US 20230103910A1

(19) **United States**

(12) **Patent Application Publication**

Ong et al.

(10) **Pub. No.: US 2023/0103910 A1**

(43) **Pub. Date: Apr. 6, 2023**

(54) **KEY PREDOMINANT SPECIES OF GUT BACTERIA COLONIZING FARM-EXPOSED INFANTS**

(71) Applicants: **Irene Ong**, Fitschburg, WI (US); **Christine Seroogy**, Madison, WI (US); **James Gern**, Middleton, WI (US); **Deborah Chasman**, Madison, WI (US)

(72) Inventors: **Irene Ong**, Fitschburg, WI (US); **Christine Seroogy**, Madison, WI (US); **James Gern**, Middleton, WI (US); **Deborah Chasman**, Madison, WI (US)

(21) Appl. No.: **17/935,863**

(22) Filed: **Sep. 27, 2022**

Related U.S. Application Data

(60) Provisional application No. 63/248,940, filed on Sep. 27, 2021.

Publication Classification

(51) **Int. Cl.**
C12Q 1/689 (2006.01)

(52) **U.S. Cl.**
CPC ***C12Q 1/689*** (2013.01); *C12Q 1/6869* (2013.01)

(57) **ABSTRACT**

A method to detect immune health status in a human infant or child, and compositions and methods to improve health status in a human fetus, infant or child, as well as compositions and methods useful to improve immune health status, are provided.

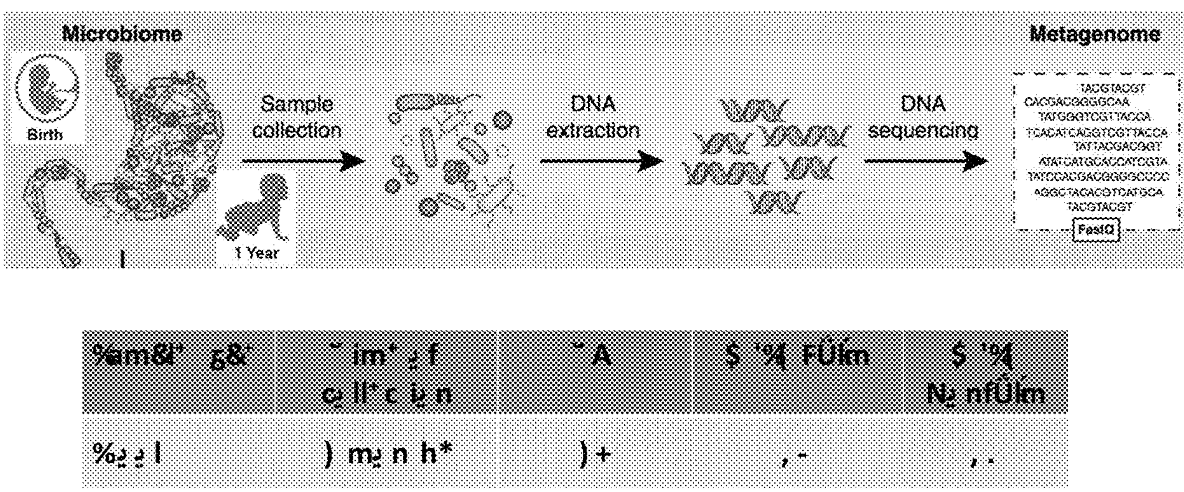
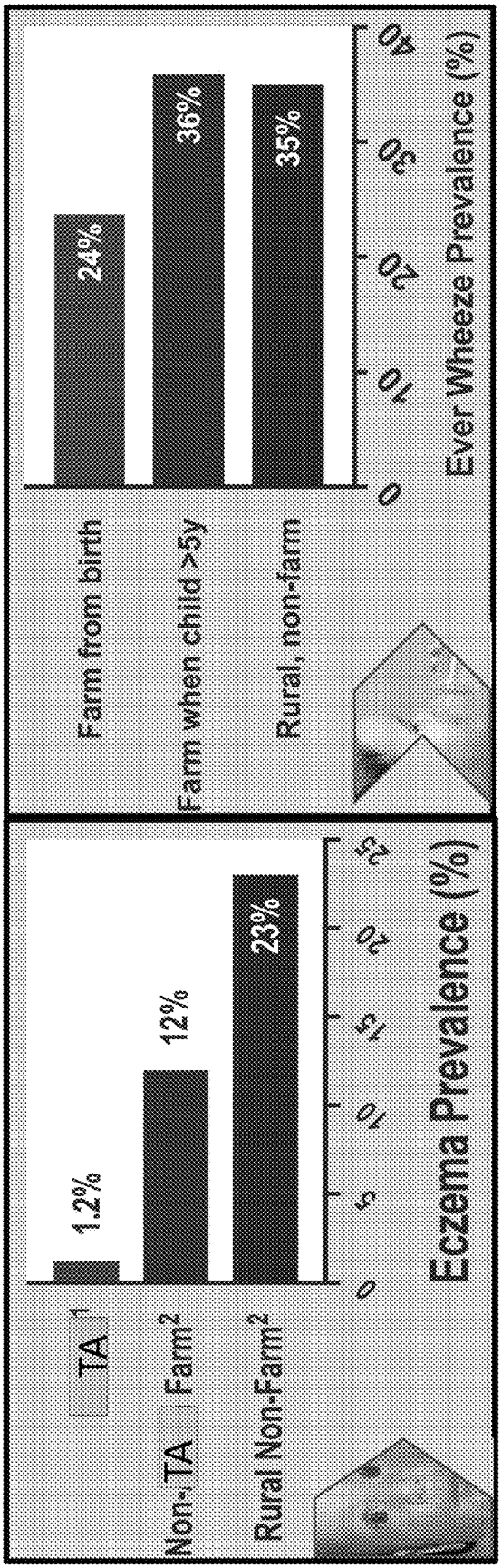


