Gestational Diabetes Mellitus and Its Impact on the Mother-Infant Gut and Breast Milk Bacteriome

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Abstract

Background

Human breast milk is a complex fluid that provides both macronutrients and micronutrients critical for the infant development. Several studies have been focused on the characterization of the human breast milk (HBM) microbiota, nowadays we know it is composed by a rich community of bacteria, viruses, protozoa and anaerobic fungi. However, it is considered that the human microbiome is not static, instead is described as a dynamic living system shaped by several factors like age, demography, malnutrition, sex, diet, sexual behavior, antibiotics and health condition. Previous studies have demonstrated that metabolic disorders such as diabetes mellitus induces dysbiosis in the gut microbiome in affected individuals. However, how gestational diabetes mellitus (GDM) affects the HBM microbiota remains poorly understood. In this study, we analyzed the bacterial diversity through the massive sequencing of the 16S rRNA in Colombian infants breastfed with HBM from GDM and control women.

Methods

In this descriptive cross-sectional study, we collected HBM and stool samples from GDM and healthy mother-infant pairs in Pereira (Colombia). The whole DNA was purified and the 16S V3-V4 region was amplified and sequenced. Reads obtained were quality filtered and classified by homology according to the Ribosomal Small Subunit SILVA.

Results

Our results revealed that the most abundant phyla in the collected samples are Firmicutes, Bacteroidetes and Proteobacteria. Interestingly, we found significant differences in the bacterial relative abundances GDM mothers' gut compared to the control group, notably *Bifidobacterium*, *Serratia* and *Sutterella* were negatively associated to GDM. In the HBM significant differences were observed in *Sutterella*, *Serratia*, *Lactococcus* were in low RA in GDM whereas *Veillonella* was in high RA. On the other hand, in the infant, *Bifidobacterium, Lactobacillus, Sutterella, Serratia, Streptococcus*, and *Veillonella* had low presence while *Lactococcus* and *Flavonifractor* were in significant high abundance in GDM, these differences in the bacterial community structure in GDM mother and their infants could be linked to vitamin K and several forms of vitamin B deficiency, inflammatory disease, and gut bacterial homeostasis.

Conclusion

Our results suggest that gut bacteriome profiles vary between healthy and GDM women, leading to gut bacterial dysbiosis in their infants.

Background

Human breast milk (HBM) is a complex biofluid produced by the mammary glands and acts as the first source of nutrition for infants. Besides the high content of macro and micronutrients present, recent evidence demonstrated that healthy HBM is also a rich source of microorganisms mainly belonging to four phyla, Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria [1]. Several studies using culture-independent techniques have encountered at genus level that the main genera in HBM are *Staphylococcus*, *Streptococcus*, *Lactobacillus, Propionibacterium Serratia, Corynebacterium*, and *Ruminococcus*, among others [2–7]. However, the definition of the "core" HBM bacteriome is still controversial since most studies around the world exhibit significant differences in the bacterial composition of healthy individuals [8].

The importance of the HBM-associated bacteriome relies on the fact that it is a viable source of mutualistic bacteria capable of vertically colonizing the infant gastrointestinal tract (GI) to deploy their metabolic and protective functions. It is noteworthy that newborns' GI is sterile, and bacterial gut colonization begins at the birth. The bacteria present correspond to mother's vaginal and fecal microbiota and this colonization continue within the first weeks through feeding [9]. In this sense, HBM is one of the main drivers shaping the GI microbiota, and this early gut colonization will have significant effects on the shape of the individual's future gut microbiota [10, 11]. In order to function as an immunomodulatory and neuromodulator factor as well to perform metabolic functions, newborns' gut bacterial colonization during breastfeeding is critical. It is known that several factors such as diet, age, gender and health status shape the bacterial populations in the gut and other human environments. However, how hyperglycemia during GDM in the mother could affect the structure of bacterial communities in the host is still unclear [12–14].

Methods

Subjects

This is a descriptive study registered and approved on July 15th, 2019 by the UAMRA-Universidad Autónoma de Tamaulipas Ethics in Research Committee (1RB00010860) under registration number 001/2019/CEI. To participate in this study, the GDM group was selected from Colombian mothers contacted through the clinical records provided by the Obstetrics Department from the Risaralda Comfamiliar Hospital. Selected women were clinically diagnosed as hyperglycemic as they measured fasting glucose between 5.1 to 6.9 mmol/L (92–125 mg/dL) or 8.5–11 mmol/L (153–199 mg/dL) two hours after meal consumption with diabetes symptoms [15]. Every selected individual was interviewed and did not reported other known additional comorbidities, including obesity, diabetes mellitus type II (DM), HIV or cancer; the infants met the age criteria established between one and three months old. The control group was selected among women who claimed to be in good health and did not reported previously known comorbidities. In both groups, if the mother had taken antibiotics treatment during pregnancy and/or if the newborn received other nourishment besides HMB subjects were excluded from

the analysis. In every case if the subject agreed to participate in this study, an informed consent was signed.

Biological samples

Infant and mother stool samples were collected by the mother and placed in a sterile plastic container. Breast milk samples were collected manually by the donors and placed directly into sterile plastic containers. All the samples were transported on refrigeration to the laboratory at the Universidad Libre, Seccional Pereira in Colombia and stored at -20 C until its processing.

