. Carnitine was the most prominently produced metabolite associated with infant formula, although it was only unique to formula for four species.

We observed that for all species, numerous metabolites were significantly consumed or generated when lactobacilli species were cultured with human milk or infant formula (**Figure 4**). Across all species, most amino acids, nucleic acids, and their derivatives were consumed, meaning that the detected levels were lower than the levels recorded in our baseline media samples. Significant decreases in response to milk and formula compared to water may be attributed to overall enhanced bacterial growth in response to extra nutrients provided by the formula and milk, leading to greater consumption of amino acids and nucleic acids to support replication. *L. acidophilus* and *L. reuteri*, however, demonstrated profound exceptions to this pattern. Rather than consumption, *L. acidophilus* yielded a net positive production of adenine, glutamine, glutamate, phenylalanine, pyroglutamic acid, and Tyr-Pro when cultured with human milk.

As for *L. reuteri*, adenine, methionine, valine, and Phe-Pro were produced rather than consumed in the context of human milk or infant formula. As previously mentioned, all six lactobacilli species produced the essential amino acid tyrosine, but only when cultured with human milk, and these concentration differences were statistically significant (**Figure 5A**). Carnitine was the most heavily associated with infant formula cultures, with five species producing significantly higher levels of carnitine compared to water and human milk cultures (**Figure 5B**). Interestingly, formula-supplemented *L. rhamnosus* yielded a large net consumption rather than generation of carnitine.

Beyond amino acids and nucleic acids, our untargeted metabolomics approach detected a wide array of other organic compounds, with numerous significant differences by culture condition (**Figure 4**). Hydroxyphenyllactic acid is a derivative of tyrosine metabolism and has been reported for its antioxidant properties[65,66]. Notably, it was exclusively produced when human milk was present, and concentration differences were statistically significant across all six species (**Figure 5C**). Given that production of hydroxyphenyllactic acid was also identified in *L. plantarum*, it is very likely that cellular machinery to generate this compound is conserved across many lactobacilli species[65]. Phosphocholine and glycerophosphocholine are both important sources of dietary choline and are precursors to critical cellular membrane components as well as the neurotransmitter choline. We observed significantly increased phosphocholine levels in the setting of human milk for all six species, while glycerophosphocholine levels were highest in infant formula cultures for all species except *L. rhamnosus* (**Figure 5D, E**). Vitamin C and riboflavin are essential micronutrients for human health, and we found that all six lactobacilli species were able to elevate vitamin C when supplemented with human

milk and formula compared to water (**Figure 5F**). Only *L. acidophilus* and *L. reuteri* produced riboflavin, however, and riboflavin production only occurred in formula-fed cultures (**Figure 5G**). Interestingly, levels of lumichrome, a byproduct of riboflavin metabolism, were significantly highest in human milk cultures for all species (**Figure 5H**).

We additionally observed notable species-conserved patterns for metabolites that were widely consumed by lactobacilli, rather than generated. 4-Vinylphenol is a metabolite of styrene and has been reported to be toxic in the liver and lungs of mice[67,68]. At baseline, all media blanks (without bacteria) contained detectable levels of 4-vinylphenol, and the addition of lactobacilli decreased its levels, indicating microbial capacity to metabolize this compound. This is consistent with previous studies documenting the ability of various microbes, including *L. plantarum*, to both synthesize and degrade this compound[69-71]. For all species, consumption of 4-vinylphenol was maximized in the context of human milk cultures (**Figure 5I**). Another compound of interest, isonicotinic acid, is a synthetic precursor for antimycobacterial and antimalarial drugs. Similar to 4-vinylphenol, we detected isonicotinic acid in our baseline media blanks, but all lactobacilli species were able to decrease its levels, suggesting microbial metabolization of the compound. For most lactobacilli species, the degradation of isonicotinic acid was most profound in water- and milk-supplemented cultures, whereas its metabolism was reduced in formula cultures. Interestingly, *L. acidophilus* produced increased levels of isonicotinic acid in response to human milk and infant formula compared to water (**Figure 5J**).

Given the known contributions of commensal microbes in modulating intestinal immune function, we further sought to examine metabolites that have documented

relevance to inflammatory processes. Our untargeted metabolomics approach detected several relevant metabolites, but only two with significant alterations in their levels by culture condition. Indolelactic acid is generated from tryptophan through the indole pathway and is well-established for its anti-inflammatory properties[72-76]. Our analyses indicate that only *L. johnsonii* and *L. reuteri* are able to produce indolelactic acid, and importantly, its production is maximized in the presence of human milk (**Figure 5K**). Vaccenic acid is a trans-fatty acid that has multiple reported beneficial properties, including suppression of intestinal inflammation and atherosclerosis[74,77-79]. Here we observed that vaccenic acid was produced exclusively by *L. reuteri,* and notably, only in the setting of human milk (**Figure 5L**).

Pathway Enrichment Analyses

To gain further insight through a functional perspective, we performed pathway enrichment analyses on our data from human milk and infant formula cultures compared to the water culture as a control (**Figure 6**). For all six lactobacilli species and regardless of culture condition, tyrosine metabolism and catecholamine biosynthesis were significantly enriched pathways. This indicates conserved lactobacilli contributions to pathways involved in production of neuroactive compounds or precursors. This is consistent with literature indicating that lactobacilli species, including *L. helveticus, L. reuteri,* and *L. rhamnosus*, can produce tyrosine, L-DOPA, dopamine, and norepinephrine[80-82]. Carnitine synthesis was significantly enriched in four species, corresponding to species in which carnitine was elevated in formula-supplemented cultures. Interestingly, for *L. acidophilus*, human milk uniquely enhanced glutamate and

glutathione metabolism, key processes in oxidative stress management[83], whereas these pathways were not enriched in formula-fed conditions.

Discussion

Immediately following birth, the infant gut becomes colonized by a diverse array of microbes that significantly influence host immune and physiological development. While lactobacilli represent a minor proportion of the neonatal gut microbiota relative to genera such as *Bifidobacterium* and *Streptococcus*[84-88], they are frequently employed as probiotics in clinical trials and even in commercially available infant formulas. Therefore, in this study we applied untargeted metabolomics to examine the metabolic output of six commonly utilized lactobacilli species in response to human milk or infant formula. Species identity was a significant contributor to metabolite production, with *L. acidophilus* displaying the most unique and diverse metabolic signatures regardless of culture condition. In contrast, other species such as *L. paracasei*, *L. johnsonii*, and *L. brevis* produced widely overlapping metabolic profiles, particularly in the presence of human milk. This species-specific variability underscores both the functional diversity and redundancy among lactobacilli members depending on nutritional context. Notably, *L. johnsonii* and *L. reuteri* produced distinctive anti-inflammatory metabolites, such as indolelactic acid and vaccenic acid, which were not detected in the other tested species (**Figure 5K, L**). These distinctions may reflect differences in probiotic functionalities and point toward the importance of selecting specific species for health-promoting purposes.

Our results revealed clear diet-dependent patterns in metabolite production. Human milk supported the biosynthesis of numerous metabolites, including important amino acids such as tyrosine, phenylalanine, and cysteine, suggesting enhanced

biosynthetic and antioxidant pathways. The elevation of bioactive compounds such as hydroxyphenyllactic acid and tyrosine in human milk cultures has implications for host antioxidant defenses and neural development, potentially aligning with clinical data linking breastfeeding with improved cognitive and immune outcomes[89,90]. Conversely, formula-fed cultures generated fewer unique metabolites, with a consistent shift toward carnitine production (**Figure 5B**). This formula-associated increase in carnitine may reflect microbial adaptation to different substrate availability, possibly contributing to infant energy metabolism during early development. Our present findings are consistent with a previous study in human infants that formula-feeding led to significantly increased levels of circulating carnitine compared to breastfeeding[91]. However, it is unclear whether elevated carnitine levels result in any physiological consequences for the infant, as clinical trials have demonstrated no differences in growth or health outcomes in human preterm infants who were supplemented carnitine[92,93].

Our untargeted metabolomics approach revealed several metabolites of interest that were significantly altered by human milk versus infant formula, however, several of these poorly understood in the context of neonatal health. For example, we observed that lumichrome was widely produced by our lactobacilli species but only in the presence of human milk (**Figure 5H**). Lumichrome is generated as a result of riboflavin metabolism, but its importance is primarily documented in the context of plant biology and soil-associated microbes[94-98]. Currently *in vitro* evidence indicates that lumichrome can act as a cytotoxic agent against cancer cell lines[99,100]. It remains uncertain, however, whether lumichrome plays a meaningful role in neonatal development. We additionally observed that 4-vinylphenol was broadly metabolized by lactobacilli, with the most profound

degradation seen in human milk cultures (**Figure 5I**). 4-Vinylphenol has been extensively documented to be hepatoxic and pneumotoxic in mouse models[67,68], however, one study has contrarily reported that 4-vinylphenol might be beneficial as an antioxidant and was cytotoxic against a human breast cancer cell line[69]. Importantly, evidence of 4-vinylphenol's effects on *in vivo* human health remains otherwise limited. Another compound of interest, isonicotinic acid, was also found to be degraded by all lactobacilli species except *L. acidophilus*, with its consumption most profound in water- and milk-supplemented cultures (**Figure 5J**). Interestingly, *L. acidophilus* produced increased levels of isonicotinic acid in response to human milk and infant formula compared to water (**Figure 5J**). It is important to note, however, that isonicotinic acid is only documented as a precursor to antimycobacterial and antimalarial drugs in the context of chemical synthesis, not necessarily endogenously within a host. Therefore, the direct physiological consequences of altered levels of isonicotinic acid in the context of human health and early life are yet to be elucidated.

Importantly, our findings suggest that the health-promoting potential of probiotics such as lactobacilli are highly contingent on the surrounding dietary context. Across all six species, human milk consistently enabled greater metabolite diversity and supported the production of several compounds with previously reported anti-inflammatory, antioxidant, and neuroactive properties. These data support the hypothesis that probiotics may not inherently be beneficial in isolation, but instead require the availability of appropriate substrates, such as those provided by human milk, to fully realize their functional potential. Therefore, the use of probiotics during infancy may be most effective when paired with human milk as compared to formula. This notion is supported by clinical

findings, such as those reported by Repa *et al*., where probiotic efficacy in reducing NEC was observed exclusively in breastfed infants and not formula-fed infants[52].

Our data further reinforces the concept that human milk is not only a direct source of nutrients but also a modulator of microbial metabolism, shaping the biochemical outputs of colonizing microbes. Accordingly, the well-established benefits of breastfeeding may be partially mediated through the enhancement of microbial metabolic pathways. This highlights a critical consideration for future probiotic-based interventions in infants, in that their efficacy may depend less on the strain itself and more on the dietary context into which they are introduced.

We recognize that there are several limitations to our study. First, we used pasteurized human milk in this study to examine microbe-milk interactions. Human milk contains a microbiome that is dominated by *Streptococcus, Staphylococcus* and *Escherichia* species[101-103] and we used pasteurized milk in this study to eliminate these endogenous microbes in our cultures. However, pasteurization is known to alter certain components of human milk[104,105], especially fatty acids, which may influence microbial metabolic outputs. As a result, we recognize that our findings may not fully recapitulate how microbes respond to fresh, unpasteurized human milk in breast fed infants. Pasteurized donor human milk is commonly provided by milk banks to infants in the neonatal intensive care settings, so it is possible that our results model this exposure for infants. Next, while our *in vitro* approach provides a controlled platform for dissecting diet-microbe interactions, it lacks key elements of the *in vivo* gut environment such as host cells, immune responses, and complex microbial communities. Examining multi-species lactobacilli communities may uncover synergistic interactions affecting metabolite output.

Additionally, we examined only one strain per species; future studies should incorporate multiple strains and consider inter-strain variability in metabolite production. We also acknowledge that metabolite production can vary across different bacterial growth phases. Our current metabolomic analysis was performed at a single time point, which may not fully capture dynamic changes in metabolite secretion. Longitudinal sampling across defined growth phases, such as lag, exponential, and stationary phase, could be incorporated in future experiments to provide a more comprehensive view of temporal metabolic shifts. Finally, the utilization of targeted metabolomic approaches is also warranted to further validate the present findings and enable more precise measurements of metabolites of interest, such as other indoles and fatty acids.

Culture medium composition is a critical determinant of microbial metabolic output, and differences in media across studies can complicate direct comparison of metabolomic findings. In the present study, we selected LDM4 because it is a chemically defined medium that provides the essential nutrients required to support Lactobacilli growth while minimizing background compounds that could confound metabolite detection. LDM4 has been used extensively in prior studies investigating lactic acid bacteria metabolism, supporting its suitability for metabolomic analyses[27,28,32,106-110]. In contrast, the most commonly used medium for cultivating lactobacilli, De Man-Rogosa-Sharpe (MRS), is nutrient-rich and contains numerous undefined components, including meat extract and yeast extract, that can directly contribute metabolites and promote high basal metabolic activity. While such rich media are well suited for maximizing biomass, they may obscure condition-specific metabolic differences. Accordingly, the use of a chemically defined medium such as LDM4 enabled clearer resolution of diet-dependent microbial metabolic

responses, which was essential for distinguishing differences between human milk- and infant formula-associated metabolite profiles in this study.

Altogether, this study highlights how infant feeding type, specifically human milk versus formula, drives distinct metabolic profiles in lactobacilli species, with implications for microbial function and host health. Human milk promotes the production of amino acids, antioxidants, and metabolites reported to modulate inflammation, while formula enriches energy-associated compounds like carnitine. The clear distinctions in metabolite profiles between human milk and formula conditions underscore the need for tailored probiotic strategies that account for feeding practices. These insights highlight that probiotics are not one-size-fits-all, but rather they must be paired with the appropriate nutritional environment to optimally support infant development.

Methods

Bacterial Culture Conditions

To investigate the impact of human milk and infant formula on lactobacilli metabolites, we cultured six species (*Lactobacillus acidophilus* ATCC 4356*, Levilactobacillus brevis* ATCC 27303*, Lactobacillus johnsonii* ATCC 33200*, Lacticaseibacillus paracasei* ATCC 25302*, Limosilactobacillus reuteri* ATCC 6475, and *Lacticaseibacillus rhamnosus* ATCC 53103) in a chemically defined medium (LDM4)[32,107] supplemented with 10% v/v pasteurized human milk, infant formula, or sterile MilliQ water. These species were chosen for their prevalence in the infant gut and in human milk and their reported probiotic potential in human infants[15,20-22,59-64]. All six species are routinely detected in infant stool[111-114], and *L. brevis, L. paracasei, L. reuteri, and L. rhamnosus* are lactobacilli commonly isolated from breastmilk[15]. All six species have also been documented to be well-tolerated in newborn and pediatric populations, with *L. acidophilus, L. reuteri,* and *L. rhamnosus* additionally demonstrating effectiveness in promoting weight gain in preterm and low birthweight infants[115-120]. Other documented clinical applications include *L. acidophilus* for prevention of sepsis and treatment acute infantile diarrhea[121], *L. reuteri* for treatment of infantile colic[122], and *L. rhamnosus* for improvement of symptoms associated with cow's milk allergy[123]. Notably, two species are included in commercially available infant formulas in the United States: *L. rhamnosus* in Enfamil Nutramigen LGG and *L. reuteri* in Dr. Brown's Soothe Pro infant formula. LDM4 medium was selected because it is chemically defined and provides essential nutrients to support growth of lactobacilli species, while minimizing background compounds. This medium is optimal for the detection of bacterial metabolites from lactic acid

bacteria[27,28,32,106-110]. The water-supplemented condition was included as a minimal nutrient control to assess metabolite production arising solely from bacterial utilization of LDM4 medium components, independent of complex nutrient sources such as milk or formula. Human milk was obtained according to an approved IRB (IRB#103782) at the Shawn Jenkin's Children's Hospital at the Medical University of South Carolina (Charleston, SC). Milk from seven healthy lactating donors was pooled to minimize inter-individual variability and ensure sample homogeneity. Samples were de-identified before receipt, and therefore specific donor demographic data were not available in accordance with ethical and privacy regulations. Expressed milk was collected and pasteurized with Holder pasteurization (HoP) for 30 minutes before experiments. Similac 360 Total Care was utilized as the infant formula in this study. After 20h incubation at 37°C in anaerobic conditions, culture supernatants were collected for untargeted metabolomics analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Baseline metabolomic profiles were determined for LDM4 supplemented with 10% v/v water, human milk, or infant formula without bacterial inoculation. These values were subtracted from corresponding bacterial culture conditions to obtain metabolite levels attributable to bacterial activity. All culture conditions were performed in biological triplicates.

LC-MS/MS Method for Reverse-Phase Non-Targeted Metabolomics

Cell free bacterial supernatants were examined by LC-MS/MS. The reverse-phase chromatographic separation for the non-targeted metabolomics measurement was performed using a Luna C18(2) and Security Guard C18 analytical and guard column combination described above, and using a MPA solution consisting of water that

contained 0.1% formic acid, a MPB solution consisting of acetonitrile that contained 0.1% formic acid, and a NW solution composed of a mixture of MeOH:Water (1:1, v:v). The mobile phase flowrate was 0.080 mL/min, the autosampler tray was chilled to 10°C, and the column oven was kept at ambient temperature. The gradient elution program was specified as follows: 0-0.5 min, 5% B; 0.5-14 min, 5-50% B; 14-14.6 min, 50-90% B; 14.6-14.9 min, 90% B; 14.9-15.0 min, 90-5% B; 15.0-20 min 5%B; and at 20.01 min a Stop command is specified, and there is a 20.4 min duty cycle for each injection.

The heated electrospray (H-ESI) ionization source parameters for the Orbitrap Fusion MS were specified in the Xcalibur-based acquisition method as follows: application mode: small molecule; method duration: 19.5 minutes; ionization mode polarity, positive mode; probe spray voltage, static at +3,500 V; gas mode, static; sheath gas (arbitrary units), 25; auxiliary gas (arbitrary units), 5; sweep gas (arbitrary units), 0; ion transfer tube temperature, 275°C; and, vaporizer temperature, 75°C. Global MS acquisition parameters were specified as follows: infusion mode, liquid chromatography; expected LC peak width (s), 30; advanced peak determination, false; mild trapping, false; default charge state, 1; enable Xcalibur AcquireX method modification, false; and, internal mass calibration, Easy-IC™.

The acquisition parameters for the HRMS/HRMS acquisition method used for the reverse-phase chromatographic method was specified as follows: detector type, Orbitrap; Orbitrap resolution, 120,000 (at m/z 200); mass range, normal; use quadrupole isolation, true; scan range (m/z), 65-650; RF lens (%), 60; AGC target, standard; maximum injection time mode, custom; maximum injection time (ms), 100; Microscans, 1; data type, profile; polarity, positive; source fragmentation, disabled; and, use Easy-IC™, true. Dynamic

exclusion filtering was enabled with the following parameters specified: exclude after n times, 1; exclusion duration (s), 6; mass tolerance, ppm; low, 10; high, 10; exclude isotopes, true; perform dependent scan on single charge state per precursor only, false; and, exclude within cycle, true. Apex detection filtering was enabled with the following parameters specified: expected peak width (FWHM, s), 10; desired apex window (%), 30. Intensity threshold filtering was enabled with the following parameters specified: filter type, intensity threshold; intensity threshold, 5.0e4. The ddMS2 OT HCD based MS2 scan function was enabled with the following parameters specified: isolation mode, quadrupole; isolation window (m/z), 2; isolation offset, off; activation type, HCD; collision energy mode, stepped; HCD collision energies (%), 25, 35, and 45; detector type, Orbitrap; Orbitrap resolution, 120,000 (at m/z 200); mass range, normal; scan range mode, auto; AGC target, standard; maximum injection time mode, custom; maximum injection time, 35 ms; Microscans, 1; data type, profile; and, Use Easy-IC™, true.

Sample Preparations for Reverse-Phase Non-Targeted Metabolomics Method

A 10 µL volume of each cell-free bacterial conditioned media sample was diluted in a 90-µL volume of water that contained 1% formic acid and the sample was vortex-mixed briefly and centrifuged at 17,000*g* for 5 minutes. Then, the extract supernatant was transferred to an autosampler vial, and a 5-µL sample volume was injected onto the high-resolution LC-MS/MS system for bioanalysis.