Figure 1


Eczema Prevalence (%)
0
5
10
15
20
25
1.2%
12%
23%
TA[1]
Non-TA Farm[2]
Rural Non-Farm[2]
Ever Wheeze Prevalence (%)
0
10
20
30
40
24%
36%
35%
Farm from birth
Farm when child >5y
Rural, non-farm

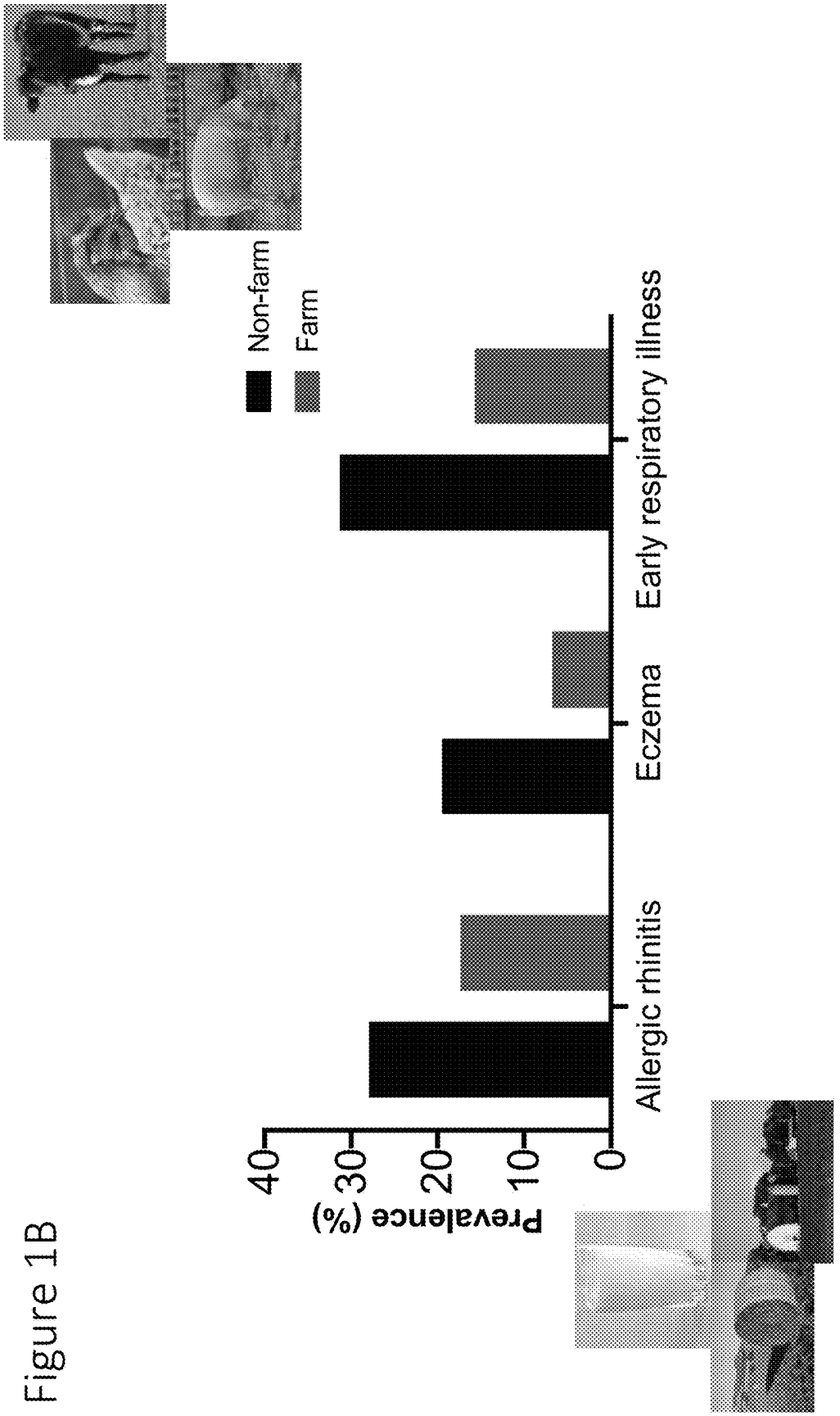

Non-farm
Farm
Prevalence (%)
0
10
20
30
40
Allergic rhinitis
Eczema
Early respiratory illness