For HBM and stool samples, genomic DNA was purified using the Invisrob Spin Universal Kit (Stratec. Berlin, Germany). DNA libraries were constructed and subjected to Illumina MiSeq pair-ended high throughput sequencing of the 16S V3-V4 region (2x300) according to Illumina's Multiplexing Sample Preparation Guide (Illumina, San Diego, CA). Sequencing was performed at the Laboratory of Genomic Services of the National Laboratory of Genomics for Biodiversity (Irapuato, Mexico) using the primers 367F (5'-CTCCTACGGGAGGCAGCAG-3') and CDR (5'-CTTGTGCGGGCCCCCGTCAATTC-3') and at the Colombian Center of Bioinformatics and Computational Biology (Manizales, Colombia) amplifying with the primers 5'-CCTAYGGGRBGCCASCAG-3' and 5'-GGACTACNNGGGTATCTAAT-3'.

Sequencing data analyses

Raw reads analyses were performed in the high performance computing cluster - Centro de Biotecnología Genómica (Instituto Politécnico Nacional, México), quality control was carried out, and then reads were assembled in contigs using the Mothur pipeline [16]. The contigs assembled were quality filtered and classified by homology according to the Ribosomal Small Subunit SILVA Database v. 132 [17]. Aligned sequences were filtered to identify and remove non-bacterial and chimeric sequences. Alpha diversity was reported as observed and estimated, accounting for less frequent species through the nonparametric method chao1 under ANOVA statistical analysis [18]. Beta diversity among the individuals was calculated through the Bray-Curtis Index distance method and tested using a permutational multivariate analysis of variance (PERMANOVA). Both metrics, alpha and beta indexes, were reported taxonomically for phylum and genus. Differential abundance was estimated through EdgeR [19] and LefSe [20]. Abundance graphs were visualized through the Microbiome Analyst Portal (https://www.microbiomeanalyst.ca) [21].

Results

Biological samples

In this study, we enrolled 20 subjects from Pereira, Colombia, in two groups of five control and five GDM mother-infant pairs, the mother ag range in the GDM and control group was 20–41 years old. All of them did not report being addicted to drugs, tobacco, or alcohol. GDM women were reportedly treated with metformin (800 mg) during their pregnancy. Body mass index (BMI) ranged from 24.7 to 31.1 in the GDM group and between 20.5 to 27.3 in the control group (supplementary table 1).

Nucleic acids purification and Illumina sequencing

Raw reads were deposited in the NCBI Sequence Read Archive (SRA) under BioProject number PRJNA728120; after QC control and filtering of non-bacterial reads, we analyzed between 4,259 and 114,731 informative reads per sample (supplementary table 2). According to the sequenced region, we identified distinct bacterial communities in BM, mother gut, and infants' gut. In either control or GDM-BM, the bacterial composition was dominated by Proteobacteria, Firmicutes and Bacteroidetes. However, the gut microbiome of the GDM mothers showed lower diversity and predominantly higher relative abundance (RA) of Firmicutes compared to the control fecal samples, whose exhibited large amounts of Bacteroidetes and higher presence of Proteobacteria. Finally, the healthy infant gut was colonized mainly with Proteobacteria compared to the GDM-fed infants that showed large relative abundance of Firmicutes (Fig. 1).

Infants gut bacteriome

We identified ~ 451 operational taxonomic units (OTUs) among the analyzed samples. The total phyla identified within the infants' bacteriome included Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes, Chloroflexi, Synergistetes, and Verrucomicrobia. Relative abundance of Firmicutes predominated in DMG fed infants (p = 4.25E-5). On the other hand, infants from the control group, in general, presented higher species diversity and a had higher abundance of Actinobacteria, Proteobacteria, and Bacteroidetes, although, it was not a statistically significant difference.

At genus level (Fig. 2), we identified ~ 103 genera. According to both, the differential abundance analysis method (edgeR) and the LEfSe analysis, *Flavonifractor* (p = 1.06E-5), *Clostridium* senso stricto (p = 0.0012), *Clostridiales* (p = 0.0022), *Paenibacillus* (p = 0.0028), *Oscillibacter* (p = 0031), *Ruminococcus* (p = 0.0035), *Gemmiger* (p = 0.0042) and unclassified Clostridiaceae species (p = 0.0048) were agents positively associated to the DMG-BM samples.

On the other side, *Escherichia/Shigella* (p = 0.047), *Streptococcus* (p = 0.028), *Serratia* (p = 0.009), *Megasphaera* (p = 0.047), *Clostridium* XVIII (p = 0.0758), *Lactobacillus* (p = 0.016), *Veillonella* (p = 0.0162), *Staphylococcus* (p = 0.028), unclassified Burkholderiales (p = 0.026), unclassified Pasteurellaceae (p = 0.044), *Sutterella* (p = 0.047), *Psychromonas* (p = 0.007), *Azoarcus* (p = 0.007), and *Aeromonas* (p = 0.016) were found in low RA in infants fed on control BM (Fig. 3A).

Alpha diversity in the gut bacteriome at OTUs level from infants fed on DMG BM was significantly higher compared with those fed with control BM (p = 0.0025) and higher in the control group at phylum level (p = 0.07). Beta diversity among the control and GDM infants was significant at OTUs level ($p < 0.01$) and barely significant at the phylum ($p < 0.033$) and genus ($p < 0.028$). The Firmicutes/Bacteroidetes ratio was significantly higher in the GDM group compared to the control.