LC-MS/MS Method for HILIC-based Non-Targeted Metabolomics

The HILIC based chromatographic separation used for non-targeted metabolomics measurement was performed using a SeQuant 5-micron polymer ZIC®-pHILIC (150 x 2.1 mm) analytical column produced by Millipore-Sigma (no guard column was used). For the positive ionization mode method, a MPA solution consisting of acetonitrile:water (95:5, *v:v*) with 10 mM ammonium formate and 2% formic acid, a MPB solution consisting of acetonitrile:water (1:1, *v:v*) with 10 mM ammonium formate and 2% formic acid were used. For the negative mode method, a MPA solution consisting of acetonitrile:water (95:5, *v:v*) with 10 mM ammonium formate (pH 5.2) and a MPB solution consisting of acetonitrile:water (1:1, *v:v*) with 10 mM ammonium formate (pH 5.2) were used. A common NW solution consisting of acetonitrile:water (1:1, *v:v*) was used for both chromatographic methods. In each instance, the mobile phase flowrate was 0.200 mL/min, the autosampler tray was chilled to 10°C, and the column oven was heated to 40°C. The gradient elution program was specified as follows: 0-3 min, 0% B; 3-25 min, 0-100% B; 25-30 min, 100% B; 30-31 min, 100-0% B; and, 31-40 min, 0% B; and at 40.01 min a Stop command is specified, and there is a 40.4 min duty cycle for each injection.

The heated electrospray (H-ESI) ionization source parameters for the Orbitrap Fusion MS were specified in the Xcalibur-based acquisition method as follows: application mode: small molecule; method duration: 39 minutes; ionization mode polarity, positive mode or negative mode; probe spray voltage, static at +3,500 V or -2,500 V; gas mode, static; sheath gas (arbitrary units), 35; auxiliary gas (arbitrary units), 7; sweep gas (arbitrary units), 0; ion transfer tube temperature, 300°C; and, vaporizer temperature, 275°C. Global MS acquisition parameters were specified as follows: infusion mode, liquid chromatography; expected LC peak width (s), 30; advanced peak determination, false;

mild trapping, false; default charge state, 1; enable Xcalibur AcquireX method modification, false; and, internal mass calibration, Easy-IC™.

The acquisition parameters for the HRMS/HRMS method for the HILIC-based chromatographic methods were specified as follows: detector type, Orbitrap; Orbitrap resolution, 120,000 (at m/z 200); mass range, normal; use quadrupole isolation, true; scan range (m/z), 65-650; RF lens (%), 60; AGC target, standard; maximum injection time mode, custom; maximum injection time (ms), 100; Microscans, 1; data type, profile; polarity, positive or negative; source fragmentation, disabled; and, use Easy-IC™, true. Dynamic exclusion filtering was enabled with the following parameters specified: exclude after n times, 1; exclusion duration (s), 6; mass tolerance, ppm; low, 10; high, 10; exclude isotopes, true; perform dependent scan on single charge state per precursor only, false; and, exclude within cycle, true. Apex detection filtering was enabled with the following parameters specified: expected peak width (FWHM, s), 10; desired apex window (%), 30. Intensity threshold filtering was enabled with the following parameters specified: filter type, intensity threshold; intensity threshold, 5.0e4. The ddMS2 OT HCD based MS2 scan function was enabled with the following parameters specified: isolation mode, quadrupole; isolation window (m/z), 2; isolation offset, off; activation type, HCD; collision energy mode, fixed; HCD collision energy type, normalized; HCD collision energies (%), 30; detector type, Orbitrap; Orbitrap resolution, 60,000 (at m/z 200); mass range, normal; scan range mode, auto; AGC target, standard; maximum injection time mode, custom; maximum injection time, 118 ms; Microscans, 1; data type, profile; and, Use Easy-IC™, true.

Sample Preparations for HILIC-based Non-Targeted Metabolomics Method

A 10 µL volume of each cell-free bacterial conditioned media sample was diluted in a 90-µL volume of acetonitrile that contained 1% formic acid and the sample was vortex-mixed briefly and centrifuged at 17,000*g* for 5 minutes. Then, the extract supernatant was transferred to an autosampler vial, and a 5-µL sample volume was injected onto the high-resolution LC-MS/MS system for bioanalysis.

Scaffold Elements Operational Parameters and Publicly Available Databases Used

Raw data files were imported into Scaffold Elements for final analysis. The Scaffold Elements workflow parameters used for non-targeted metabolomics data analysis were specified with the following search parameters: mass range (m/z), 70-700; retention time range, full range; match type, mass only; parent tolerance, 20.0 ppm; fragment tolerance, 0.1 Da; retention time (RT) tolerance, 0.5 min; treat MS1 peak group as single analyte, FALSE; Perform RT alignment, TRUE; perform feature reextraction, TRUE; report unknown analytes, TRUE; all analytes have MS2 data, FALSE. Feature finding parameters were specified as follows: noise threshold, 0.1% of max signal; minimum delta scan time, 0.5 sec. Reverse-phase and HILIC-based positive mode database searches used the following positively charged precursor ions including $[M+2H]^{2+}$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+H-NH_3]^+$, $[2M+H]^+$, $[M+H]^+$, and, $[M+H-H_2O]^+$. Negative mode HILIC database searches used the following negatively charged precursor ions including $[M-2H]^{2-}$, $[M-H]^-$, $[3M-H]^-$, $[2M-H]^-$, and, $[M-H-H_2O]^-$.

Publically available databases searched include the following: MoNA-export-MassBank.msp.libdb; MoNA-export-HMDB.msp.libdb; hmdb_library_elements.libdb; lipidmaps_library_elements.libdb; MoNA-export-Fiehn_HILIC.msp.libdb;

NIST_msms_v2017.11.27.libdb. Advanced search setting were specified as follows: ID score retention threshold: 0.7; ISF intensity threshold, 0.1 (10% spectrum max intensity); RT reproducibility threshold, 0.75 (75% reproducibility); RT Inclusion Threshold, 1.0 sec; RT cross-charge inclusion threshold, 1.0 sec; max aligned RT diff, 1 min; max unaligned RT diff, 5 min; ignore experimental MS2s, FALSE. Metabolite score thresholds include: ID score, 0.7; Log10Intensity, 0; reproducibility, 1. No statistical comparisons were performed using Scaffold Elements. All metabolomics data was manually filtered at a Tier 2 level of scrutiny for inclusion into downstream data analysis and association networking[124].

Tier 2 Metabolomics Data Filtering Procedures

All metabolomics data contained in the Excel-based results table were sorted according to their computed MS2 score (highest to lowest), and all metabolite features lacking an acquired MS2 spectra were removed from the data set. Metabolite features were then sorted by their analyte names, and any metabolite feature designated as a “cluster” was removed from the data set. Metabolite features were sorted by their retention time (RT), and all “pre-void” early eluting metabolites with RTs less than the computed void time (~1.4 mins) of the system were removed from the data set. Further, all metabolite features eluting after the gradient max (> 16.5 mins for the reverse phase method and >31.5 min for the HILIC methods) were also removed from the data set. Then, publically available databases were consulted to determine if each metabolite was to be classified as: isobaric bile acids, or isobaric mono-, di-, or tri-saccharides or hexose-amine sugars that require specialized chromatography for identification – these

metabolite features were removed were removed from the data set. All therapeutic drugs, drug metabolites, or exposome-based environmental compound (i.e., artificial sweeteners, flame retardants, or plasticizers) were flagged as such and were interrogated individual for possible in inclusion in the data set.

The remaining metabolite features are searched within Scaffold Elements for compliance with the following inclusion criteria: i) database matched based precursor (MS1) ions; ii) m/z and ion abundance of fragment ions (n≥3) match reference spectra; iii) consistent RTs observed in extracted-ion current (XIC) chromatogram overlays for all experimental data; and, iv) good agreement between empirical and reference isotope ratios[124]. All ions that do not satisfy the above exclusion criteria spelled out above are removed from downstream data processing, statistical testing, and association networking.

Statistical Analysis

Statistical analyses for overall metabolomic profiles were performed using the One-Factor Statistical Analysis module in MetaboAnalyst (version 6.0), a web-based platform dedicated for comprehensive metabolomics data interpretation. Data was normalized using the auto scaling function (mean-centering and division by the standard deviation of each variable). Significant differences were calculated by one-way analysis of variance (ANOVA) where metabolites with adjusted p-values (false discovery rate; FDR) less than 0.05 were determined to be statistically significant. Principal component analysis (PCA) plots were also generated using this MetaboAnalyst module. The Over Representation Enrichment Analysis module in MetaboAnalyst was utilized for pathway

analyses. Normalization was performed in the same manner as described above. We utilized SMPDB as the metabolite set library. The top seven hits for each species-diet combination were visualized.

Acknowledgements: We would like to express our deepest appreciation to the families that participated in this study. The Texas Children's Research Institute provides salary support to staff members for who work in the Virginia and L.E. Simmons Family Foundation Mass Spectrometry Laboratory Lab and purchased all of the reagents, consumables and durable supplies described. The graphical abstract was generated in Biorender.com

Data Availability: The raw data supporting the conclusions of this manuscript are openly available in the NIH Common Fund's National Metabolomics Data Repository (NMDR).

Competing Interests: TDH is an Editorial Board Member and is contracted as an Associate Academic Editor for Cell Press – STAR Protocols. TDH is a voluntary participant in the SCIEX Global Thought Leaders in Mass Spectrometry Program. All other authors have no competing interests to declare.

Author Contributions: Concept and design (AG, BP, MAE); intellectual contribution (KEC, TDH, MAE); data acquisition and analysis (AG, BP), interpretation (AG, KEC, MAE); drafting manuscript (AG), editing manuscript (BP, KEC, TDH, MAE); funding (KEC, TDH, MAE).

References

1. Hill, C.J.*, et al.* Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* **5**, 4 (2017).
2. Tanaka, M. & Nakayama, J. Development of the gut microbiota in infancy and its impact on health in later life. *Allergol Int* **66**, 515-522 (2017).
3. Yang, I.*, et al.* The Infant Microbiome: Implications for Infant Health and Neurocognitive Development. *Nurs Res* **65**, 76-88 (2016).
4. Zhang, H., Zhang, Z., Liao, Y., Zhang, W. & Tang, D. The Complex Link and Disease Between the Gut Microbiome and the Immune System in Infants. *Front Cell Infect Microbiol* **12**, 924119 (2022).
5. Underwood, M.A., Mukhopadhyay, S., Lakshminrusimha, S. & Bevins, C.L. Neonatal intestinal dysbiosis. *J Perinatol* **40**, 1597-1608 (2020).
6. Hoskinson, C.*, et al.* Delayed gut microbiota maturation in the first year of life is a hallmark of pediatric allergic disease. *Nat Commun* **14**, 4785 (2023).
7. Sanidad, K.Z. & Zeng, M.Y. Neonatal gut microbiome and immunity. *Curr Opin Microbiol* **56**, 30-37 (2020).
8. Stokholm, J.*, et al.* Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun* **9**, 141 (2018).
9. Depner, M.*, et al.* Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. *Nat Med* **26**, 1766-1775 (2020).
10. Carlson, A.L.*, et al.* Infant Gut Microbiome Associated With Cognitive Development. *Biol Psychiatry* **83**, 148-159 (2018).
11. Tzitiridou-Chatzopoulou, M., Kountouras, J. & Zournatzidou, G. The Potential Impact of the Gut Microbiota on Neonatal Brain Development and Adverse Health Outcomes. *Children (Basel)* **11**(2024).
12. Lu, J.*, et al.* Effects of Intestinal Microbiota on Brain Development in Humanized Gnotobiotic Mice. *Sci Rep* **8**, 5443 (2018).
13. Gregory, K.E.*, et al.* Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants. *Microbiome* **4**, 68 (2016).
14. Yi, D.Y. & Kim, S.Y. Human Breast Milk Composition and Function in Human Health: From Nutritional Components to Microbiome and MicroRNAs. *Nutrients* **13**(2021).
15. Zhang, X.*, et al.* The Composition and Concordance of Lactobacillus Populations of Infant Gut and the Corresponding Breast-Milk and Maternal Gut. *Front Microbiol* **11**, 597911 (2020).
16. Martín, R.*, et al.* Human milk is a source of lactic acid bacteria for the infant gut. *J Pediatr* **143**, 754-758 (2003).
17. Martín, R., Heilig, G.H., Zoetendal, E.G., Smidt, H. & Rodríguez, J.M. Diversity of the Lactobacillus group in breast milk and vagina of healthy women and potential role in the colonization of the infant gut. *J Appl Microbiol* **103**, 2638-2644 (2007).
18. Łubiech, K. & Twarużek, M. Lactobacillus Bacteria in Breast Milk. *Nutrients* **12**(2020).

19. Satokari, R.M.*, et al.* Diversity of Bifidobacterium and Lactobacillus spp. in Breast-Fed and Formula-Fed Infants as Assessed by 16S rDNA Sequence Differences. *Microbial Ecology in Health and Disease* **14**, 97-105 (2002).
20. Chau, K.*, et al.* Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating Lactobacillus reuteri DSM 17938. *J Pediatr* **166**, 74-78 (2015).
21. Baldassarre, M.E.*, et al.* Effectiveness and Safety of a Probiotic-Mixture for the Treatment of Infantile Colic: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial with Fecal Real-Time PCR and NMR-Based Metabolomics Analysis. *Nutrients* **10**(2018).
22. Indrio, F.*, et al.* The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. *J Pediatr* **152**, 801-806 (2008).
23. Zhang, W.*, et al.* Clinical efficacy of probiotics on feeding intolerance in preterm infants: a systematic review and meta-analysis. *Transl Pediatr* **11**, 229-238 (2022).
24. Dempsey, E. & Corr, S.C. Lactobacillus spp. for Gastrointestinal Health: Current and Future Perspectives. *Front Immunol* **13**, 840245 (2022).
25. Tang, H., Huang, W. & Yao, Y.F. The metabolites of lactic acid bacteria: classification, biosynthesis and modulation of gut microbiota. *Microb Cell* **10**, 49-62 (2023).
26. Krishna, M.*, et al.* Maternal Lactobacillus reuteri supplementation shifts the intestinal microbiome in mice and provides protection from experimental colitis in female offspring. *FASEB Bioadv* **4**, 109-120 (2022).
27. Engevik, M.*, et al.* Limosilactobacillus reuteri ATCC 6475 metabolites upregulate the serotonin transporter in the intestinal epithelium. *Benef Microbes* **12**, 583-599 (2021).
28. Engevik, M.A.*, et al.* Immunomodulation of dendritic cells by Lactobacillus reuteri surface components and metabolites. *Physiol Rep* **9**, e14719 (2021).
29. Chamberlain, M., O'Flaherty, S., Cobián, N. & Barrangou, R. Metabolomic Analysis of Lactobacillus acidophilus, L. gasseri, L. crispatus, and Lacticaseibacillus rhamnosus Strains in the Presence of Pomegranate Extract. *Front Microbiol* **13**, 863228 (2022).
30. Engevik, M.A.*, et al.* Reuterin disrupts Clostridioides difficile metabolism and pathogenicity through reactive oxygen species generation. *Gut Microbes* **12**, 1788898 (2020).
31. Hall, A.E., Engevik, M.A., Oezguen, N., Haag, A. & Versalovic, J. ClC transporter activity modulates histidine catabolism in Lactobacillus reuteri by altering intracellular pH and membrane potential. *Microb Cell Fact* **18**, 212 (2019).
32. Engevik, M.A.*, et al.* Microbial Metabolic Capacity for Intestinal Folate Production and Modulation of Host Folate Receptors. *Front Microbiol* **10**, 2305 (2019).
33. Engevik, K.A.*, et al.* Phylogenetically diverse bacterial species produce histamine. *Syst Appl Microbiol* **47**, 126539 (2024).
34. Miao, H.*, et al.* Lactobacillus species ameliorate membranous nephropathy through inhibiting the aryl hydrocarbon receptor pathway via tryptophan-produced indole metabolites. *Br J Pharmacol* **181**, 162-179 (2024).

35. Zhang, Q.*, et al.* Lactobacillus plantarum-derived indole-3-lactic acid ameliorates colorectal tumorigenesis via epigenetic regulation of CD8(+) T cell immunity. *Cell Metab* **35**, 943-960 e949 (2023).
36. Fong, W.*, et al.* Lactobacillus gallinarum-derived metabolites boost anti-PD1 efficacy in colorectal cancer by inhibiting regulatory T cells through modulating IDO1/Kyn/AHR axis. *Gut* **72**, 2272-2285 (2023).
37. Le, B., Anh, P.T.N. & Yang, S.H. Enhancement of the Anti-Inflammatory Effect of Mustard Kimchi on RAW 264.7 Macrophages by the Lactobacillus plantarum Fermentation-Mediated Generation of Phenolic Compound Derivatives. *Foods* **9**(2020).
38. Menard, S.*, et al.* Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut* **53**, 821-828 (2004).
39. Guo, S.*, et al.* Secreted Metabolites of Bifidobacterium infantis and Lactobacillus acidophilus Protect Immature Human Enterocytes from IL-1beta-Induced Inflammation: A Transcription Profiling Analysis. *PLoS One* **10**, e0124549 (2015).
40. Gu, Q.*, et al.* Lactobacillus plantarum ZJ316 alleviates ulcerative colitis by inhibiting inflammation and regulating short-chain fatty acid levels and the gut microbiota in a mouse model. *Food Funct* **14**, 3982-3993 (2023).
41. Vemuri, R.*, et al.* Lactobacillus acidophilus DDS-1 Modulates Intestinal-Specific Microbiota, Short-Chain Fatty Acid and Immunological Profiles in Aging Mice. *Nutrients* **11**(2019).
42. Xie, Z., Li, M., Qian, M., Yang, Z. & Han, X. Co-Cultures of Lactobacillus acidophilus and Bacillus subtilis Enhance Mucosal Barrier by Modulating Gut Microbiota-Derived Short-Chain Fatty Acids. *Nutrients* **14**(2022).
43. Xu, B.*, et al.* Milk-derived Lactobacillus with high production of short-chain fatty acids relieves antibiotic-induced diarrhea in mice. *Food Funct* **15**, 5329-5342 (2024).
44. Makki, K., Deehan, E.C., Walter, J. & Backhed, F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. *Cell Host Microbe* **23**, 705-715 (2018).
45. Stewart, M.L., Savarino, V. & Slavin, J.L. Assessment of dietary fiber fermentation: effect of Lactobacillus reuteri and reproducibility of short-chain fatty acid concentrations. *Mol Nutr Food Res* **53 Suppl 1**, S114-120 (2009).
46. Sinha, A.K.*, et al.* Dietary fibre directs microbial tryptophan metabolism via metabolic interactions in the gut microbiota. *Nat Microbiol* **9**, 1964-1978 (2024).
47. Huang, Z., Boekhorst, J., Fogliano, V., Capuano, E. & Wells, J.M. Impact of High-Fiber or High-Protein Diet on the Capacity of Human Gut Microbiota To Produce Tryptophan Catabolites. *J Agric Food Chem* **71**, 6956-6966 (2023).
48. Filannino, P., Bai, Y., Di Cagno, R., Gobbetti, M. & Ganzle, M.G. Metabolism of phenolic compounds by Lactobacillus spp. during fermentation of cherry juice and broccoli puree. *Food Microbiol* **46**, 272-279 (2015).
49. Odiase, E.*, et al.* The Gut Microbiota Differ in Exclusively Breastfed and Formula-Fed United States Infants and are Associated with Growth Status. *The Journal of Nutrition* **153**, 2612-2621 (2023).