Figure 1B

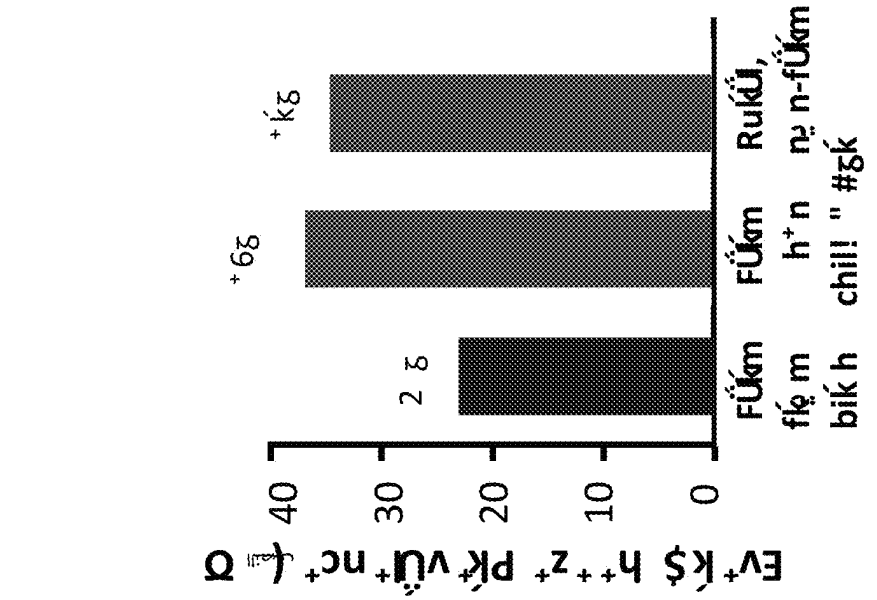

0
10
20
30
40
FŰkm
fkę m
bik h
FŰkm
h+n
chil! "
Ruk Ű,
nų n-fŰkm
#ɡk

Figure 1C

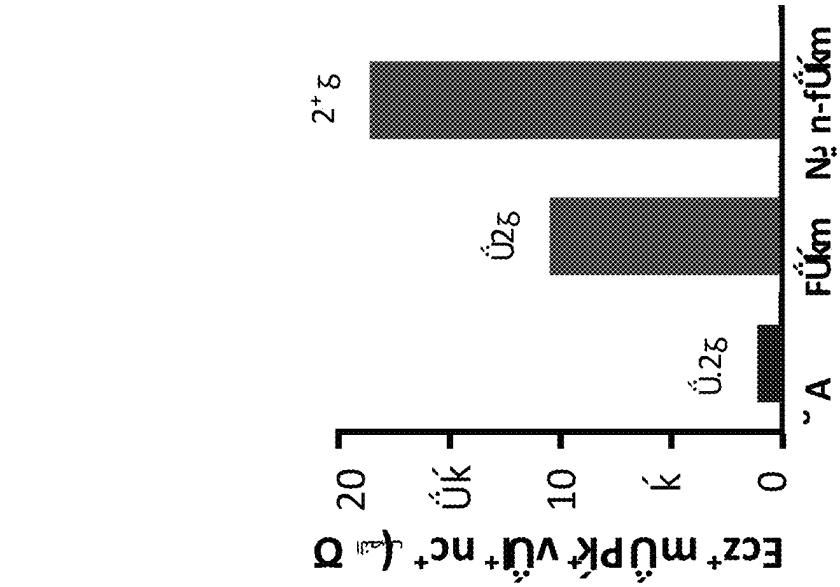