Mothers gut and BM bacteriome

Gut bacteriome in GDM and control mothers showed ~ 136 OTUs distributed in 62 identified genera. Found bacteria belong mainly to three phyla, Firmicutes, Bacteroidetes, and Actinobacteria. Comparing the two groups, we found that Firmicutes were significantly associated with the GDM group (p = 0.0015). At genus level (Fig. 2), unclassified Paenibacillaceae (p = 2.9E-5), Paenibacillus (p = 5.82E-5), *Mitsuokella* (p = 4.32E-4), *Haemophilus* (p = 8.48E-4), *Staphylococcus* (p = 0.006), *Brevibacillus* (p = 0.01), *Bacillus* (p = 0.01) were differentially associated to GDM women whereas *Alistipes* (p = 2.92E-6), *Phascolartobacterium* (p = 3.11E-5), Bacteroides (p = 6.35E-4), *Coprobacillus* (p = 8.11E-4), *Allobaculum* (p = 0.001), *Megamonas* (p = 0.005), *Bilophila* (p = 0.009), *Parabacteroides* (p = 0.01) had higher RA within the control group (Fig. 3C). Alpha diversity did not show significant differences at the taxonomic levels tested, while beta diversity among the groups was significant only at genus level (p < 0.018).

In GDM BM, we found the analyzed samples composed by ~ 189 OTUs from 47 identified bacterial genera belonging to three phyla, Proteobacteria, Firmicutes, and Bacteroidetes. However, we did not find any significant difference in RA of any phylum among the samples. At genus level (Fig. 2), unclassified *Bacteroidetes* (p = 2.82E-4), *Stentrophomonas* (p = 9.63E-4), Veillonella (p = 0.001), *Syntrophorhabdus* (p = 0.001), *Bacillales* (p = 0.0021) in higher RA within the GDM samples, whereas *Enterococcus* (p = 0.005) and Serratia (p = 0.005) were found with significant higher RA in control samples (Fig. 3B). We did not find significant alpha diversity among the control and GDM samples. However, a slightly higher alpha diversity index was observed in the genus-level control samples. No significant beta diversity among the tested groups was registered (p < 0.422).

Discussion

Lactobacillus and *Bifidobacterium*

Our results showed that the abundance of *Lactobacillus* (p = 0.016) and *Bifidobacterium* tend to decrease in the infant gut fed on GDM BM. We found, additionally, that *Lactobacillus* abundance is decreased in the GDM mother gut and BM. *Lactobacillus* is a gram-positive genus that encompasses approximately 140 species. The species associated to human fecal samples are *L. acidophilus*, *L. casei*, *L. crispatus*, *L. gasseri*, *L. paracasei*, *L. plantarum*, *L. reuteri*, and *L. ruminis*. It is believed that this genus play a role maintaining the microbiota homeostasis [9, 22].

Species within the *Bifidobacterium* genus, including *B. bifidum*, *B. breve* and subspecies of *B. longum*, such as *B. longum* subsp. *longum* and *B. longum* subsp. *infantis* are human-specialized and coexist in the human gut [23]. In the infant, *Bifidobacterium* participate in the metabolism of human milk oligosaccharides (HMOs) and mucin glycosylated proteins [24]. It is known that *Bifidobacterium* is the dominant microorganism in the breast-fed infant gut microbiota under normal conditions. Since, during the breast-fed period, the infant does not have additional sources of colonizing *Bifidobacterium*, metabolism of main HMOs such as 2-fucosyllactose (2-FL) and lacto-N-neonatrose (LNnT) could be compromised affecting the bacterial strains community in the gut [14].

Flavonifractor sp.

In this study, we found presence of the gram-positive *Flavonifractor sp.* that is highly associated with the gut bacteriome of GDM-fed infants (p = 1.06E-5). The resolution obtained did not allow to identify it to species level. Interestingly, we have not found evidence of *Flavonifractor* in the mothers' group. However, it is known that *Flavonifractor plautii* is a human gut microbiome species able to degrade naturally produced flavonoids present in fruit and vegetables commonly consumed in diet, specifically the most abundant, quercetin. Previously, *Flavonifractor* was considered a possible biomarker since it is reported as one of the microorganisms heavily associated with colorectal cancer in two recent studies [25, 26]. *Flavonifractor sp.* was also found in high RA in diabetes mellitus affected mice [27]. In contrast appears to be associated with lower GDM risk when diet is rich in polyphenols and flavonoids [28]. The high RA and strong association of *Flavonifractor* in GDM infants could be effectively linked and serve as a potential marker for gestational hyperglycemia.

Paenibacillus spp.

It is noteworthy that *Paenibacillus spp*. reads are present in very high RA across the analyzed samples (Supplementary table 3). *Paenibacillus* is a facultative anaerobic, rod-shaped gram-positive. This species has been barely described in previous studies. To date, only two species have been reported as isolated from human gut, *P. faecis* and *P. phocaensis* [29, 30]. Our results showed *Paenibacillus* in a high RA in GDM maternal gut and their children. However, further analyses are required to determine the species found in this study.