50. Inchingolo, F.*, et al.* Difference in the Intestinal Microbiota between Breastfeed Infants and Infants Fed with Artificial Milk: A Systematic Review. *Pathogens* **13**(2024).
51. Mai, T.T., Tran, D.Q., Roos, S., Rhoads, J.M. & Liu, Y. Human Breast Milk Promotes the Secretion of Potentially Beneficial Metabolites by Probiotic Lactobacillus reuteri DSM 17938. *Nutrients* **11**(2019).
52. Repa, A.*, et al.* Probiotics (Lactobacillus acidophilus and Bifidobacterium infantis) prevent NEC in VLBW infants fed breast milk but not formula [corrected]. *Pediatr Res* **77**, 381-388 (2015).
53. Klopp, A.*, et al.* Modes of Infant Feeding and the Risk of Childhood Asthma: A Prospective Birth Cohort Study. *J Pediatr* **190**, 192-199 e192 (2017).
54. El-Heneidy, A., Abdel-Rahman, M.E., Mihala, G., Ross, L.J. & Comans, T.A. Milk Other Than Breast Milk and the Development of Asthma in Children 3 Years of Age. A Birth Cohort Study (2006(-)2011). *Nutrients* **10**(2018).
55. McGuire, W. & Anthony, M.Y. Donor human milk versus formula for preventing necrotising enterocolitis in preterm infants: systematic review. *Arch Dis Child Fetal Neonatal Ed* **88**, F11-14 (2003).
56. Altobelli, E., Angeletti, P.M., Verrotti, A. & Petrocelli, R. The Impact of Human Milk on Necrotizing Enterocolitis: A Systematic Review and Meta-Analysis. *Nutrients* **12**(2020).
57. Boyd, C.A., Quigley, M.A. & Brocklehurst, P. Donor breast milk versus infant formula for preterm infants: systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed* **92**, F169-175 (2007).
58. Cortez, J.*, et al.* Maternal milk feedings reduce sepsis, necrotizing enterocolitis and improve outcomes of premature infants. *J Perinatol* **38**, 71-74 (2018).
59. Fernandez, L.*, et al.* The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res* **69**, 1-10 (2013).
60. Yang, B.*, et al.* Bifidobacterium and Lactobacillus Composition at Species Level and Gut Microbiota Diversity in Infants before 6 Weeks. *Int J Mol Sci* **20**(2019).
61. Urbanska, M. & Szajewska, H. The efficacy of Lactobacillus reuteri DSM 17938 in infants and children: a review of the current evidence. *Eur J Pediatr* **173**, 1327-1337 (2014).
62. Roggero, P.*, et al.* Analysis of immune, microbiota and metabolome maturation in infants in a clinical trial of Lactobacillus paracasei CBA L74-fermented formula. *Nat Commun* **11**, 2703 (2020).
63. Murakami, K., Habukawa, C., Nobuta, Y., Moriguchi, N. & Takemura, T. The effect of Lactobacillus brevis KB290 against irritable bowel syndrome: a placebo-controlled double-blind crossover trial. *Biopsychosoc Med* **6**, 16 (2012).
64. Manzoni, P.*, et al.* Routine Lactobacillus rhamnosus GG administration in VLBW infants: a retrospective, 6-year cohort study. *Early Hum Dev* **87 Suppl 1**, S35-38 (2011).
65. Suzuki, Y.*, et al.* Identification of antioxidants produced by Lactobacillus plantarum. *Biosci Biotechnol Biochem* **77**, 1299-1302 (2013).
66. Gu, X.*, et al.* The antioxidant activity and metabolomic analysis of the supernatant of Streptococcus alactolyticus strain FGM. *Sci Rep* **14**, 8413 (2024).

67. Vogie, K., Mantick, N. & Carlson, G. Metabolism and toxicity of the styrene metabolite 4-vinylphenol in CYP2E1 knockout mice. *J Toxicol Environ Health A* **67**, 145-152 (2004).
68. Carlson, G.P., Ullman, M., Mantick, N.A. & Snyder, P.W. 4-Vinylphenol-induced pneumotoxicity and hepatotoxicity in mice. *Toxicol Pathol* **30**, 565-569 (2002).
69. Girawale, S.D., Meena, S.N., Yadav, A.A. & Kodam, K.M. Biocatalysis at its finest: Bacillus paralicheniformis produces 4-vinylphenol with potent biological activities. *Biocatalysis and Agricultural Biotechnology* **52**(2023).
70. Rodriguez, A., Meadows, J.A., Sun, N., Simmons, B.A. & Gladden, J.M. Evaluation of bacterial hosts for conversion of lignin-derived p-coumaric acid to 4-vinylphenol. *Microb Cell Fact* **20**, 181 (2021).
71. Santamaria, L., Reveron, I., de Felipe, F.L., de Las Rivas, B. & Munoz, R. Ethylphenol Formation by Lactobacillus plantarum: Identification of the Enzyme Involved in the Reduction of Vinylphenols. *Appl Environ Microbiol* **84**(2018).
72. Ehrlich, A.M.*, et al.* Indole-3-lactic acid associated with Bifidobacterium-dominated microbiota significantly decreases inflammation in intestinal epithelial cells. *BMC Microbiol* **20**, 357 (2020).
73. Meng, D.*, et al.* Indole-3-lactic acid, a metabolite of tryptophan, secreted by Bifidobacterium longum subspecies infantis is anti-inflammatory in the immature intestine. *Pediatr Res* **88**, 209-217 (2020).
74. Cristina, M.O.*, et al.* Mechanisms and Therapeutic Potential of Key Anti-inflammatory Metabiotics: Trans-Vaccenic Acid, Indole-3-Lactic Acid, Thiamine, and Butyric Acid. *Probiotics Antimicrob Proteins* (2025).
75. Kim, K., Kim, H. & Sung, G.Y. Effects of Indole-3-Lactic Acid, a Metabolite of Tryptophan, on IL-4 and IL-13-Induced Human Skin-Equivalent Atopic Dermatitis Models. *Int J Mol Sci* **23**(2022).
76. Qian, X.*, et al.* Bifidobacteria with indole-3-lactic acid-producing capacity exhibit psychobiotic potential via reducing neuroinflammation. *Cell Rep Med* **5**, 101798 (2024).
77. Bassett, C.M.*, et al.* Dietary vaccenic acid has antiatherogenic effects in LDLr-/- mice. *J Nutr* **140**, 18-24 (2010).
78. Valenzuela, C.A., Baker, E.J., De Souza, C.O., Miles, E.A. & Calder, P.C. Differential Effects of Ruminant and Industrial 18-Carbon trans-Monounsaturated Fatty Acids (trans Vaccenic and Elaidic) on the Inflammatory Responses of an Endothelial Cell Line. *Molecules* **26**(2021).
79. Jacome-Sosa, M.*, et al.* Vaccenic acid suppresses intestinal inflammation by increasing anandamide and related N-acylethanolamines in the JCR:LA-cp rat. *J Lipid Res* **57**, 638-649 (2016).
80. Ory, R.L., Hood, D.W. & Lyman, C.M. The role of glutamine in the synthesis of arginine by Lactobacillus arabinosus. *J Biol Chem* **207**, 267-273 (1954).
81. Oleskin, A.*, et al.* Lactic-Acid Bacteria Supplement Fermented Dairy Products with Human Behavior-Modifying Neuroactive Compounds. *Journal of Pharmacy and Nutrition Sciences* **4**, 199-206 (2014).
82. Miller, C. & Jimenez-Flores, R.J. The Ohio State University (2023).
83. Wu, G., Fang, Y.Z., Yang, S., Lupton, J.R. & Turner, N.D. Glutathione metabolism and its implications for health. *J Nutr* **134**, 489-492 (2004).

84. Barker-Tejeda, T.C.*, et al.* Comparative characterization of the infant gut microbiome and their maternal lineage by a multi-omics approach. *Nature Communications* **15**, 3004 (2024).
85. Vallès, Y.*, et al.* Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. *PLoS genetics* **10**, e1004406 (2014).
86. Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A. & Brown, P.O. Development of the human infant intestinal microbiota. *PLoS biology* **5**, e177 (2007).
87. Hill, C.J.*, et al.* Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* **5**, 4 (2017).
88. Hattoufi, K.*, et al.* Association of gut microbiota and type of feeding: Molecular analysis of a cohort of preterm moroccan newborns. *Pediatr Neonatol* (2025).
89. Dawod, B., Marshall, J.S. & Azad, M.B. Breastfeeding and the developmental origins of mucosal immunity: how human milk shapes the innate and adaptive mucosal immune systems. *Curr Opin Gastroenterol* **37**, 547-556 (2021).
90. Lopez, D.A.*, et al.* Breastfeeding Duration Is Associated With Domain-Specific Improvements in Cognitive Performance in 9-10-Year-Old Children. *Front Public Health* **9**, 657422 (2021).
91. Bene, J.*, et al.* Differences in circulating carnitine status of preterm infants fed fortified human milk or preterm infant formula. *J Pediatr Gastroenterol Nutr* **57**, 673-676 (2013).
92. Whitfield, J., Smith, T., Sollohub, H., Sweetman, L. & Roe, C.R. Clinical effects of L-carnitine supplementation on apnea and growth in very low birth weight infants. *Pediatrics* **111**, 477-482 (2003).
93. Shortland, G.J.*, et al.* Randomised controlled trial of L-carnitine as a nutritional supplement in preterm infants. *Arch Dis Child Fetal Neonatal Ed* **78**, F185-188 (1998).
94. Rajamani, S.*, et al.* The vitamin riboflavin and its derivative lumichrome activate the LasR bacterial quorum-sensing receptor. *Mol Plant Microbe Interact* **21**, 1184-1192 (2008).
95. Lopez, B.R., Palacios, O.A., Bashan, Y., Hernandez-Sandoval, F.E. & de-Bashan, L.E. Riboflavin and lumichrome exuded by the bacterium Azospirillum brasilense promote growth and changes in metabolites in Chlorella sorokiniana under autotrophic conditions. *Algal Research* **44**(2019).
96. Yamamoto, K. & Asano, Y. Efficient production of lumichrome by Microbacterium sp. strain TPU 3598. *Appl Environ Microbiol* **81**, 7360-7367 (2015).
97. Phillips, D.A.*, et al.* Identification of lumichrome as a sinorhizobium enhancer of alfalfa root respiration and shoot growth. *Proc Natl Acad Sci U S A* **96**, 12275-12280 (1999).
98. Dakora, F.D., Matiru, V.N. & Kanu, A.S. Rhizosphere ecology of lumichrome and riboflavin, two bacterial signal molecules eliciting developmental changes in plants. *Front Plant Sci* **6**, 700 (2015).
99. Robledo, D.A. & Muhammad, G. Evaluation of the Cytotoxic Properties of Lumichrome-Derived Compounds on Breast Adenocarcinoma, Colorectal Cancer, and Liver Carcinoma. *Central Asian Journal of Medicine and Natural Sciences* **2**(2022).

100. Chantarawong, W., Kuncharoen, N., Tanasupawat, S. & Chanvorachote, P. Lumichrome Inhibits Human Lung Cancer Cell Growth and Induces Apoptosis via a p53-Dependent Mechanism. *Nutr Cancer* **71**, 1390-1402 (2019).
101. Seferovic, M.D.*, et al.* Maternal diet alters human milk oligosaccharide composition with implications for the milk metagenome. *Sci Rep* **10**, 22092 (2020).
102. Xu, R.*, et al.* The human milk microbiome is minimally associated with breastfeeding practices. *Scientific Reports* **15**, 19308 (2025).
103. Moossavi, S.*, et al.* Composition and Variation of the Human Milk Microbiota Are Influenced by Maternal and Early-Life Factors. *Cell Host & Microbe* **25**, 324-335.e324 (2019).
104. Tran, L.C.*, et al.* The metabolome of human milk is altered differentially by Holder pasteurization and high hydrostatic pressure processing. *Front Nutr* **10**, 1107054 (2023).
105. Ten-Domenech, I.*, et al.* The effect of Holder pasteurization on the lipid and metabolite composition of human milk. *Food Chem* **384**, 132581 (2022).
106. Waller, M.E.*, et al.* Analyzing the Responses of Enteric Bacteria to Neonatal Intensive Care Supplements. *Int J Microbiol* **2024**, 3840327 (2024).
107. Horvath, T.D.*, et al.* Interrogation of the mammalian gut–brain axis using LC–MS/MS-based targeted metabolomics with in vitro bacterial and organoid cultures and in vivo gnotobiotic mouse models. *Nature Protocols* **18**, 490-529 (2023).
108. Engevik, M.A., Stripe, L.K., Baatz, J.E., Wagner, C.L. & Chetta, K.E. Identifying single-strain growth patterns of human gut microbes in response to preterm human milk and formula. *Food Funct* **13**, 5571-5589 (2022).
109. Engevik, M.A.*, et al.* The metabolic profile of Bifidobacterium dentium reflects its status as a human gut commensal. *BMC Microbiol* **21**, 154 (2021).
110. Engevik, M.A.*, et al.* Bifidobacterium dentium-derived y-glutamylcysteine suppresses ER-mediated goblet cell stress and reduces TNBS-driven colonic inflammation. *Gut Microbes* **13**, 1-21 (2021).
111. Davoodabadi, A., Dallal, M., Ebrahimi, M. & Rajabi, Z. Colonization of Lactobacilli among the Fecal Microflora of Healthy Iranian Breast-Fed Infants. *Journal of Clinical Gastroenterology and Treatment* **6**(2020).
112. Demirok, N., Durak, M. & Arici, M. Probiotic lactobacilli in faeces of breastfed babies. *Food Science and Technology* **42**(2021).
113. Haarman, M. & Knol, J. Quantitative real-time PCR analysis of fecal Lactobacillus species in infants receiving a prebiotic infant formula. *Appl Environ Microbiol* **72**, 2359-2365 (2006).
114. Al Kassaa, I.*, et al.* Characterization of lactobacilli strains isolated from baby's feces for their potential immunobiotic application. *Iran J Microbiol* **11**, 379-388 (2019).
115. Fortmann, I.*, et al.* Lactobacillus Acidophilus/Bifidobacterium Infantis Probiotics Are Beneficial to Extremely Low Gestational Age Infants Fed Human Milk. *Nutrients* **12**(2020).
116. Waki, N., Matsumoto, M., Fukui, Y. & Suganuma, H. Effects of probiotic Lactobacillus brevis KB290 on incidence of influenza infection among schoolchildren: an open-label pilot study. *Lett Appl Microbiol* **59**, 565-571 (2014).

117. Haschke-Becher, E.*, et al.* Urinary D-lactate excretion in infants receiving Lactobacillus johnsonii with formula. *Ann Nutr Metab* **53**, 240-244 (2008).
118. Wang, S.*, et al.* A randomized, double blind, parallel, placebo-controlled study to investigate the efficacy of Lactobacillus paracasei N1115 in gut development of young children. *Food Sci Nutr* **9**, 6020-6030 (2021).
119. Spreckels, J.E.*, et al.* Lactobacillus reuteri Colonisation of Extremely Preterm Infants in a Randomised Placebo-Controlled Trial. *Microorganisms* **9**(2021).
120. Vendt, N.*, et al.* Growth during the first 6 months of life in infants using formula enriched with Lactobacillus rhamnosus GG: double-blind, randomized trial. *J Hum Nutr Diet* **19**, 51-58 (2006).
121. Vivatvakin, B. & Kowitdamrong, E. Randomized control trial of live Lactobacillus acidophilus plus Bifidobacterium infantis in treatment of infantile acute watery diarrhea. *J Med Assoc Thai* **89 Suppl 3**, S126-133 (2006).
122. Savino, F.*, et al.* Lactobacillus reuteri DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. *Pediatrics* **126**, e526-533 (2010).
123. Basturk, A., Isik, I., Atalay, A. & Yilmaz, A. Investigation of the Efficacy of Lactobacillus rhamnosus GG in Infants With Cow's Milk Protein Allergy: a Randomised Double-Blind Placebo-Controlled Trial. *Probiotics Antimicrob Proteins* **12**, 138-143 (2020).
124. Schymanski, E.L.*, et al.* Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. *Environmental Science & Technology* **48**, 2097-2098 (2014).

Figure 1. Metabolomic profiles demonstrate interspecies differences among six tested lactobacilli species when supplemented with human milk, infant formula, or water. (**A**) PCA plot and (**B**) metabolite intersection plot for lactobacilli species grown in chemically defined medium supplemented with 10% v/v water as a control. (**C**) PCA plot and (**D**) metabolite intersection plot for lactobacilli species grown in chemically defined medium supplemented with 10% v/v human milk. Lactobacilli Intersection plots were generated using the UpSetR package in R.

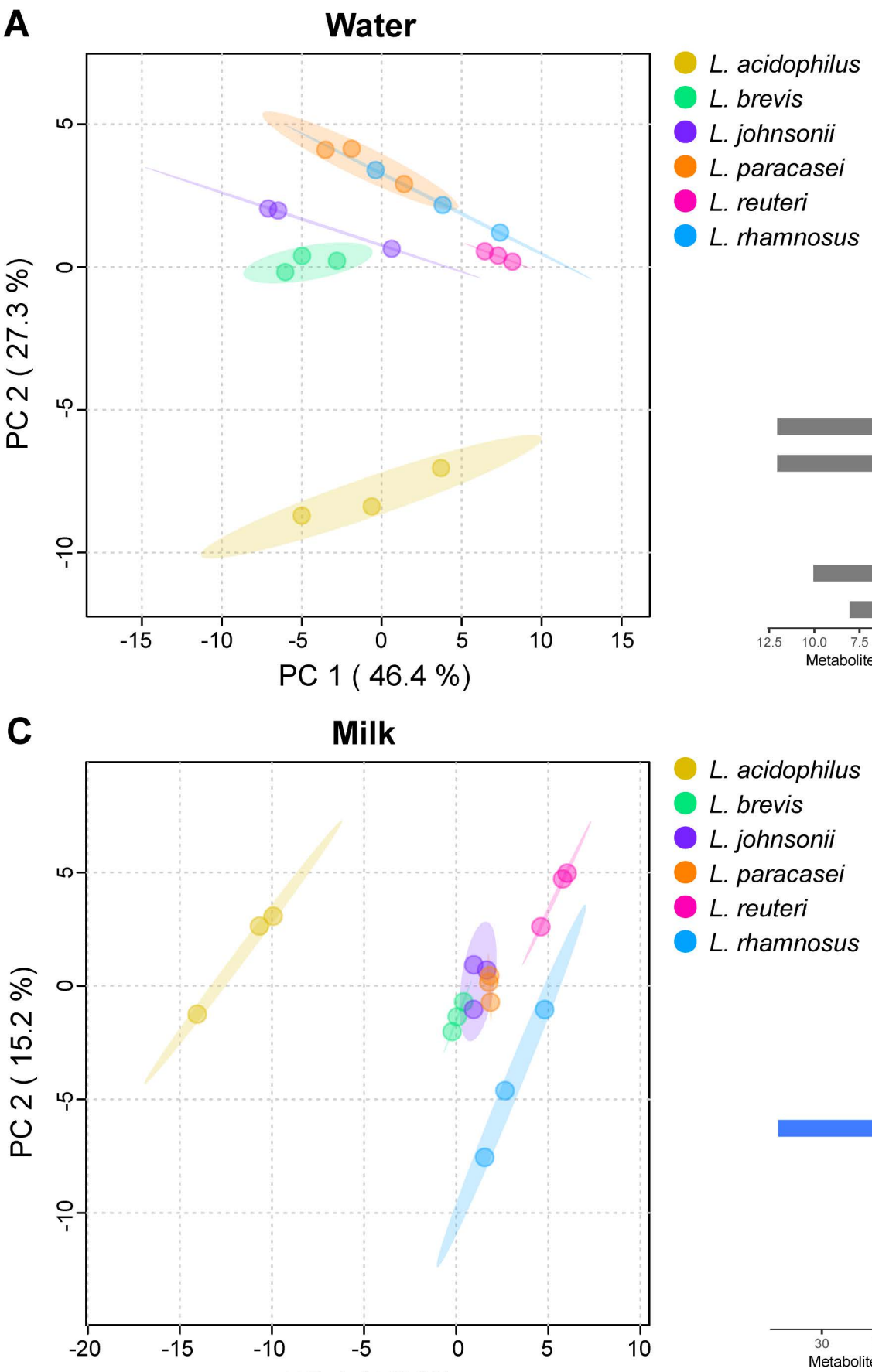
Figure 2. Lactobacilli species produce distinct metabolomic signatures when supplemented with human milk, infant formula, or water. PCA plots for each species (*L. acidophilus*, *L. brevis*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*) demonstrating clusters by media condition (water, human milk, or infant formula). Data were visualized using MetaboAnalyst.