0
ǩ
Ük
20
A
FŰkm
Nų n-fŰkm

Figure 2

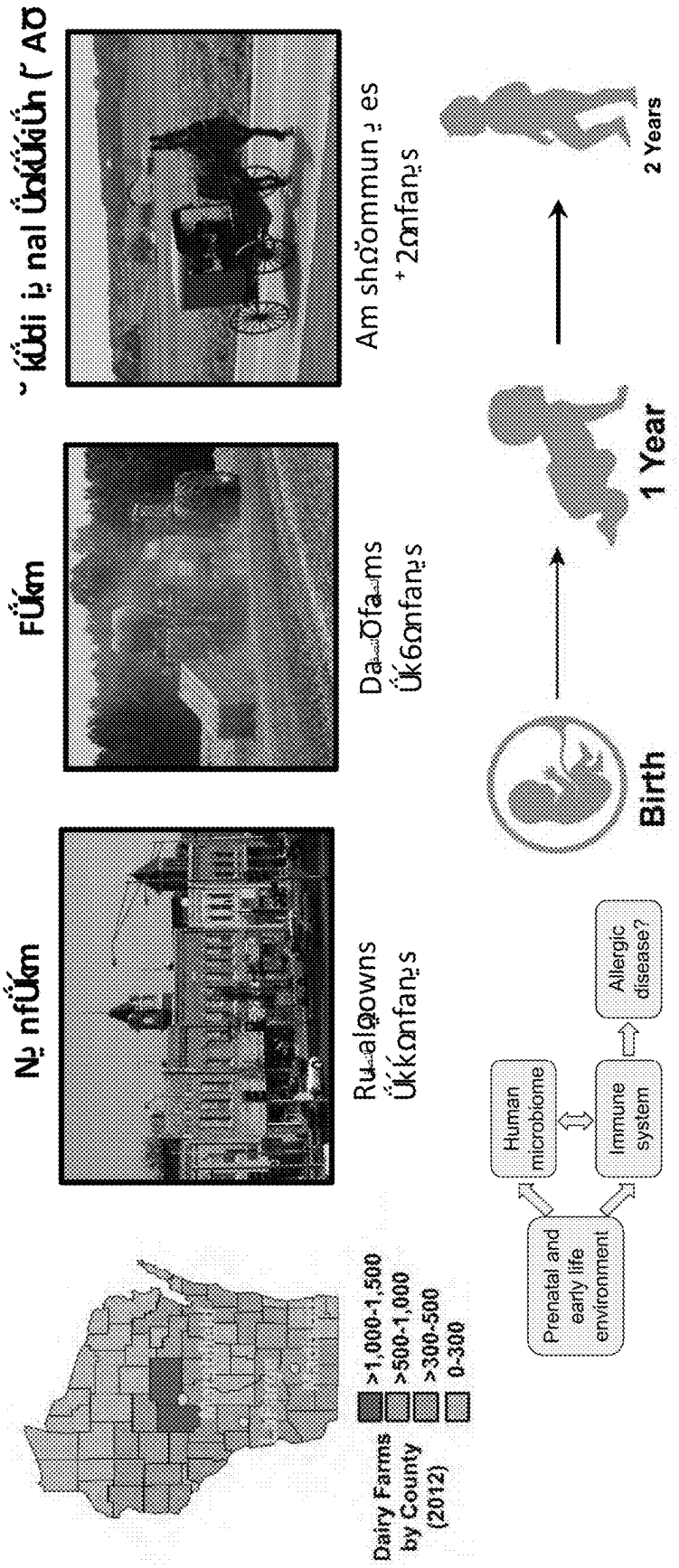

Birth
1 Year
2 Years
Prenatal and early life environment
Human microbiome
Immune system