Vitamin K producers

It is known that gut bacteria have an essential role in human metabolism, including the production of essential nutrients such as vitamin K_2, complex B vitamins, and short chain fatty acids (SCFA). Vitamin K_2 are menaquinones (MKs) (2-methyl-3-multipreny-1,4-naphtoquinone), a fat-soluble vitamin produced by human gut microbiota synthesized during bacterial anaerobic respiration. There are several forms of MKs that vary in the number of their isoprene-5-carbon prenyl units ranging from 2 to 15 repeats [31]. MKs biological importance resides in the fact that they serve as electron transport chains during the prokaryotic respiration, carrying electrons to the cytoplasmic membrane, functioning as antioxidants, protecting cellular membranes from lipid oxidation [32]. Major MKs in human gut are produced by members of the genera *Bacteroides* (MK-10, MK-11), *Enterobacteria* (MK-8), *Serratia* (MK-4), *Lactococcus* (MK-7, MK-8, MK-9) and *Lactobacillus* (MK-4) [31, 33–36]. We found Enterobacteriaceae, *Serratia* sp., and *Lactobacillus* sp., in a low RA in gut samples of GDM-fed infants. Remarkably, *Lactococcus* had increased RA in control samples, within BM samples, GDM BM had significantly low RA of *Serratia* (p = 0.009). Singularly, *Lactococcus* had a low RA in GDM BM samples in contrast to the observed results in the GDM infants' gut. Here, we hypothesized that the high levels of glucose occurring under GDM could affect the abundance of this species in the mother breast milk. However, their number increases again once they are established in the infant colon. Our findings suggest that GDM infants could have a diminished supplementation of MKs at least during the first months of life. However, further research

measuring MKs concentration under hyperglycemic conditions is needed to elucidate the impact of GDM in women and their infants.

Complex B producers

It is known that besides niacin (vitamin B_3), host mammalians do not synthesize vitamin B *de novo*, and, in consequence, these compounds should be obtained either from diet or synthesized by the gut microbiota [37].

Thiamine (vitamin B_1) function as cofactor for several important enzymatic reactions including the citric acid cycle reactions catalyzed by the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [38]. Thiamine is produced by several gut and probiotic bacterial species such as *Bacteroides fragilis*, *Ruminococcus lactaris*, *Prevotella copri* and some *Lactobacillus spp.*, *Bifidobacterium spp.*, and *Fusobacterium spp*. According to our findings, at least *Lactobacillus spp*. and *Bifidobacterium spp*. are in low RA in GDM women and infants, and this could lead to a reduced amount of bacterial synthesized thiamine. In consequence, the competition for thiamine between gut bacteria and the host could be increased.

Riboflavin (vitamin B_2) is a precursor of the redox coenzymes flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) essential in TCA and fatty oxidation pathways. Although diet is an important source of riboflavin, it is also synthesized by several members of the human gut microbiota, including *Lactobacillus spp.*, *Lactococcus spp.*, and *Weisella spp.* [38, 39]. In this study *Lactobacillus* sp. RA is reduced in GDM BM-fed infants ($p = 0.028$) while *Lactococcus* sp. and *Weissella sp.* increase ($p = 0.009$).

Folates (vitamin B_9) are anionic hydrophilic carrier molecules that are not naturally synthesized by human cells; thus, they must be acquired through diet components or produced by the gut microbiota. Bacterial folate biosynthesis in the form of tetrahydrofolate (THF) is carried out mainly by members of the phyla Bacteroidetes (*Bacteroides spp*. and *Prevotella spp*.), Firmicutes (*Lactobacillus spp*. and *Streptococcus spp*.) and Actinobacteria (*Bifidobacterium spp*.) [38, 40]. We found that *Bifidobacterium spp.* is a bacterial genus affected by GDM. Its low RA in the GDM BM fed infant could be traced to maternal BM and to the gut in hyperglycemic women (Fig. 3). Additionally, there is a low count of *Lactobacillus* and *Streptococcus* in GDM individuals could lead to stress levels of THF in affected infants since BM is the only folate source at that stage, and folate levels in BM are maintained by the BM microbiota [41, 42]

Cobalamin (Vitamin B_{12}) is produced exclusively by microorganisms, animals, plants, and fungi that do not have the ability to biosynthesize it. In the human gut, *Lactobacillus reuteri* is the indigenous bacteria that supply the daily requirements [43]. A study by Boran, Baris [44] compared the gut microbiota between healthy vitamin B_{12} sufficient and insufficient infants and found no difference between the two groups. However, vitamin B_{12} insufficiency could be related to several factors, including GDM. Our results suggest that low RA of *Lactobacillus* spp. could be linked to low RA of *L. reuteri*.

The bacteriome structure in the infants analyzed here suggests that GDM infants have a reduced RA of members of the genera *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* compared with control samples and could have a reduced biosynthesis of several complex B vitamins.

Inflammatory bowel disease

Bacterial dysbiosis of gut species has been associated with inflammatory bowel disease (IBD). Several studies revealed that most common pattern associated with IBD is a reduced amount of Firmicutes and Bacteroides and an increase of *Enterobacteriaceae* species [45]. Our results revealed an increased RA of *Ruminococcus spp*. and a significantly decreased abundance of *Sutterella spp.*, *Clostridium* XVIII, *Lactobacillus spp.*, and a non-statistically significant but reduced RA of *Bifidobacterium spp.* These results are similar to those presented by Joossens, Huys [46] and Gevers, Kugathasan [47].

Overall findings of this study suggest that gut bacteriome profiles vary between healthy and GDM women, leading to gut bacterial dysbiosis in their infants. A hypothesis for the entero-mammary translocation of internal bacteria hypothesis was previously proposed by Fernández, Langa [48]. Consistently, we observed low RA of several bacterial genera in the infants' gut (Fig. 4), such as *Bifidobacterium*, *Sutterella*, and *Serratia*, that can be traced to the maternal gut, and breast milk from GDM affected women.