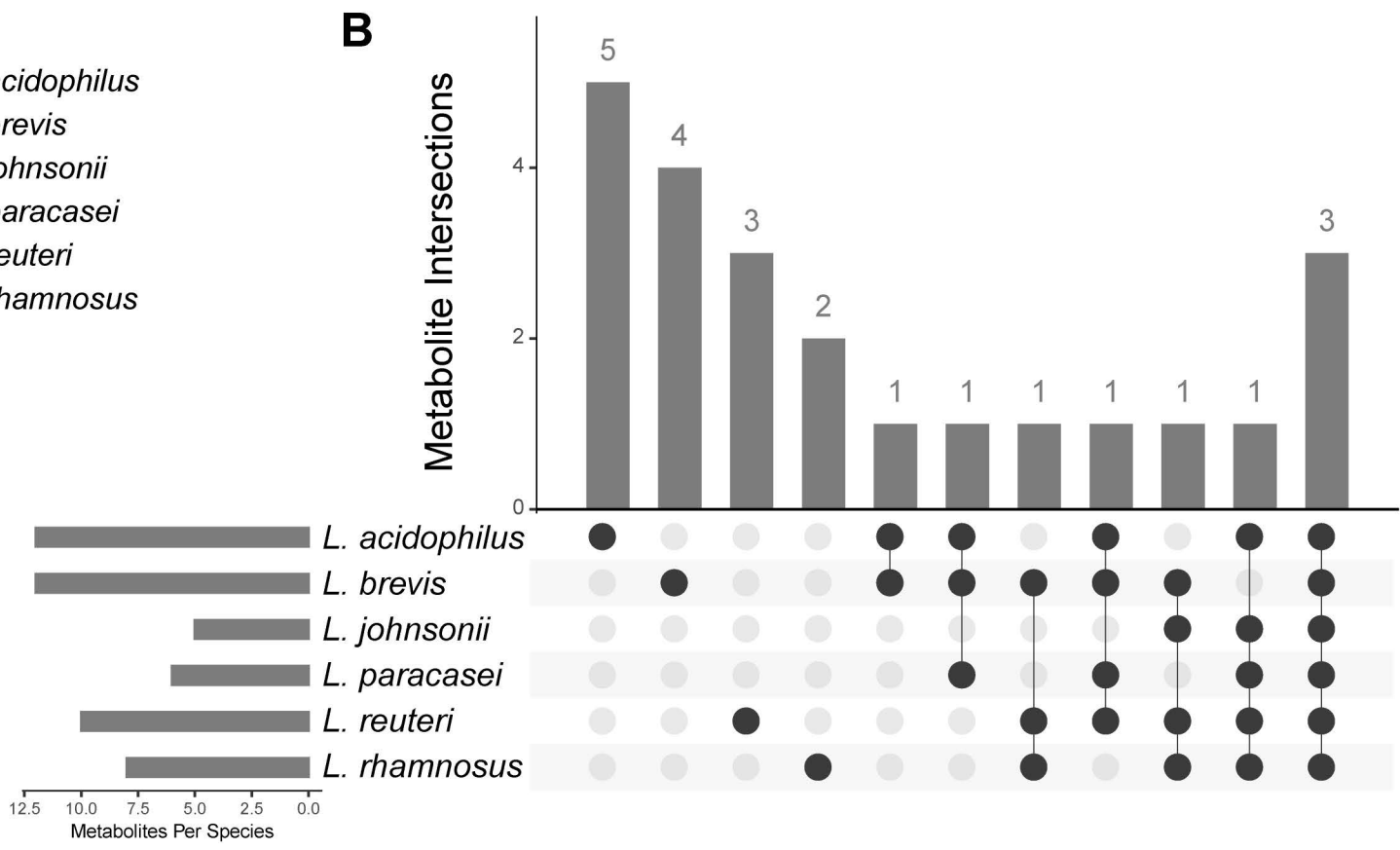
Figure 3. Lactobacilli-produced metabolites differ depending on media condition, with human milk cultures containing the highest number of uniquely produced metabolites. Venn diagrams for each species (*L. acidophilus*, *L. brevis*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*) demonstrating number of shared or uniquely produced metabolites per media condition. Metabolites of interest are labeled for each Venn diagram.

Figure 4. Results from untargeted metabolomics reveal that compared to water as a control, numerous metabolites are significantly consumed or generated when lactobacilli are cultured with human milk or infant formula. Dot plots for each species (*L. acidophilus*, *L. brevis*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*) demonstrating metabolite abundances (counts) by color scale with significant differences compared to the water control denoted by dot size. For each species, the left-hand list contains amino acids, nucleic acids, and their derivates, while the right-hand list contains other organic compounds.

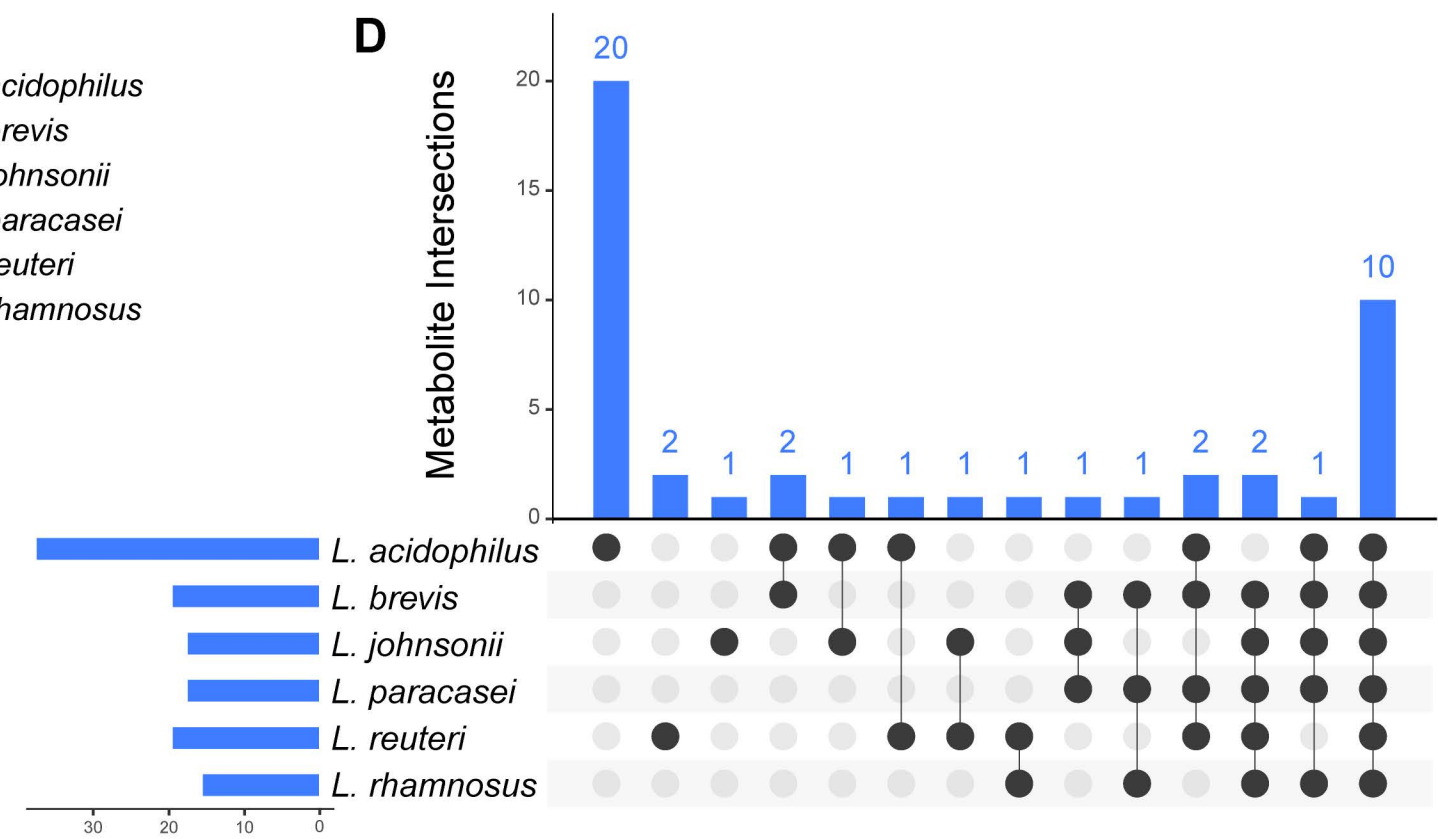
Figure 5. Levels of numerous metabolites with physiological relevance are significantly increased or decreased when lactobacilli are supplemented with human milk or infant formula. Heat plots of metabolite abundance (Counts) by the six tested lactobacilli species for (**A**) hydroxyphenyllactic acid, (**B**) tyrosine, (**C**) lumichrome, (**D**) 4-vinylphenol, (**E**) indolelactic acid, (**F**) vaccenic acid, (**G**) isonicotinic acid, and (**H**) carnitine. Significant differences were determined using two-way ANOVA (*<0.05, **<0.01, ***<0.001, ****<0.0001).

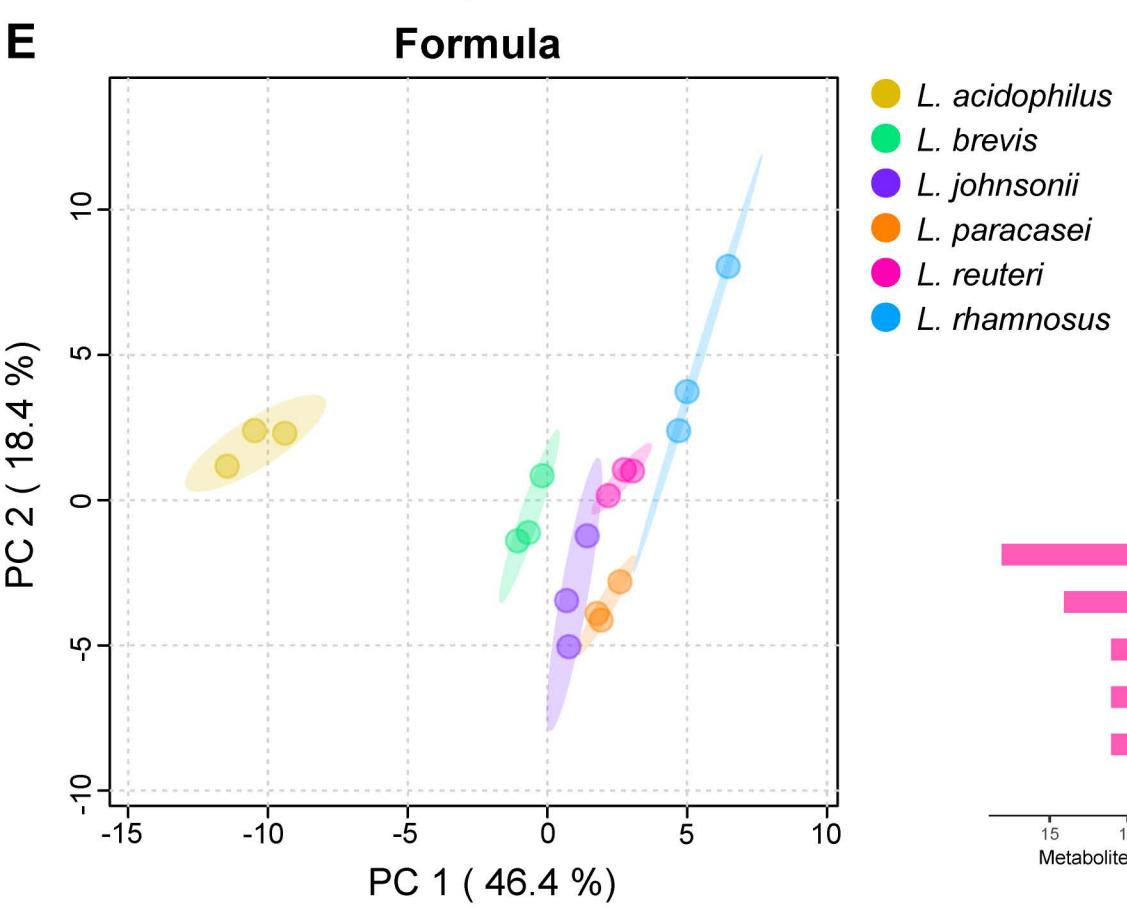
Figure 6. Pathway enrichment analyses demonstrate similarities and differences between lactobacilli cultured with human milk versus infant formula. For each species (*L. acidophilus*, *L. brevis*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*), the top seven hits for enriched pathways for human milk and infant formula are presented in order of p-value, and those that were significant ($p<0.05$) are denoted by an asterisk. Pathway enrichment analyses were performed using MetaboAnalyst.

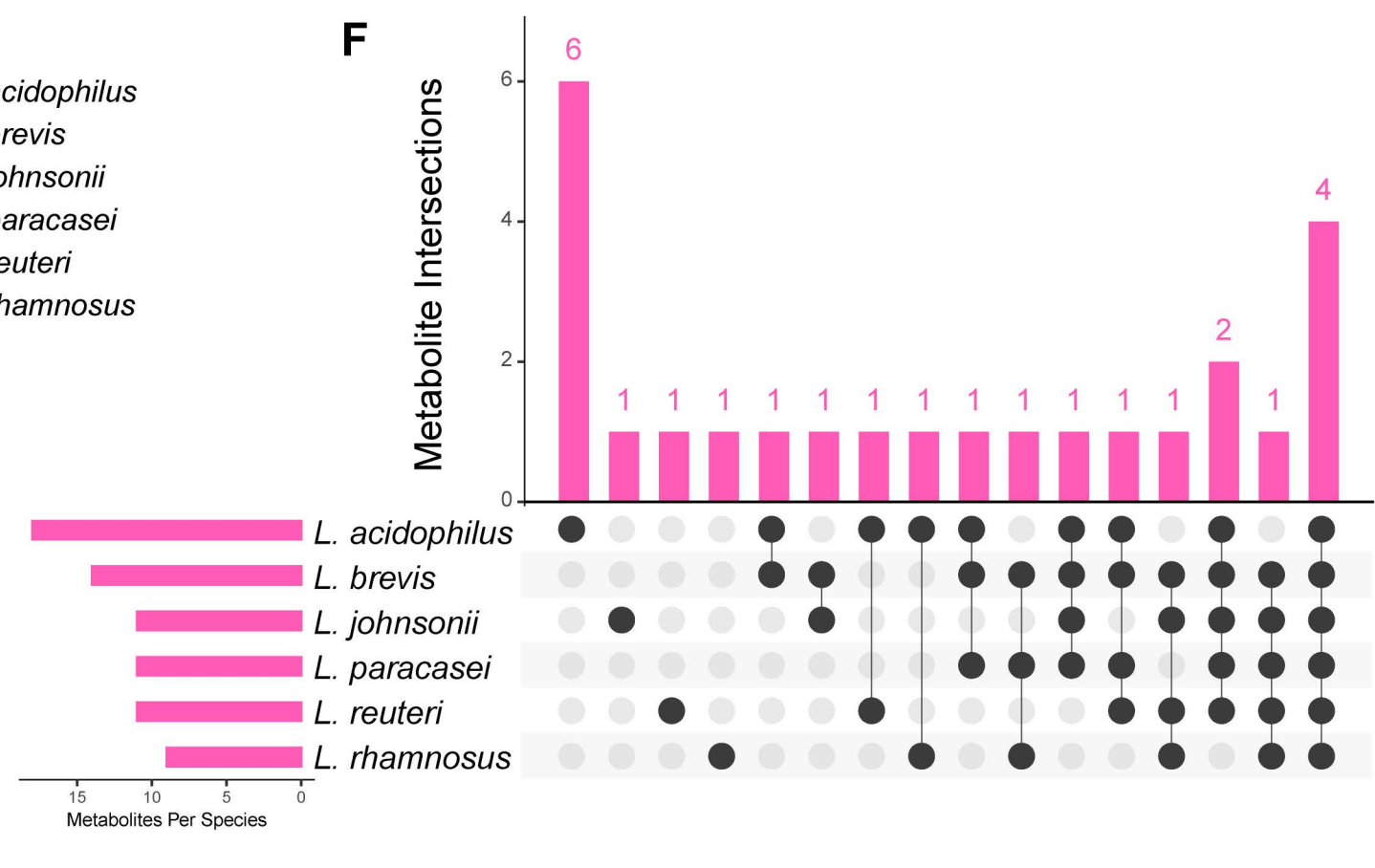

A
Water
PC 2 (27.3 %)
PC 1 (46.4 %)
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus


B
Metabolite Intersections
Metabolites Per Species

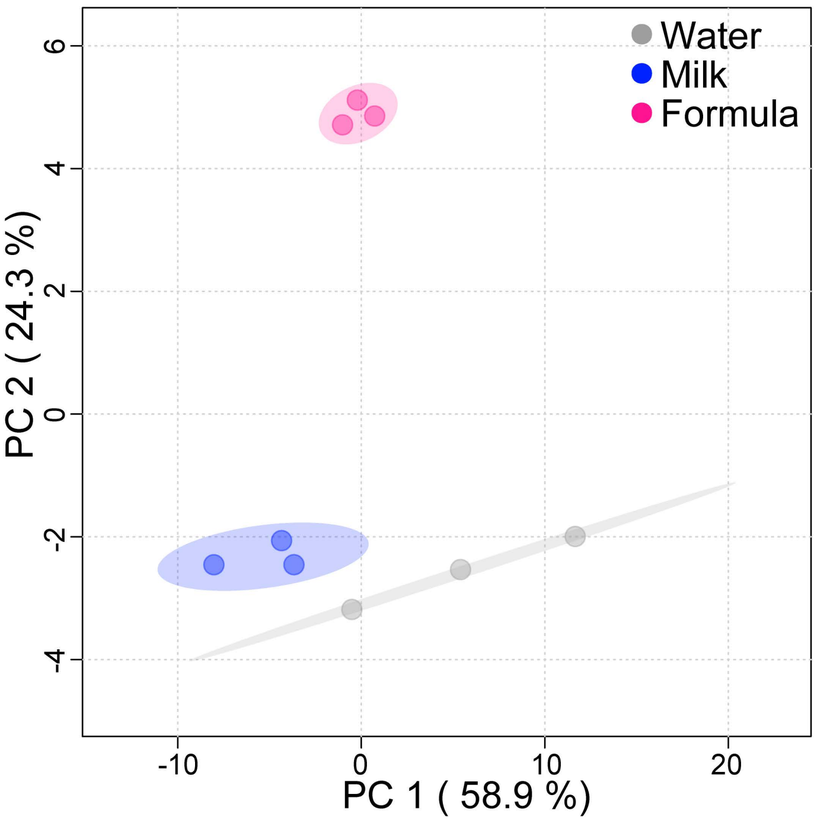
C
Milk
PC 2 (15.2 %)
PC 1 (52 %)
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus


D
Metabolite Intersections
Metabolites Per Species

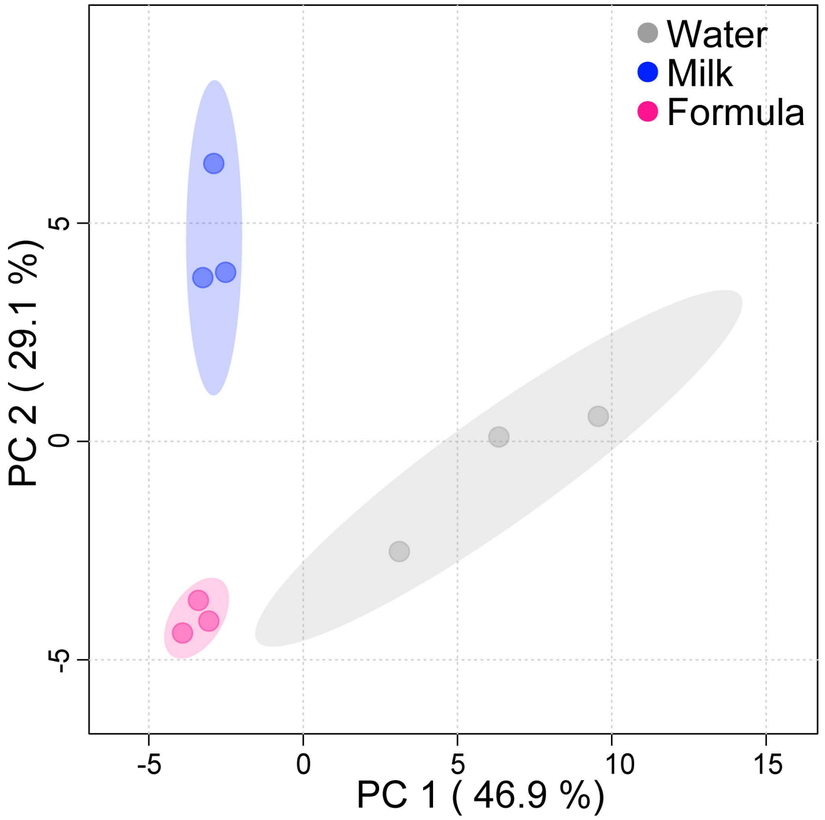

E
Formula
PC 2 (18.4 %)
PC 1 (46.4 %)
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus


F
Metabolite Intersections
Metabolites Per Species

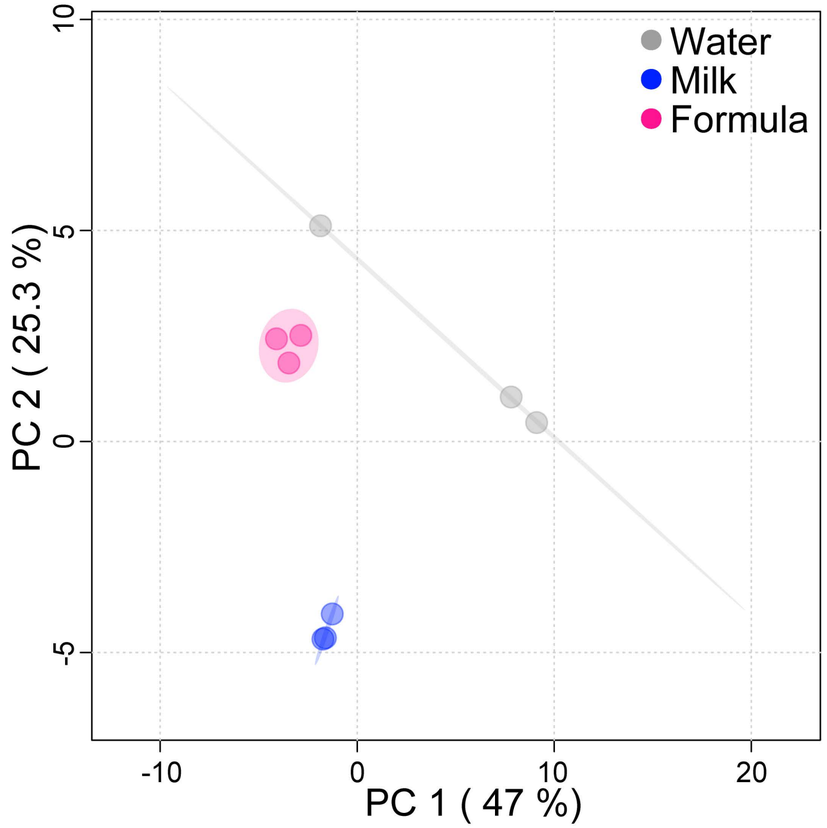
L. acidophilus



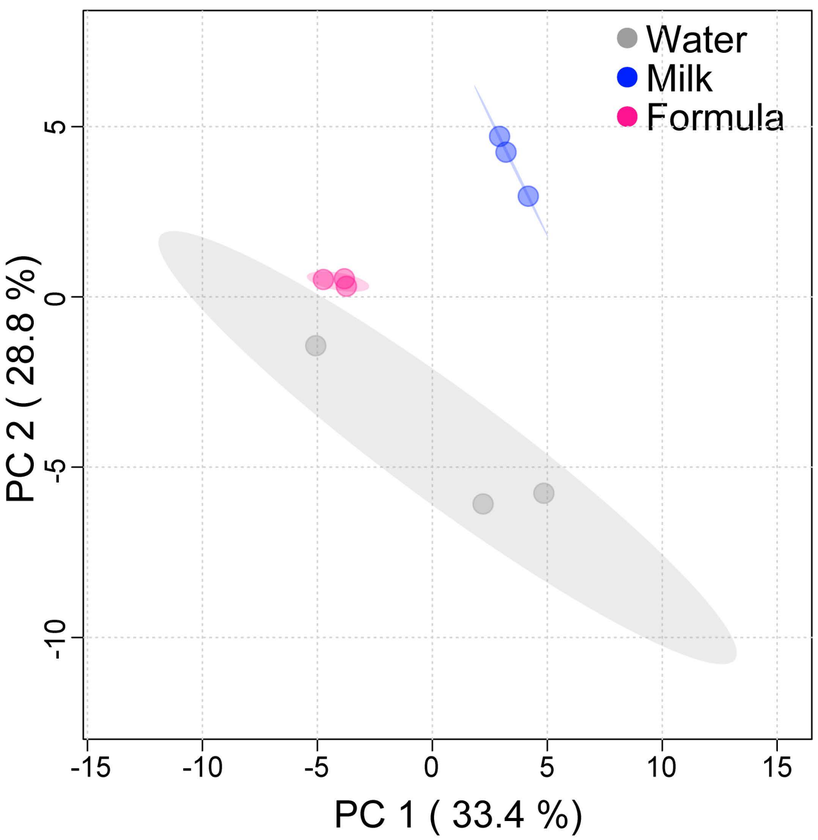
L. brevis



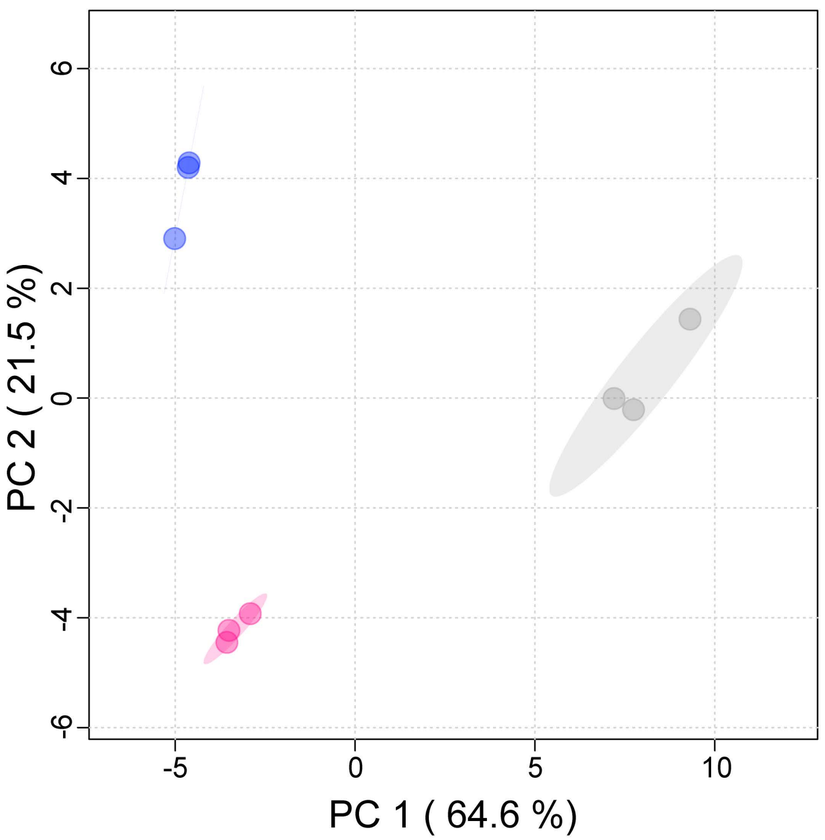
L. johnsonii



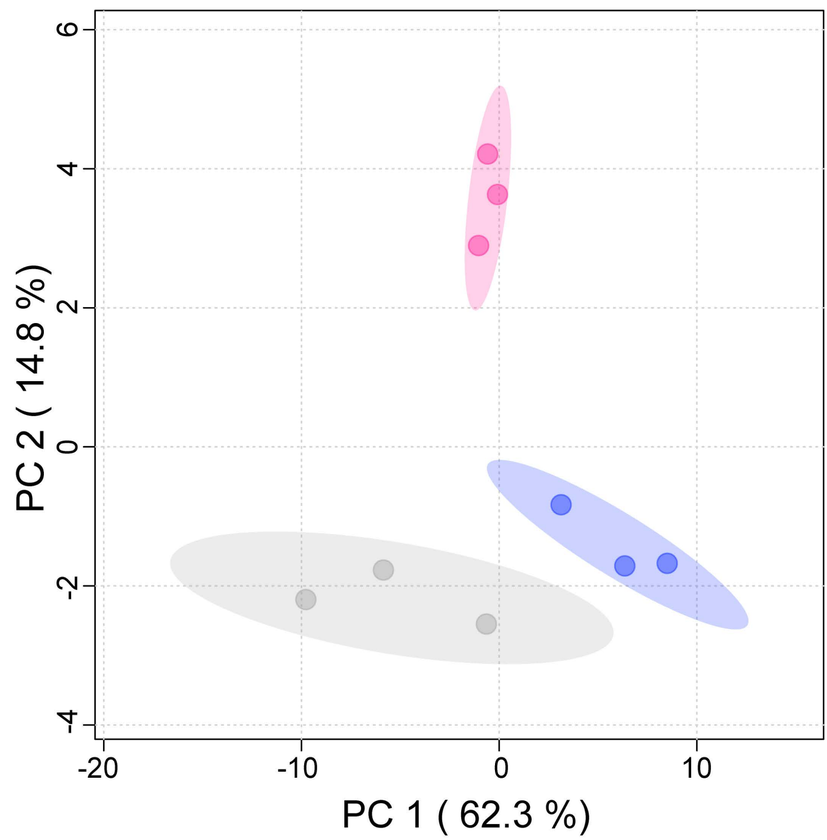
L. paracasei



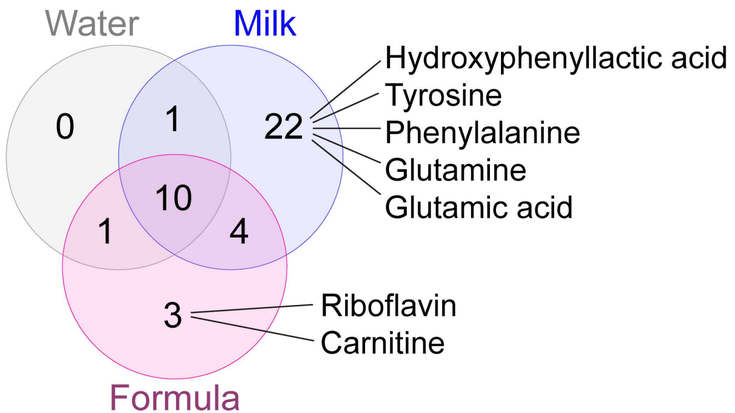
L. reuteri



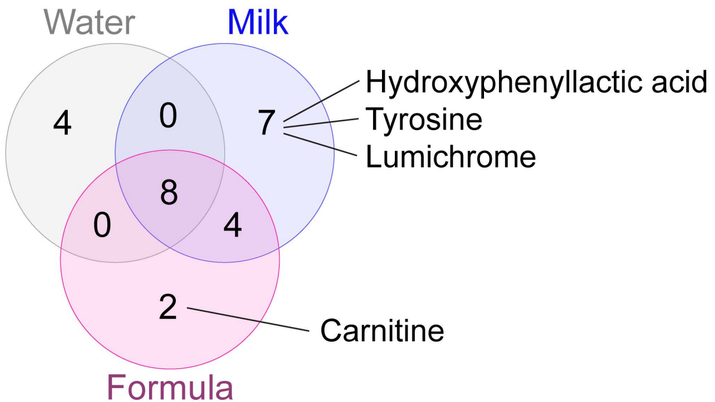
L. rhamnosus



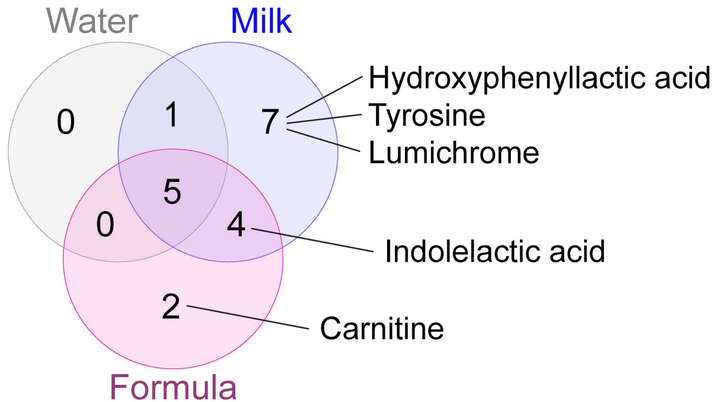
L. acidophilus



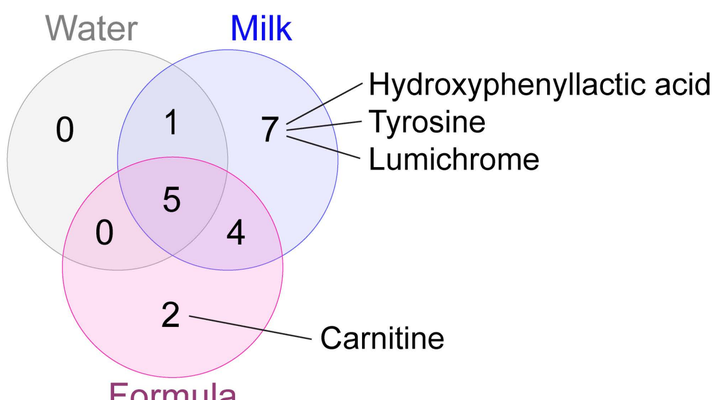
L. brevis



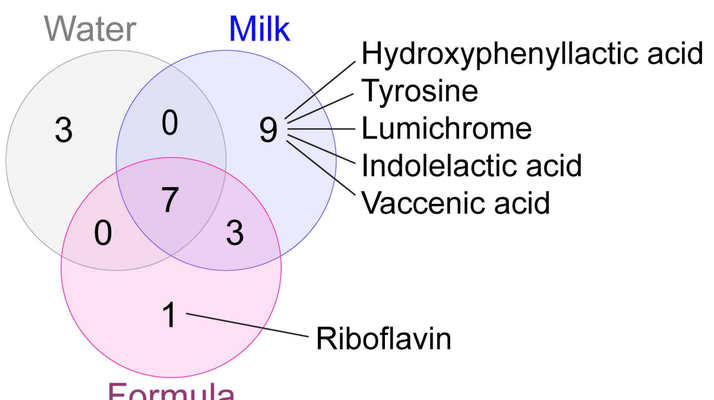
L. johnsonii



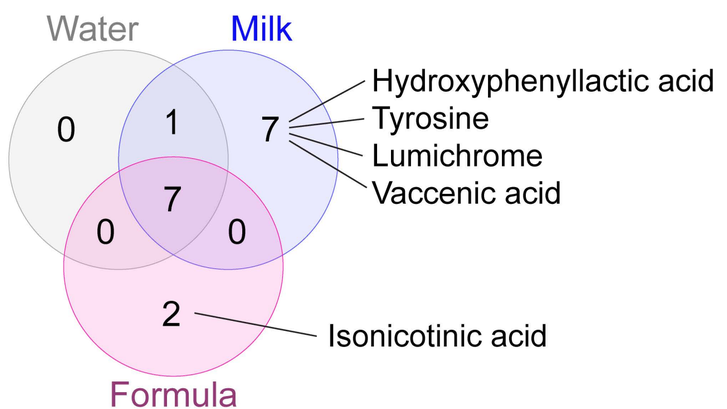
L. paracasei



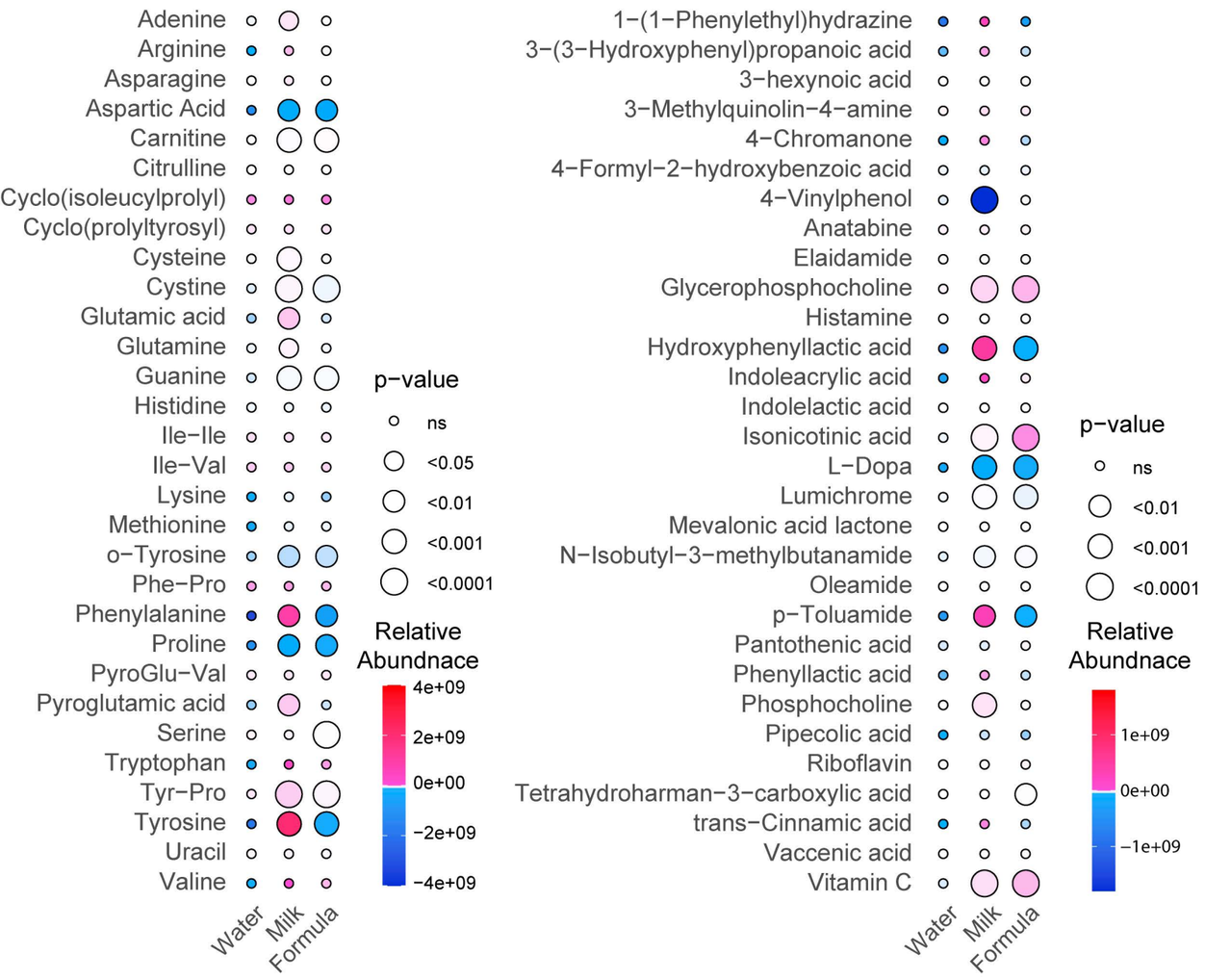
L. reuteri



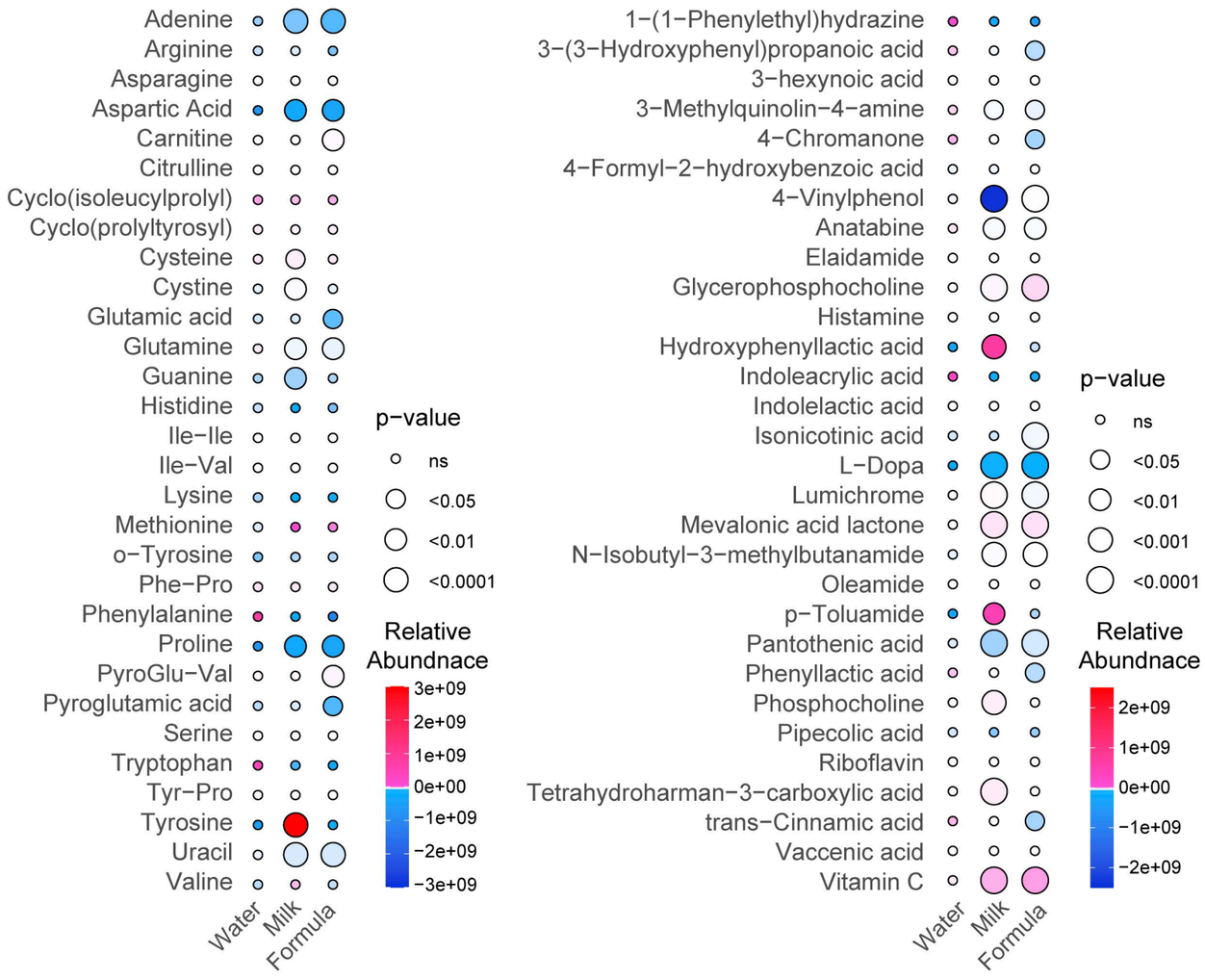
L. rhamnosus