Allergic disease?
Dairy Farms by County (2012)
>1,000-1,500
>500-1,000
>300-500
0-300

Figure 2B

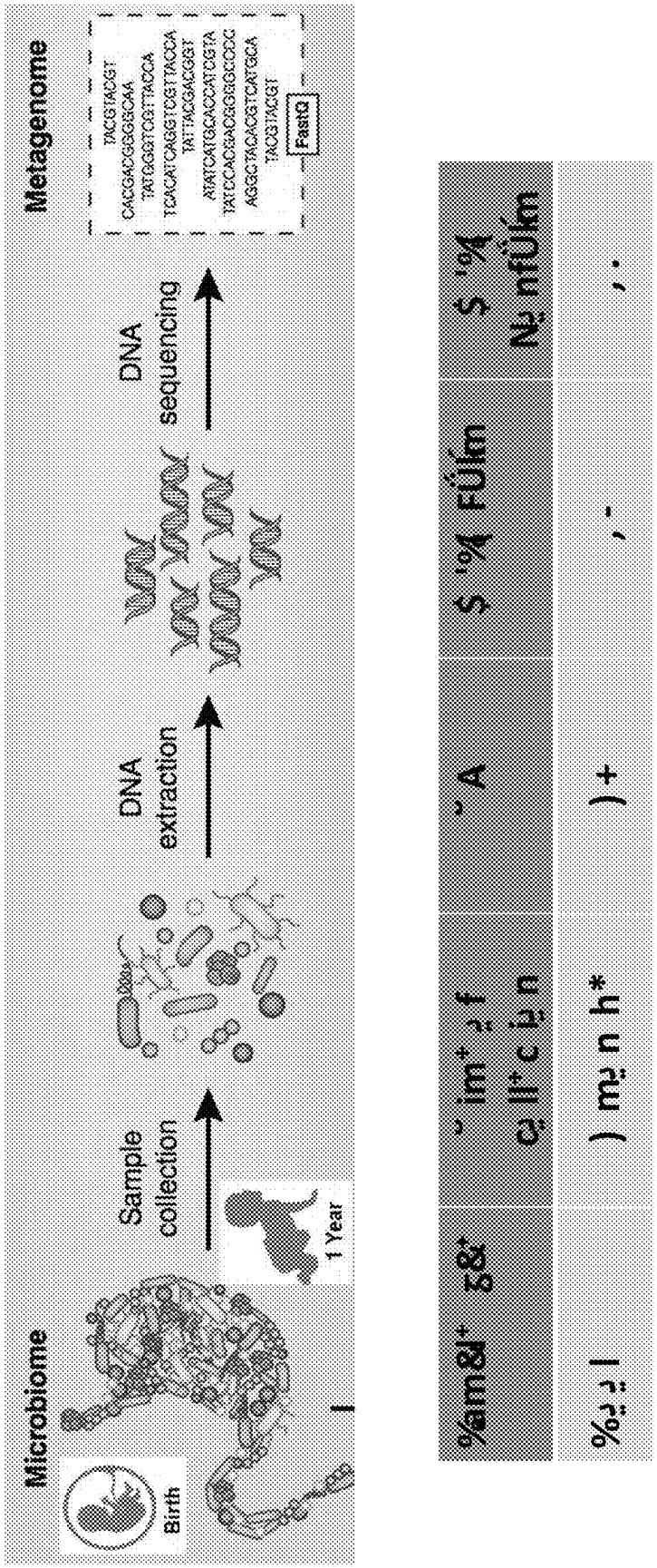
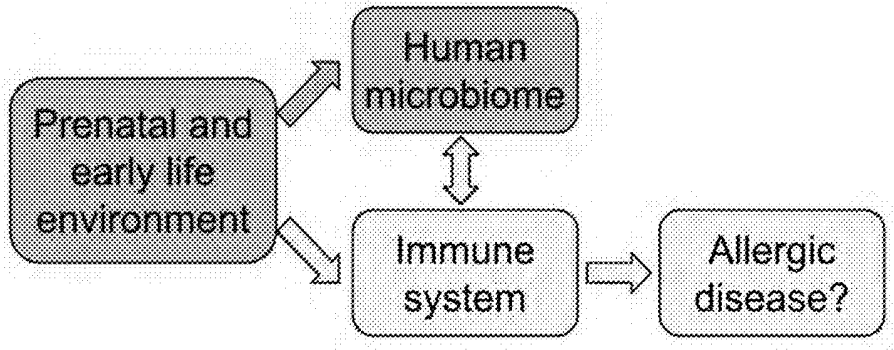