Abbreviations

2-FL
2-fucosyllactose
dL
deciliter
DM
diabetes mellitus type II GI:gastrointestinal tract
GDM
gestational diabetes mellitus
GutL
gut infant
GutM
gut mother
HBM
human breast milk
HMOs
human milk oligosaccharides
HIV
human immunodeficiency virus
IBD
inflammatory bowel disease

LNnT
lacto-N-neonatrose
mg
milligram
MKs
menaquinones
mmol
millimol
NCBI
national center for biotechnology information
OTU
operational taxonomic unit
RA
relative abundance
SCFA
short chain fatty acids
SRA
sequence read archive
THF
tetrahydrofolate.

Declarations

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Authors' contributions

HMM, SYVC and FSL conceptualized the study. SYVC collected and processed biological samples. MJHB, IPC and HMM interpreted data and performed bioinformatics analyses. HMM, SYVC, FSL, EAC and GCRC contributed in the data analyses, reviewed and approved the final version of the manuscript.

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Ethics approval and consent to participate

Ethical approval was given by the UAMRA-Universidad Autónoma de Tamaulipas Ethics in Research Committee (1RB00010860) under registration number 001/2019/CEI.

Consent for publication

Not applicable

Competing interests

Authors declare that they have no conflict of interest

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Supplementary Material

Table S1. Metadata of women included in this study

ID	Status	Age	Familiar antecedents	Physical activity	Addictions	Medication	Weight (kg)	Height (cm)	BMI
W1	GDM	22	No	No	No	Metformin	55	146	25.8
W2	GDM	41	No	No	No	Metformin	72	152	31.1
W3	GDM	39	No	No	No	Metformin	68	150	30.2
W4	GDM	35	CVD	No	No	Metformin	58	160	22.6
W5	GDM	35	Diabetes	No	No	Metformin	65	162	24.7
W6	Control	25	No	No	No	No	60	165	22
W7	Control	38	CVD	No	No	No	56	165	20.5
W8	Control	19	No	No	No	No	72	165	26.4
W9	Control	20	No	No	No	No	70	160	27.3
W10	Control	40	No	No	No	No	70	168	24.8

Table S2. Reads used for bacteriome identification.

Sample	Type	Status	#Reads
BM1	Breast milk	Control	8,792
BM2	Breast milk	Control	114,731
BM3	Breast milk	Control	26,082
BM4	Breast milk	Control	105,956
BM5	Breast milk	Control	97,864
GDMBM1	Breast milk	GDM	78,214
GDMBM2	Breast milk	GDM	4,259
GDMBM3	Breast milk	GDM	11,221
GDMBM4	Breast milk	GDM	37,179
GDMBM5	Breast milk	GDM	113,264
FM1	Stool mother	Control	63,331
FM2	Stool mother	Control	15,909
FM3	Stool mother	Control	26,558
FM4	Stool mother	Control	14,164
FM5	Stool mother	Control	23,916
GDMFM1	Stool mother	GDM	29,432
GDMFM2	Stool mother	GDM	92,245
GDMFM3	Stool mother	GDM	75,556
GDMFM4	Stool mother	GDM	53,736
GDMFM5	Stool mother	GDM	99,768
FIn1	Stool infant	Control	105,910
FIn2	Stool infant	Control	118,097
FIn3	Stool infant	Control	110,573
FIn4	Stool infant	Control	124,267
FIn5	Stool infant	Control	109,668
DMGFIn1	Stool infant	GDM	104,658
DMGFIn2	Stool infant	GDM	115,104
DMGFIn3	Stool infant	GDM	111,572

DMGFIn4	Stool infant	GDM	118,473
DMGFIn5	Stool infant	GDM	111,690

Table S3. Linear discriminant analysis (LDA) effect size (LEfSe) analysis for infants

Genus	p value	FDR	LDA score
Bifidobacterium	0.0758	0.169	6.1
Clostridiaceae unclassified	0.0005	0.066	-2.48
Psychromonas	0.007	0.066	2.63
Azoracus	0.007	0.066	2.32
Nakamurella	0.008	0.066	2.36
Desulfovibrionaceae unclassified	0.008	0.066	-2.1
Clostridium sensu stricto	0.009	0.066	-5.84
Serratia	0.009	0.066	5.02
Flavonifractor	0.009	0.066	-4.3
Clostridiales unclassified	0.009	0.066	-3.55
Burkholderia	0.009	0.066	-2.79
Ruminococcus	0.009	0.066	-3.75
Faecalibacterium	0.009	0.066	-4.15
Lactococcus	0.009	0.066	-2.58
Paenibacillus	0.009	0.066	-6.43
Weissella	0.013	0.069	-2.2
Desulfovibrionales unclassified	0.015	0.069	-2.8
Veillonella	0.016	0.069	4.5
Lactobacillus	0.016	0.069	4.66
Ruminococcacceae unclassified	0.016	0.069	-4.27
Gemmiger	0.016	0.069	-3.37
Methylobacterium	0.016	0.069	-2.91
Aeromonas	0.016	0.069	2.07
Enterobacteriaceae unclassified	0.016	0.069	4.84
Pantoea	0.016	0.069	3.65
Burkholderiales unclassified	0.026	0.088	3.29
Staphylococcus	0.028	0.088	4.38
Collinsella	0.028	0.088	4.76

Oscillibacter	0.028	0.088	-3.67
Rothia	0.028	0.088	3.06
Streptococcus	0.028	0.088	5.12
Lachnospiraceae	0.028	0.088	-3.99
Clostridium XIVb	0.028	0.088	-2.23
Pasteurellaceae unclassified	0.044	0.127	3.48
Megasphaera	0.472	0.127	4.96
Sutterella	0.472	0.127	2.94
Escherichia/Shigella	0.472	0.127	5.77
Citrobacter	0.472	0.127	4.36