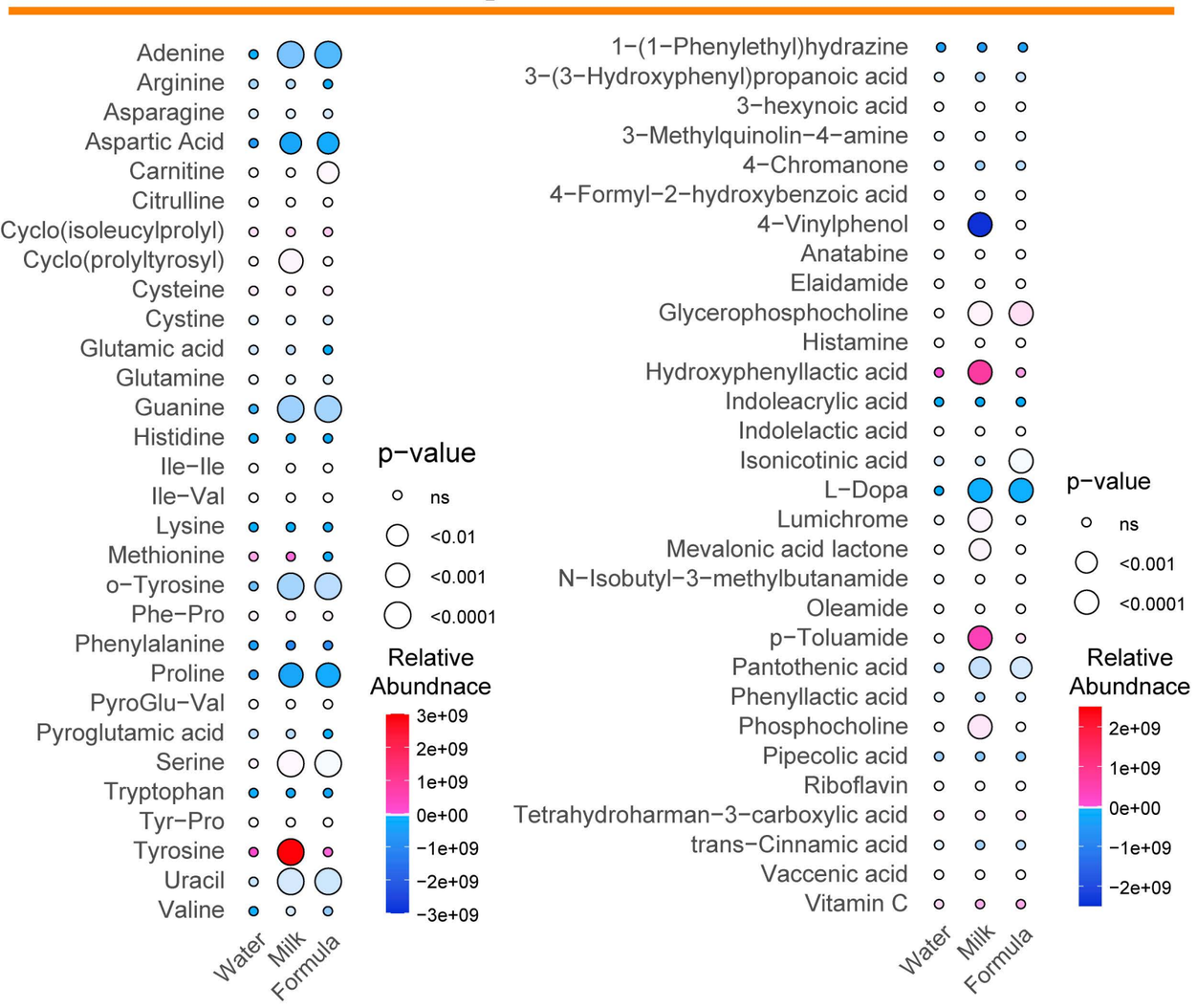
L. acidophilus



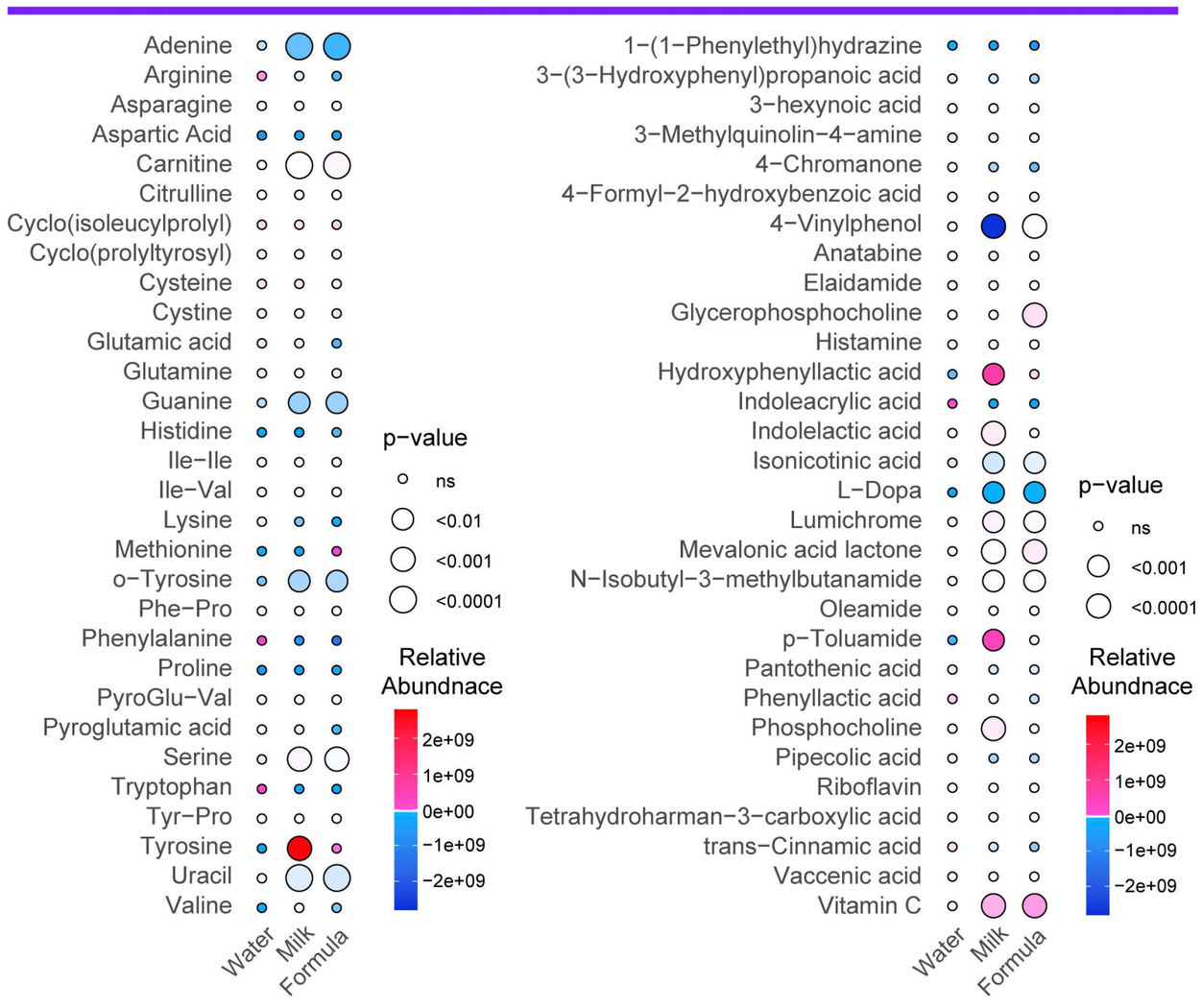
L. brevis



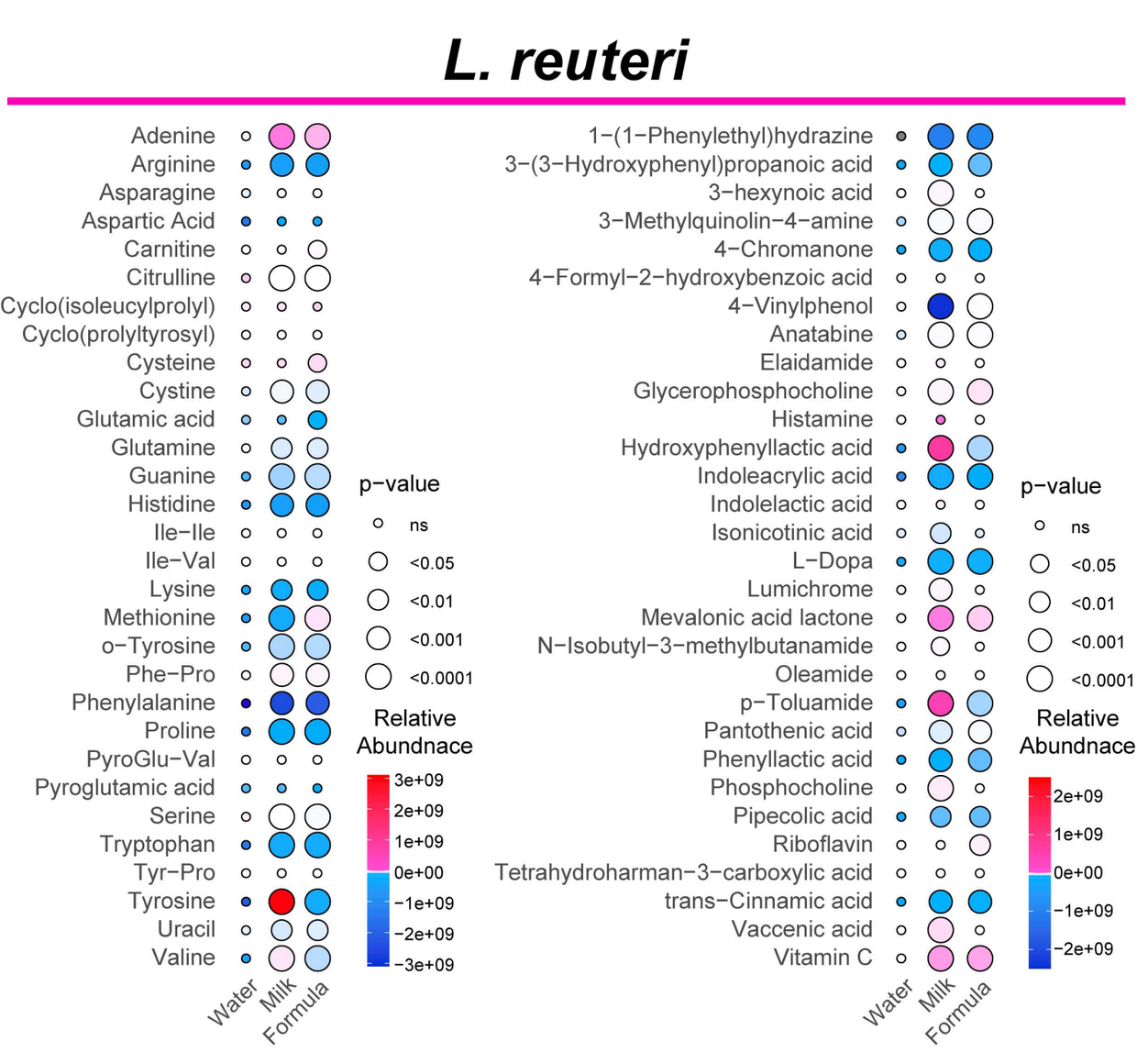
L. paracasei



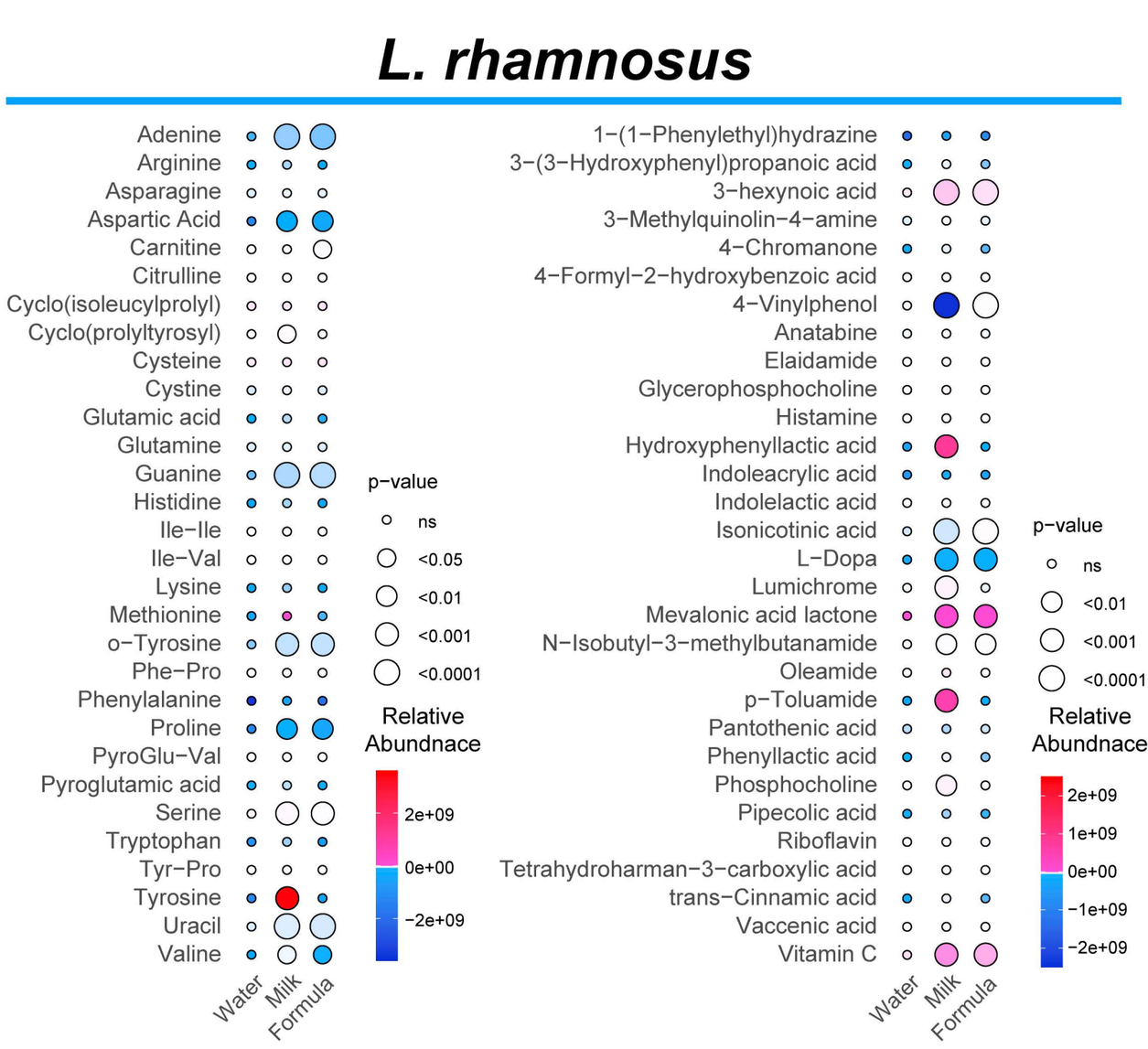
L. johnsonii

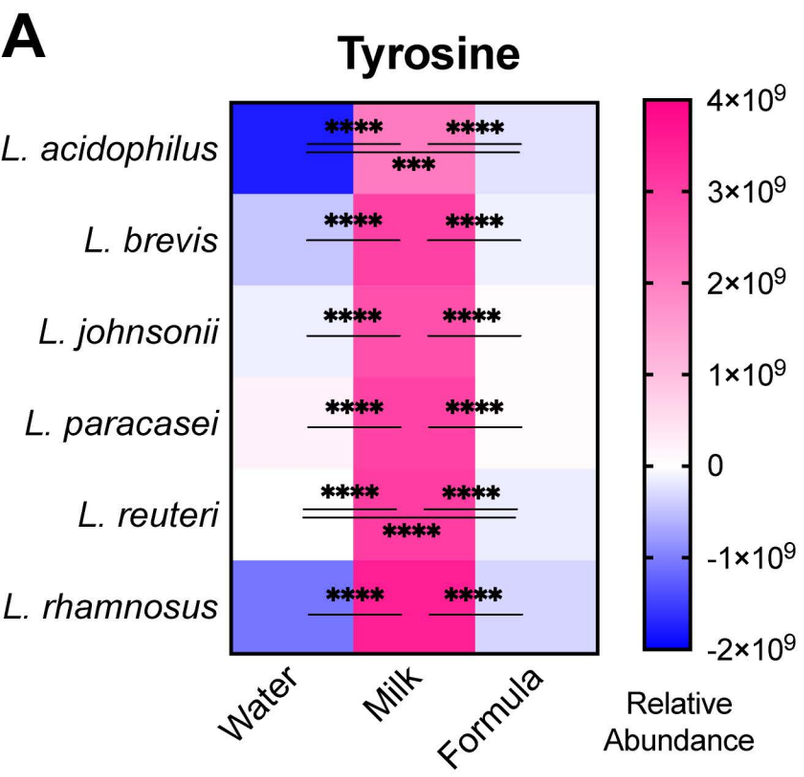


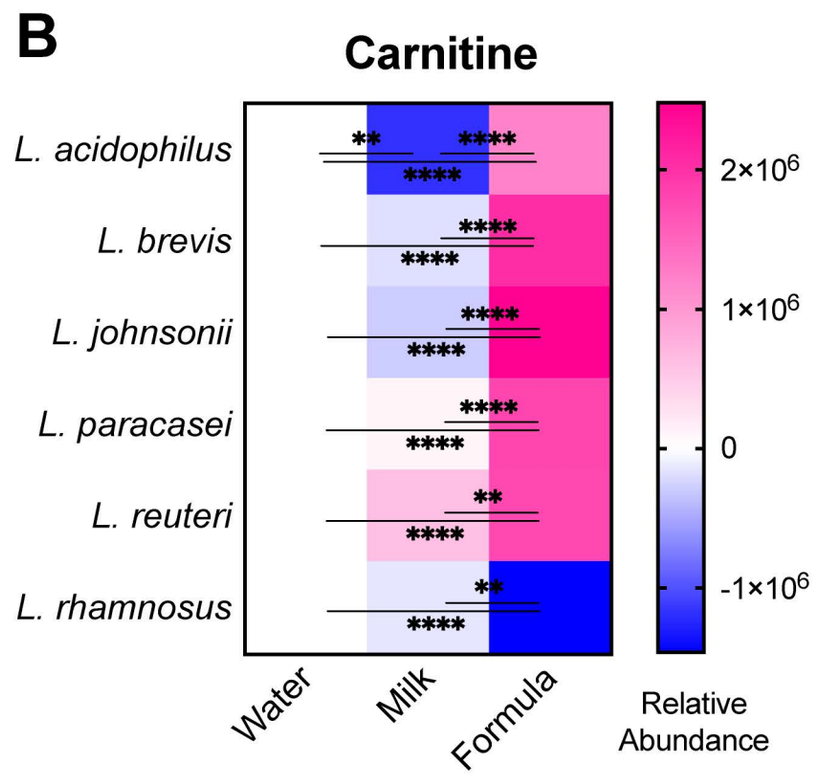
L. reuteri

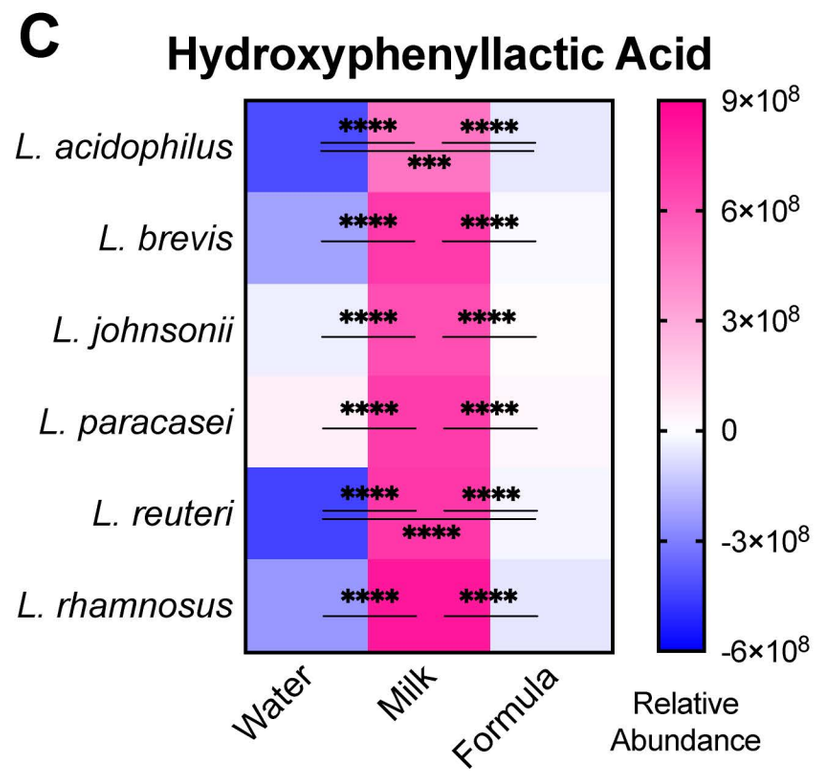


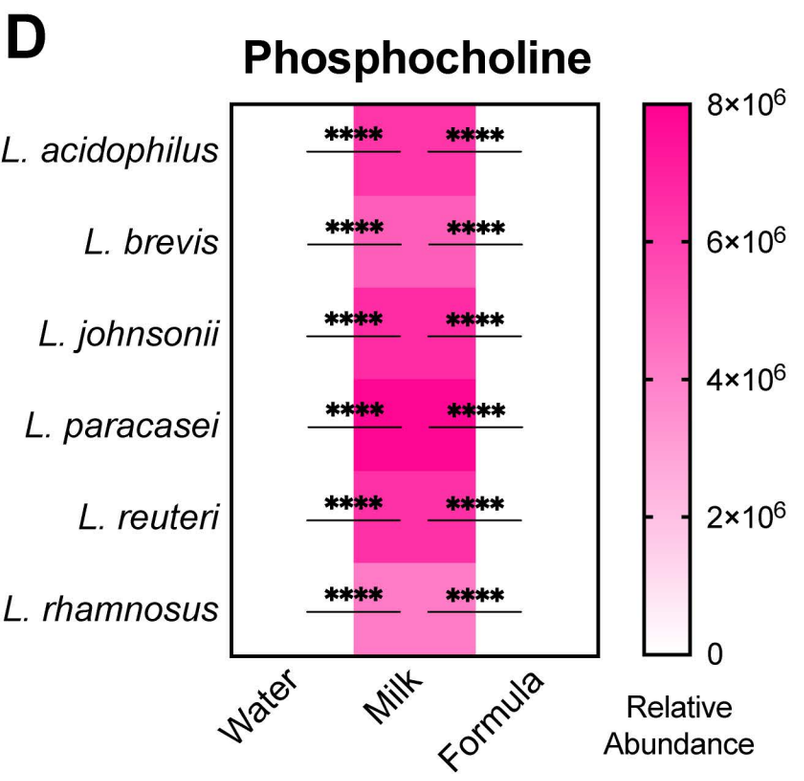
L. rhamnosus

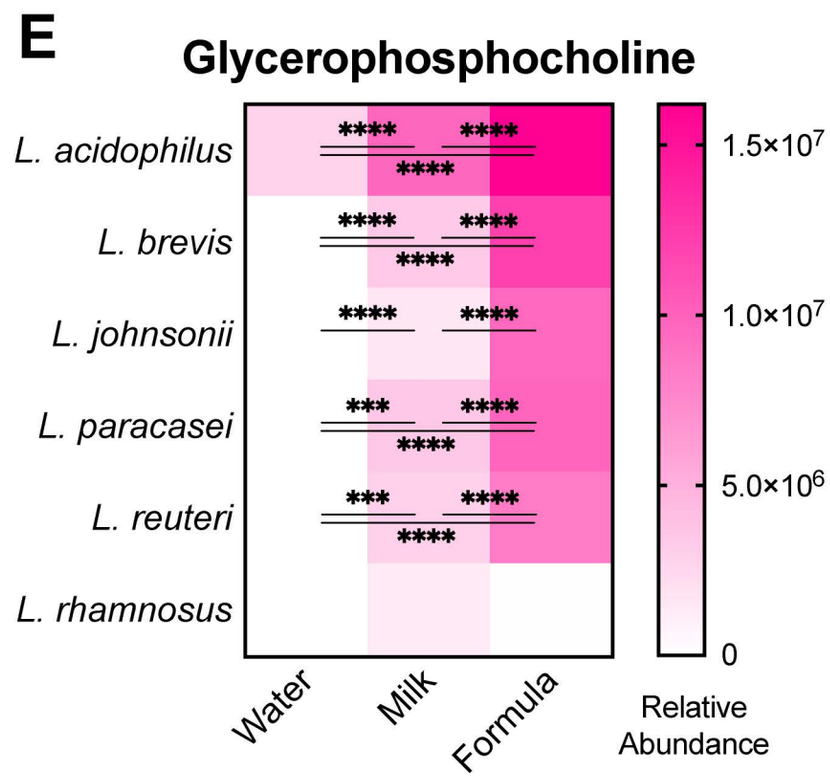


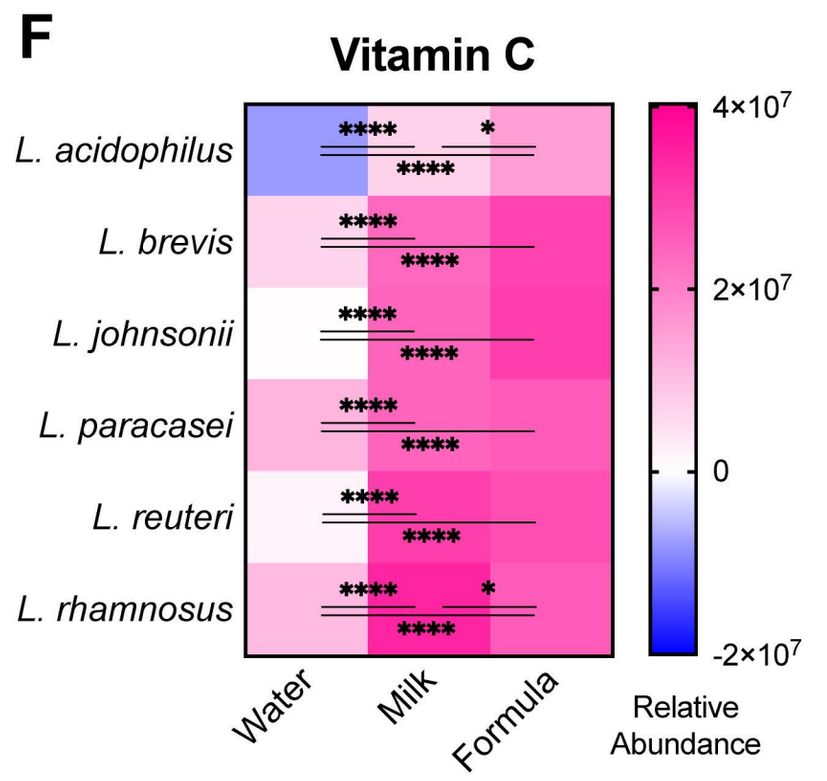

A
Tyrosine
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
4×10^9
3×10^9
2×10^9
1×10^9
0
-1×10^9
-2×10^9
Relative Abundance

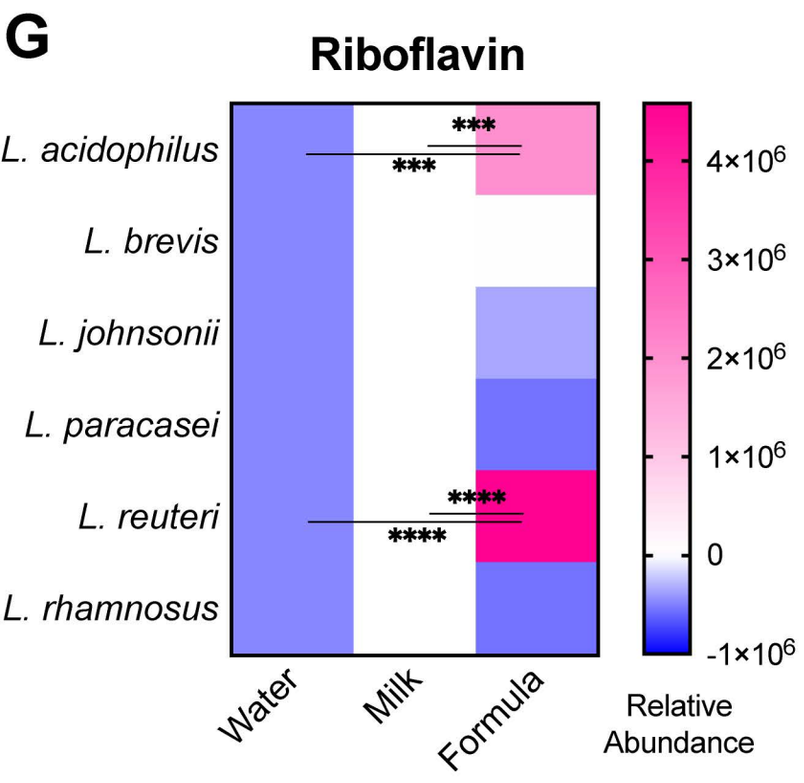

B
Carnitine
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