Microbiome
Birth
1 Year
Sample collection
DNA extraction
DNA sequencing
Metagenome
FastQ

Figure 2C

	# TA	# Farm	# Nonfarm
Male	0.67 (18)	0.59 (27)	0.44 (19)
Vaginal delivery	1.00 (27)	0.85 (39)	0.93 (40)
Exclusively breastfeeding	1.00 (27)	0.54 (25)	0.67 (29)
Total	**27**	**46**	**43**



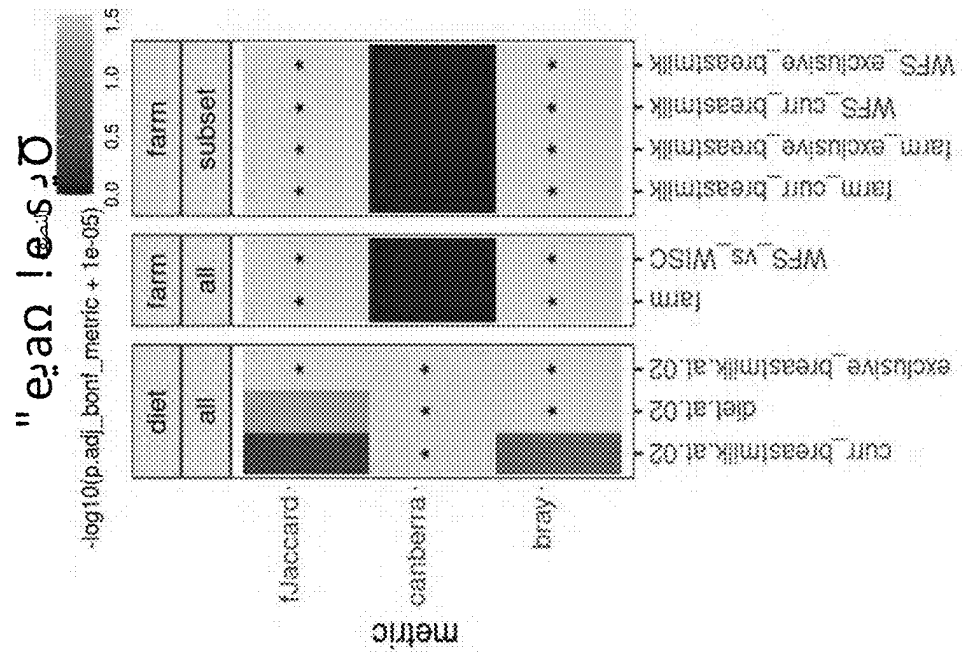


Figure 3

Figure 4

Figure 5

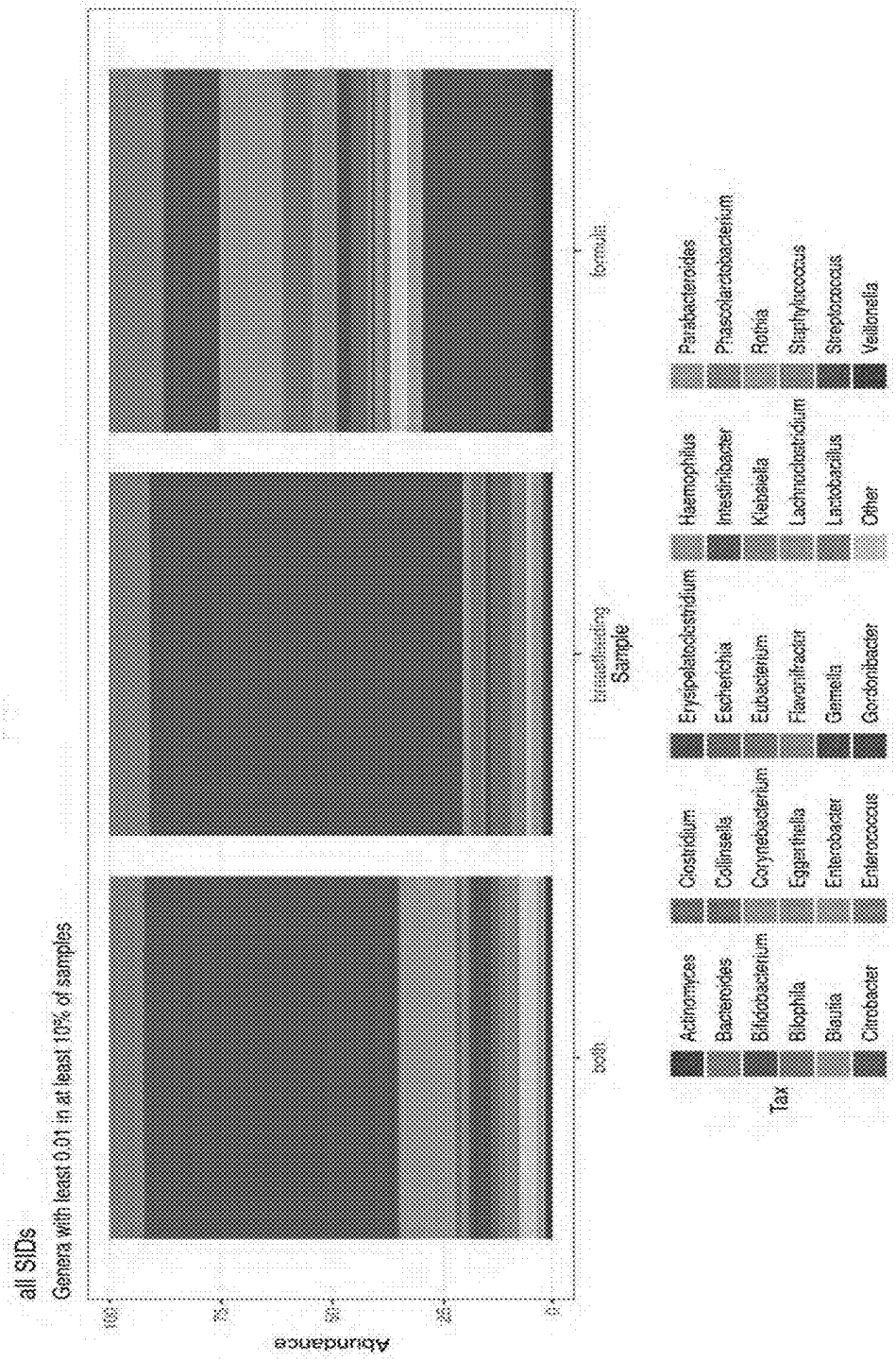

all SIDs
Genera with least 0.01 in at least 10% of samples
Abundance
100
75
50
25
0
both
breastfeeding
formula
Sample
Tax
Actinomyces
Bacteroides
Bifidobacterium
Blautia
Citrobacter
Clostridium
Collinsella
Corynebacterium
Eggerthella
Enterobacter
Enterococcus
Erysipelatoclostridium
Escherichia
Eubacterium
Flavonifractor
Gemella
Gordonibacter
Haemophilus
Intestinibacter
Klebsiella
Lachnoclostridium
Lactobacillus
Other
Parabacteroides
Phascolarctobacterium
Rothia
Staphylococcus
Streptococcus
Veillonella