Figures

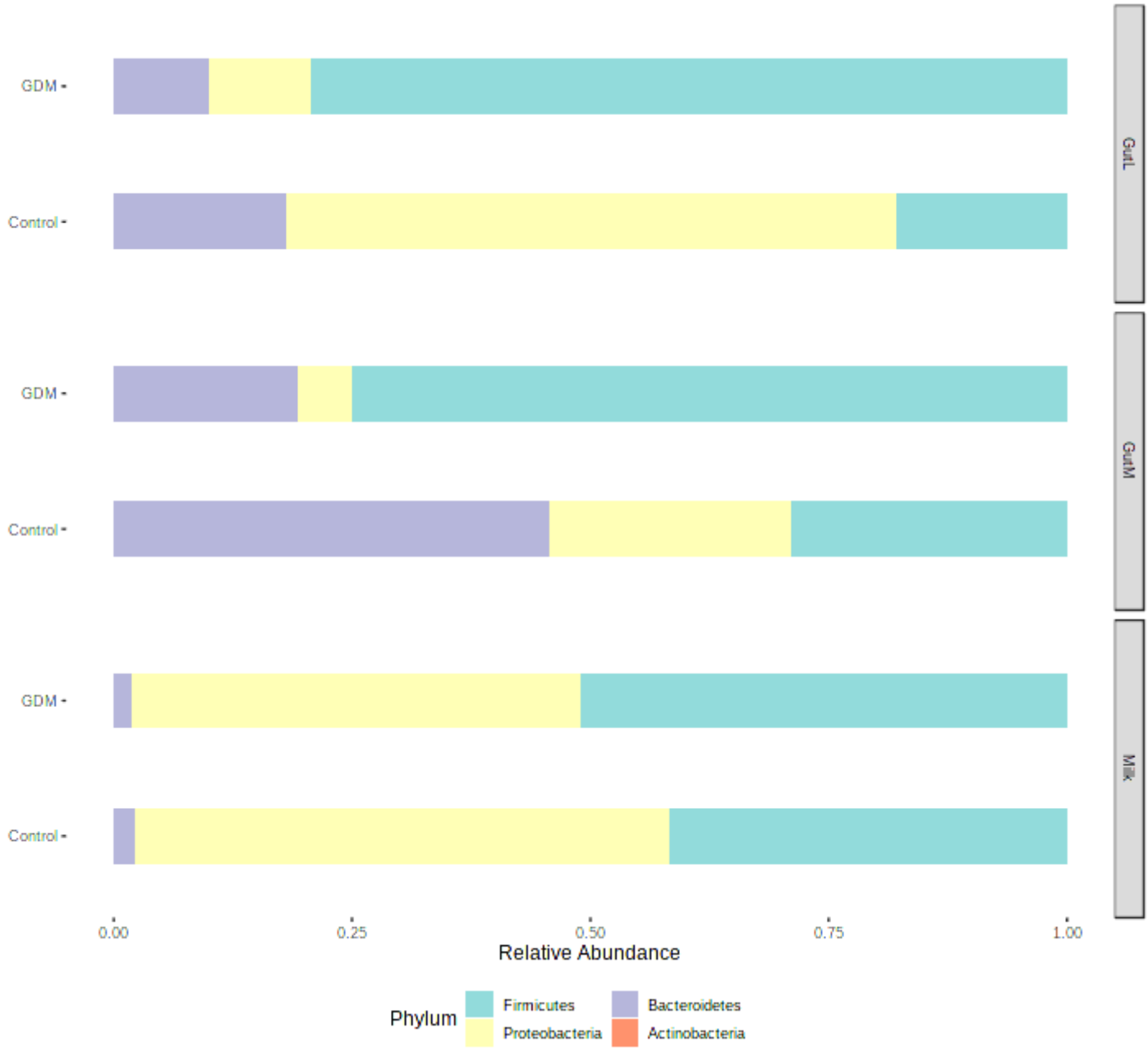

Figure 1

Relative abundance of phyla in the analyzed samples concatenated by health condition. GDM: gestational diabetes mellitus; GutL: gut infant; GutM: gut mother; HBM: Breast milk