2×10^6
1×10^6
0
-1×10^6
Relative Abundance

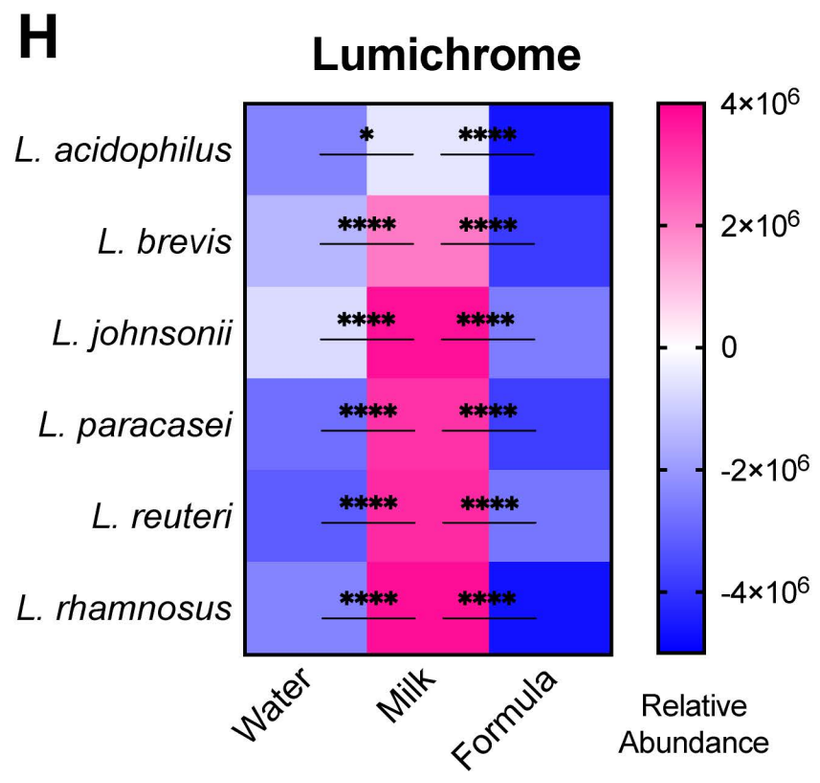

C
Hydroxyphenyllactic Acid
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
9×10^8
6×10^8
3×10^8
0
-3×10^8
-6×10^8
Relative Abundance

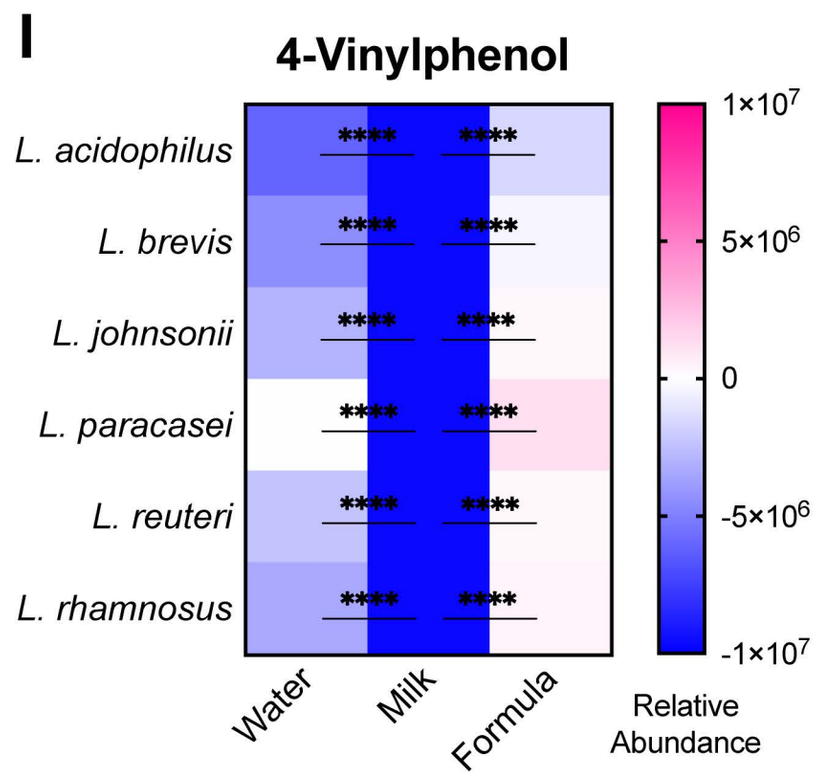

D
Phosphocholine
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
8×10^6
6×10^6
4×10^6
2×10^6
0
Relative Abundance

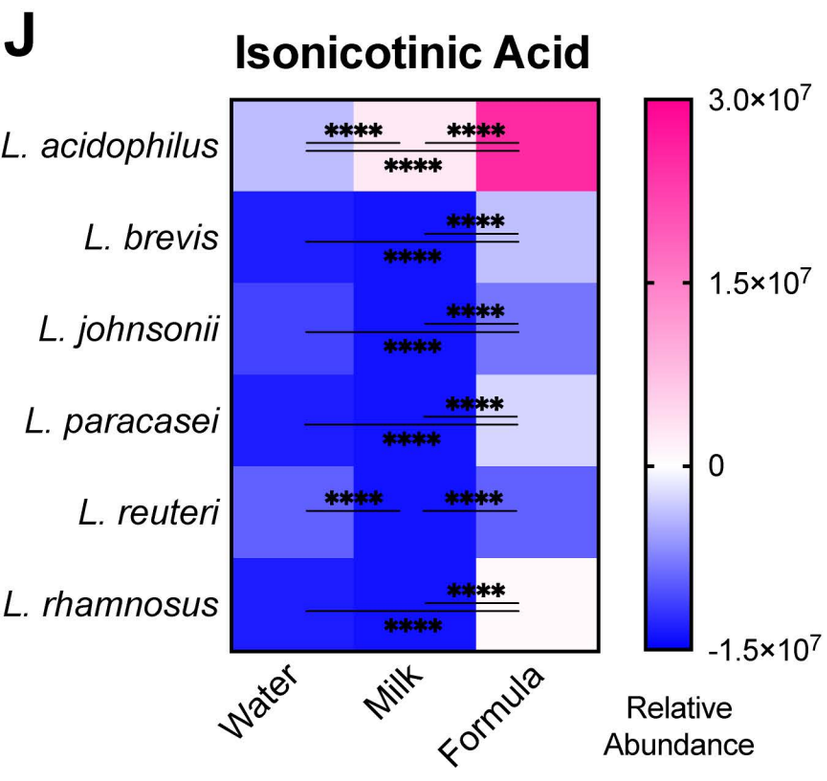

E
Glycerophosphocholine
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
1.5×10^7
1.0×10^7
5.0×10^6
0
Relative Abundance

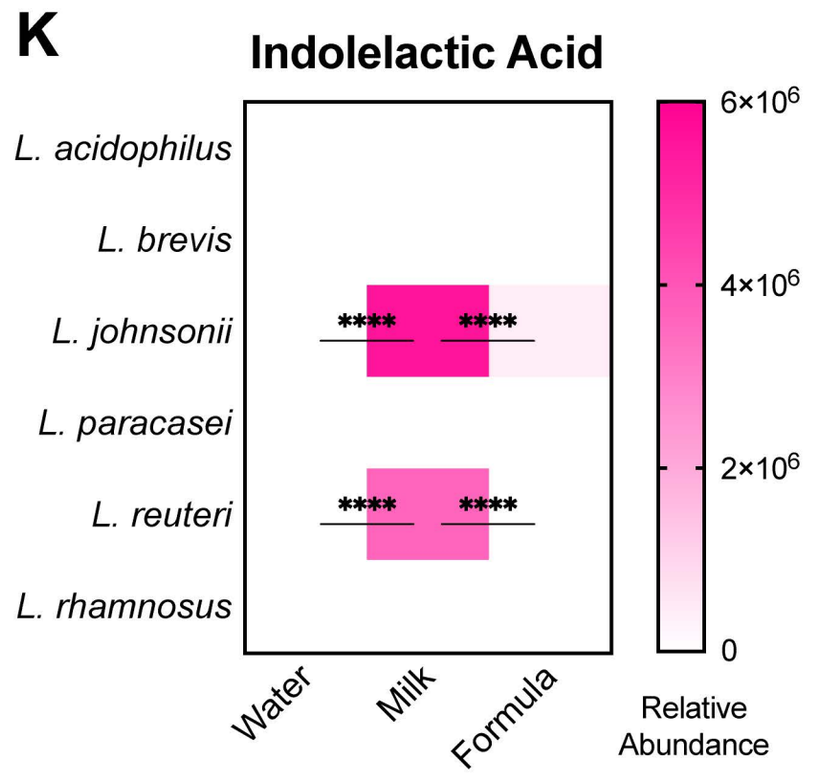

F
Vitamin C
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
4×10^7
2×10^7
0
-2×10^7
Relative Abundance

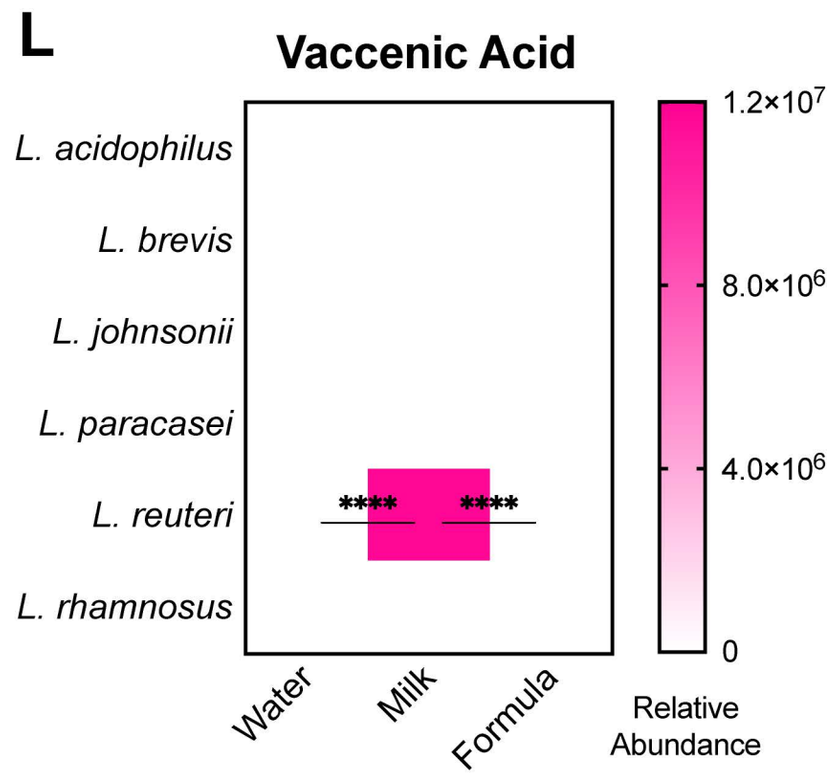

G
Riboflavin
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
4×10^6
3×10^6
2×10^6
1×10^6
0
-1×10^6
Relative Abundance


H
Lumichrome
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
4×10^6
2×10^6
0
-2×10^6
-4×10^6
Relative Abundance

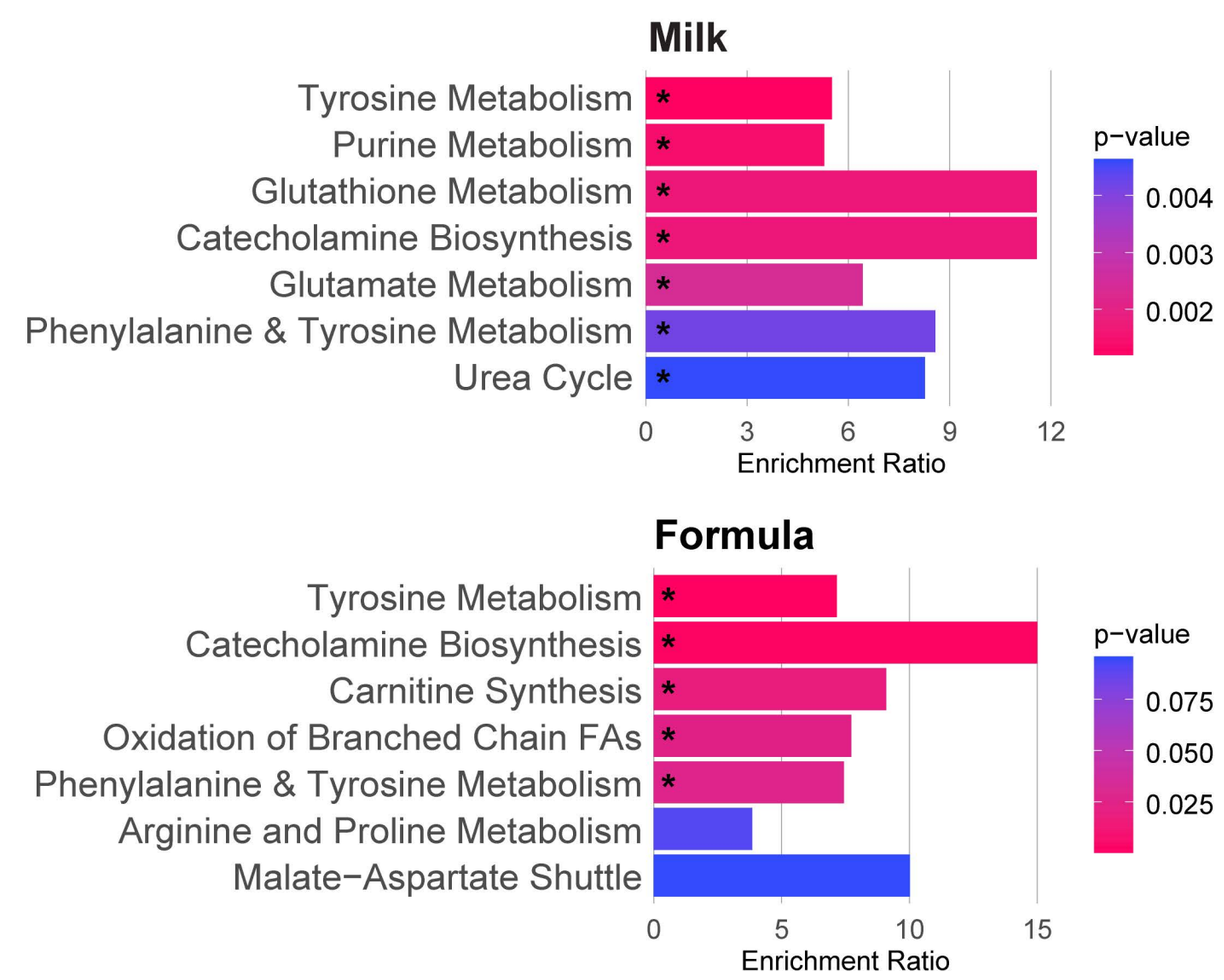

I
4-Vinylphenol
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
1×10^7
5×10^6
0
-5×10^6
-1×10^7
Relative Abundance


J
Isonicotinic Acid
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
3.0×10^7
1.5×10^7
0
-1.5×10^7
Relative Abundance

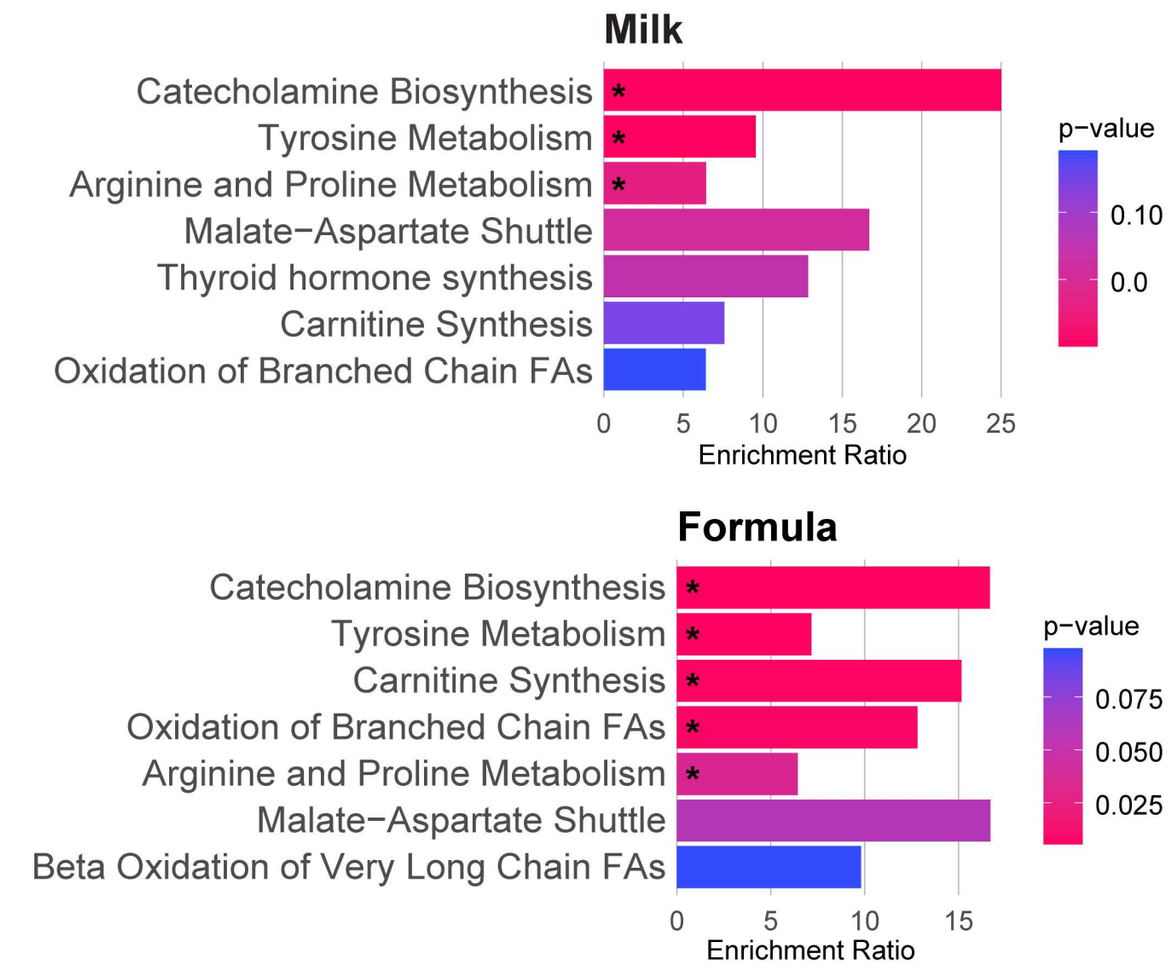

K
Indolelactic Acid
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
6×10^6
4×10^6
2×10^6
0
Relative Abundance


L
Vaccenic Acid
L. acidophilus
L. brevis
L. johnsonii
L. paracasei
L. reuteri
L. rhamnosus
Water
Milk
Formula
1.2×10^7
8.0×10^6
4.0×10^6
0
Relative Abundance

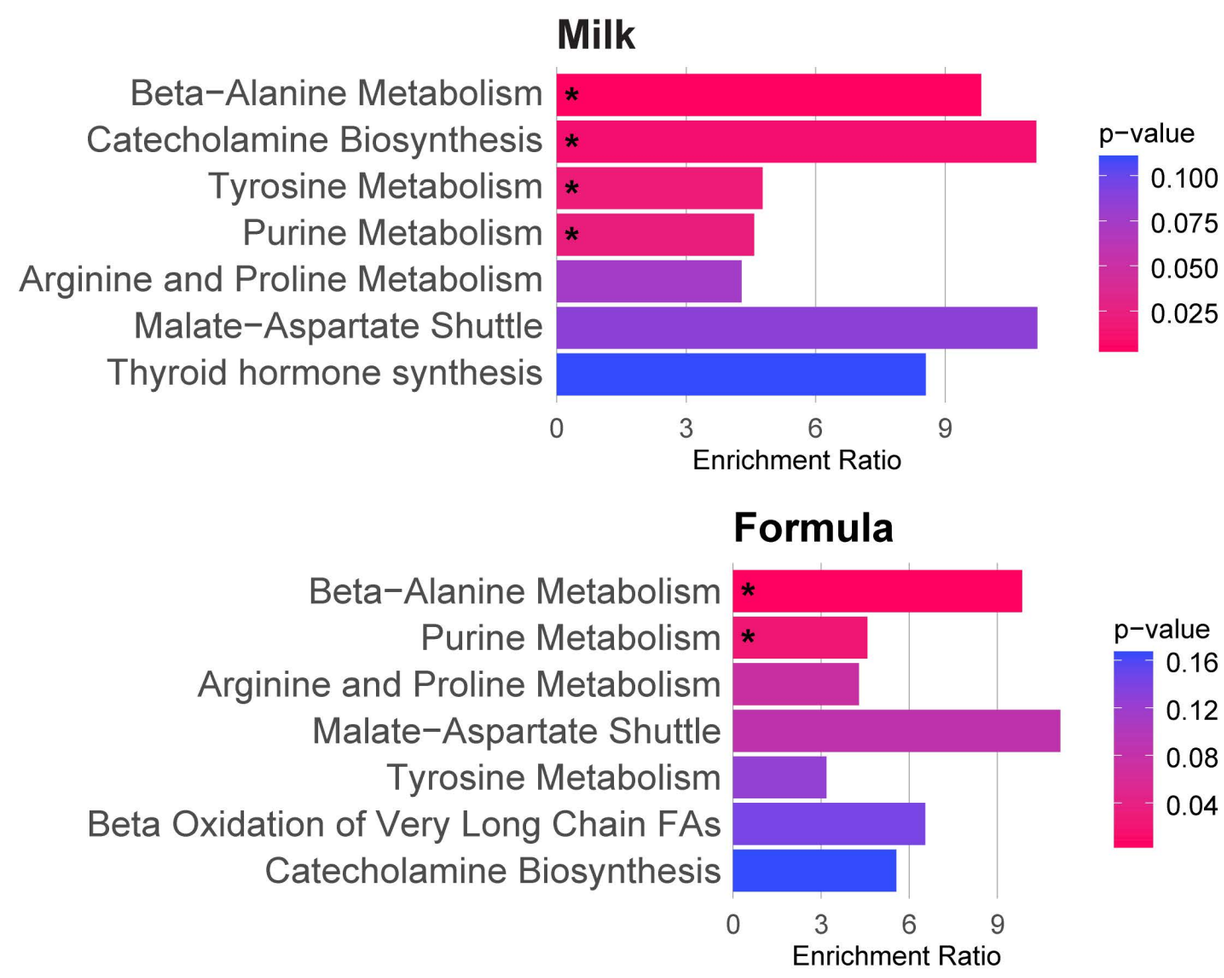
L. acidophilus



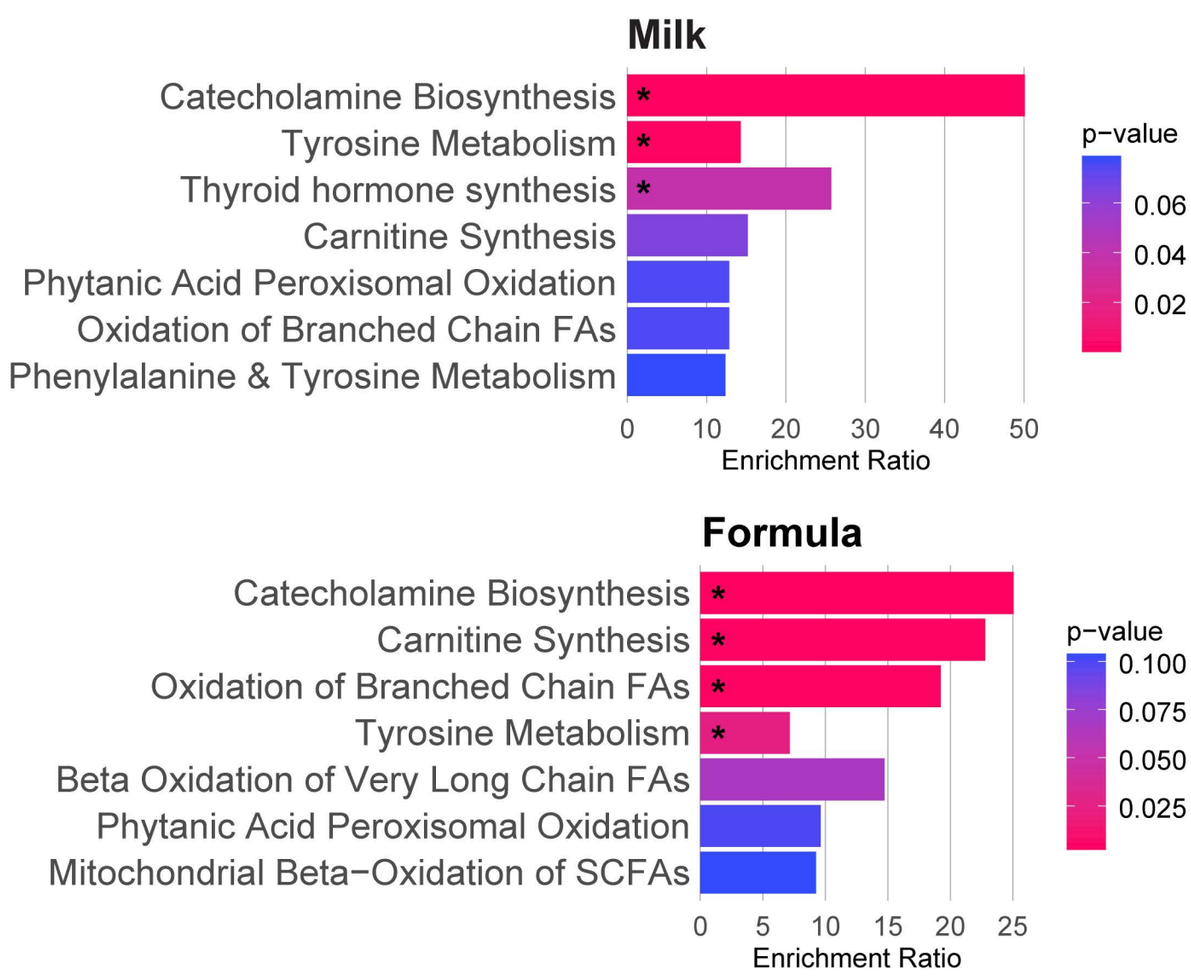
L. brevis



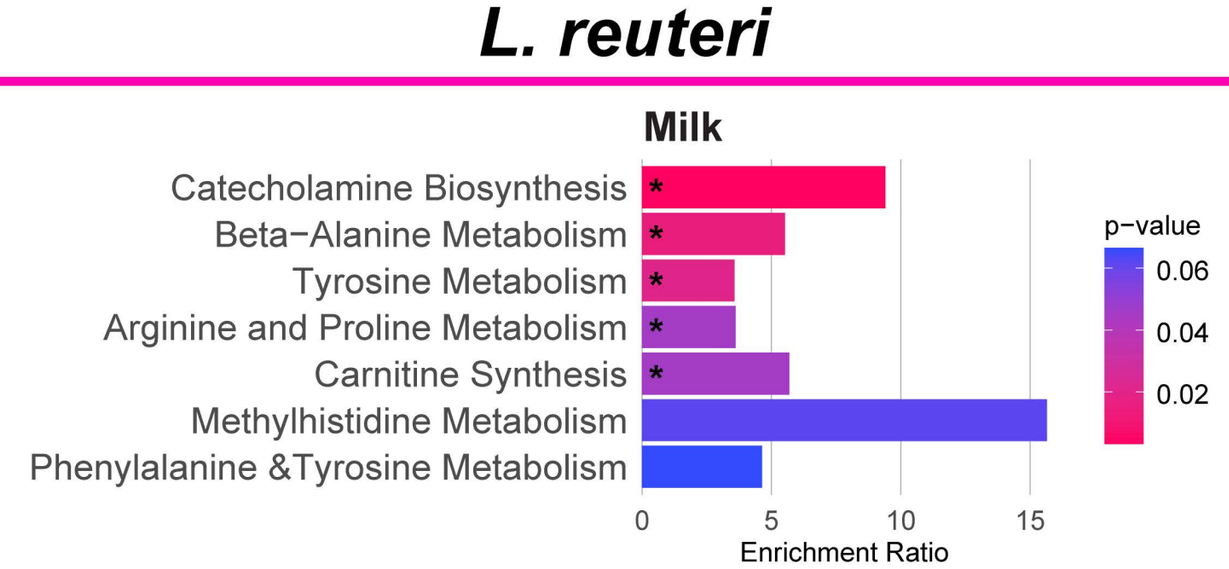
L. paracasei



L. johnsonii



L. reuteri



L. rhamnosus