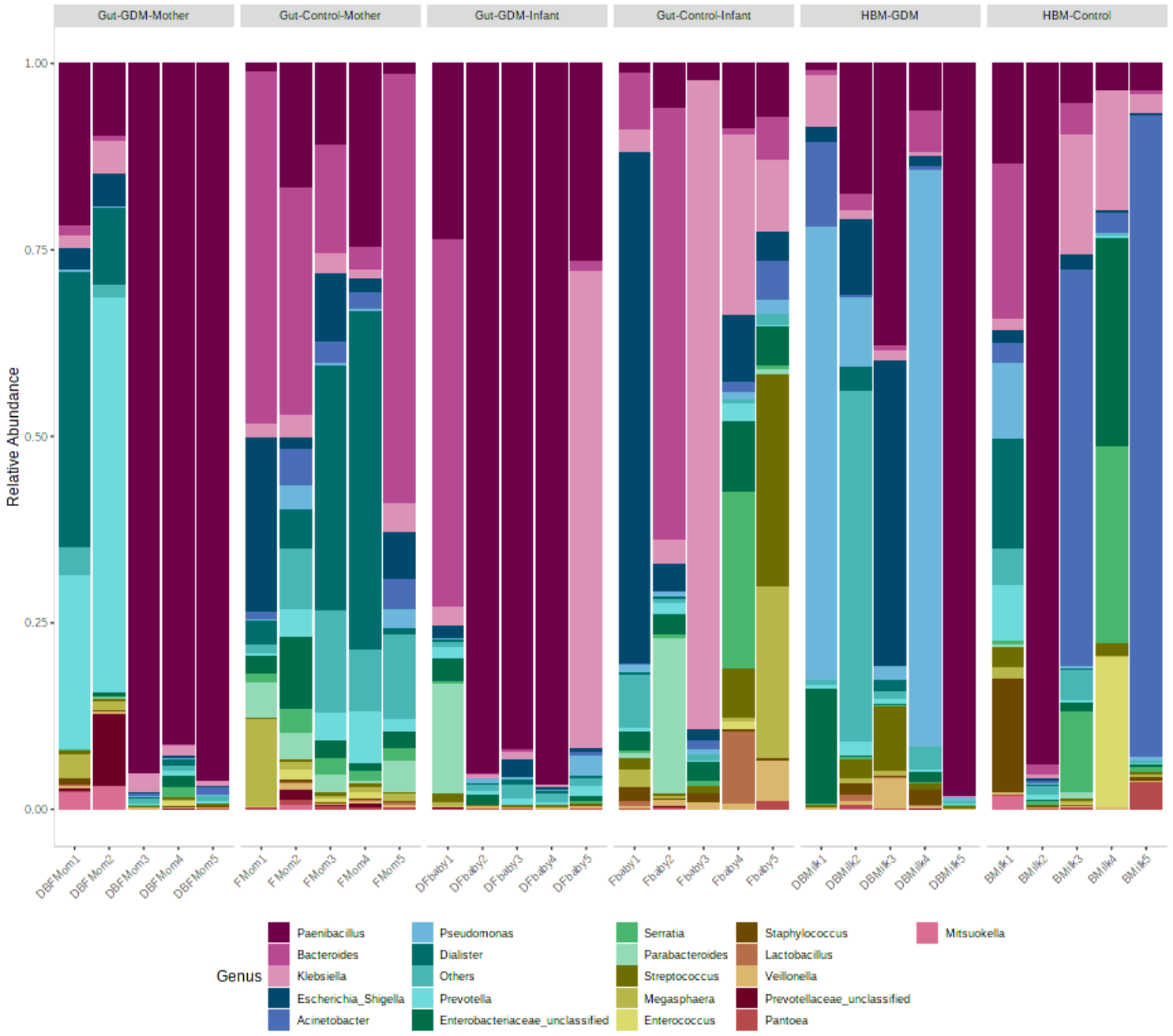

Figure 2

Bar chart of RA at genus level for each sample. DBFMom1-5: DMG gut mother, FMom1-5: control gut mother, DFbaby1-5: DMG gut infant, Fbaby1-5: control gut infant, DBMilk1-5: DMG breast milk, BMilk1-5: control breast milk.

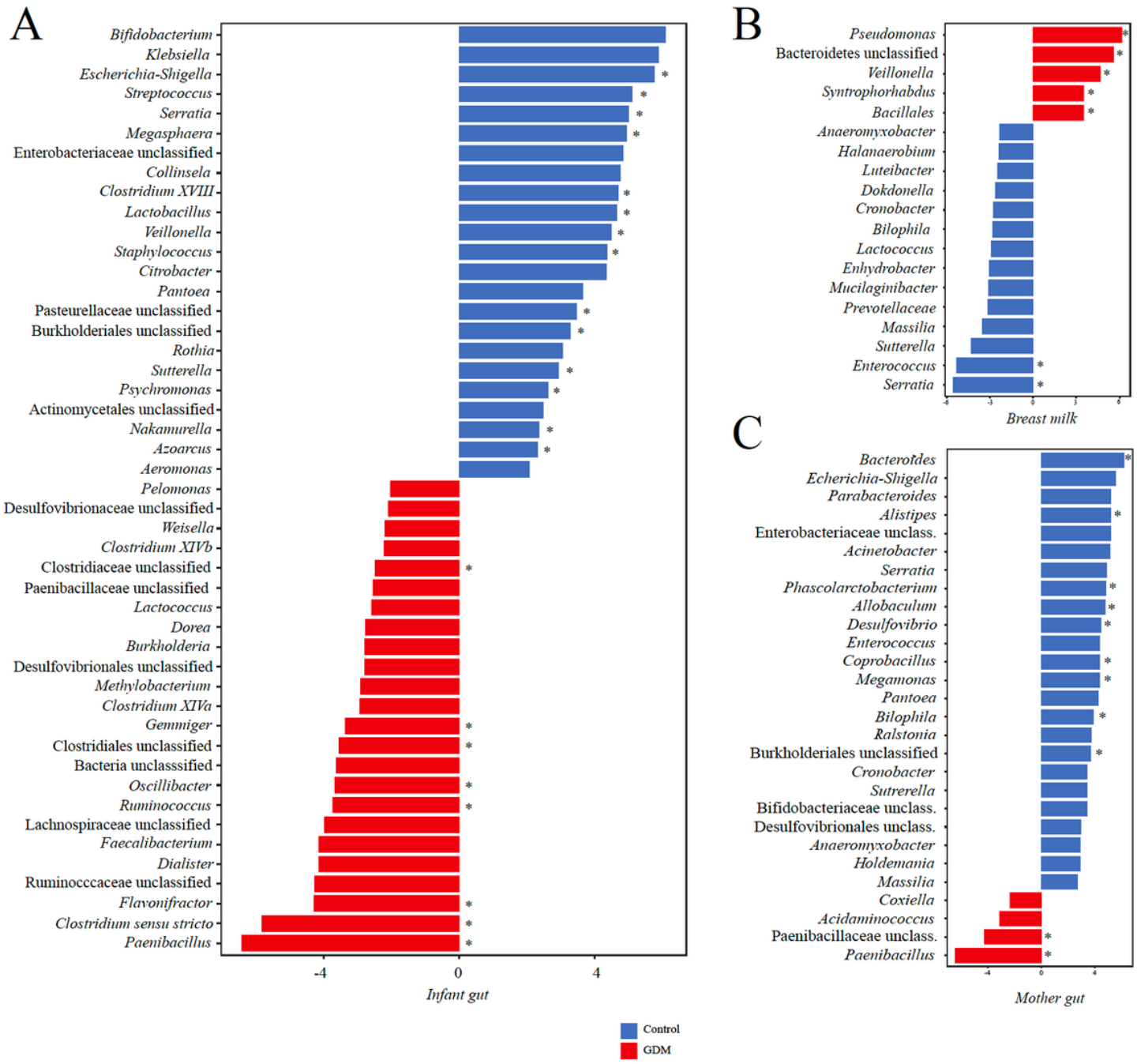

Figure 3

Linear discriminant analysis graph showing significant differential genus abundance among GDM and control infant gut bacteriome.

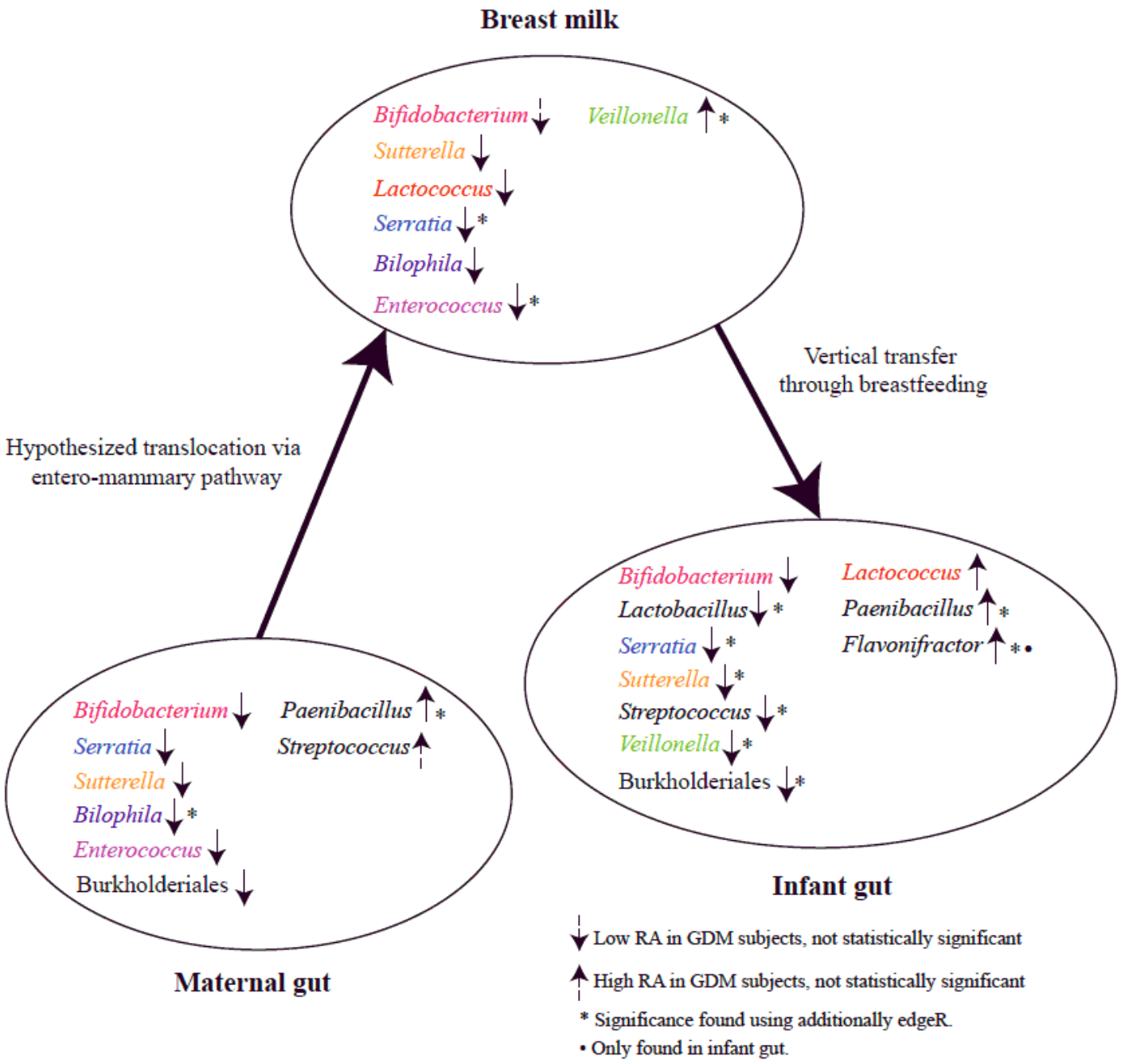

Figure 4

Bacterial differential abundance in GDM subjects. Statistical significance was calculated through a linear discriminant analysis (LDA) with effect size (LEfSe).