Figure 6

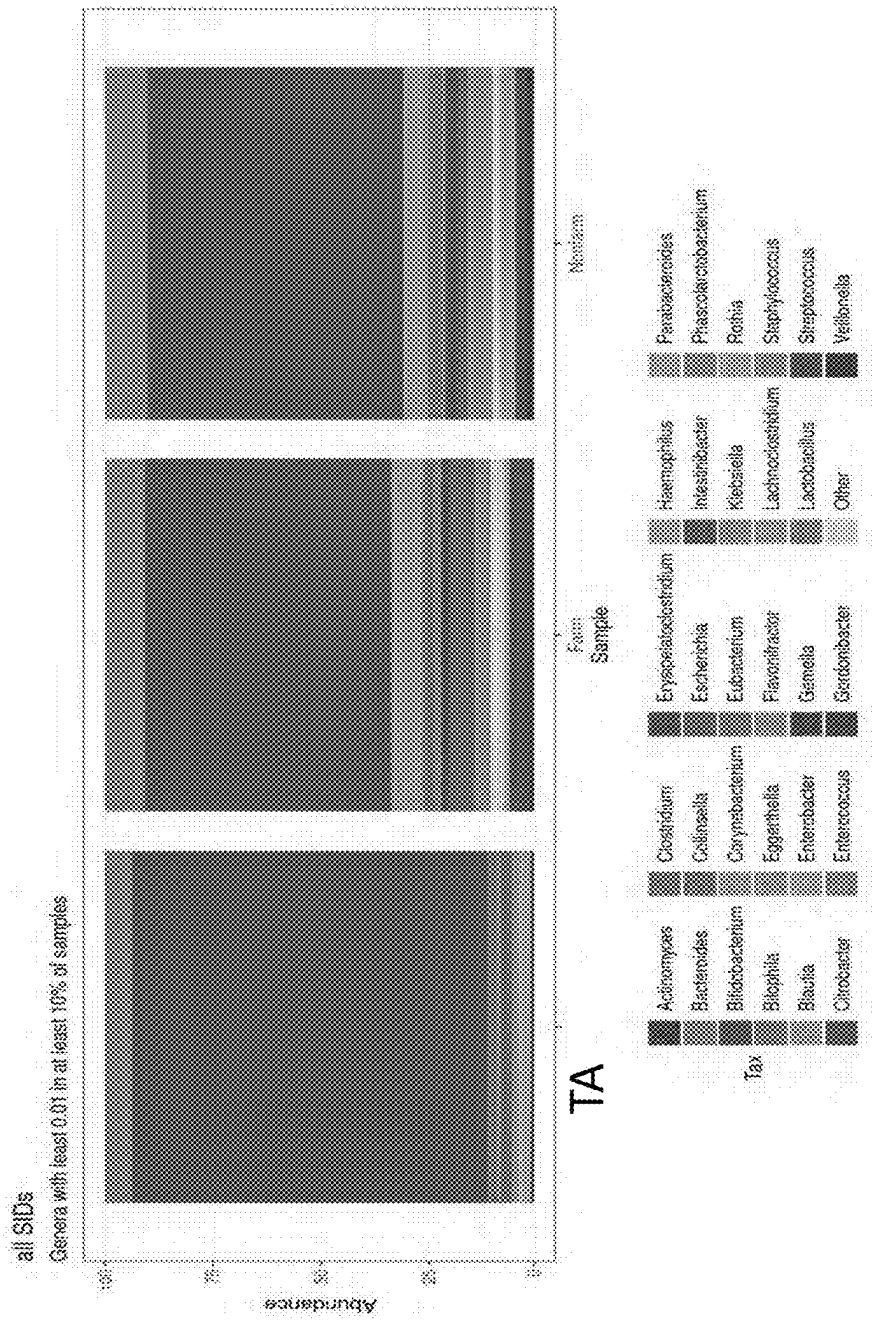

all SIDs
Genera with least 0.01 in at least 10% of samples
Abundance
100
75
50
25
0
TA
Mexico
Sample
Tax
Actinomyces
Bacteroides
Bifidobacterium
Bilophila
Blautia
Citrobacter
Clostridium
Collinsella
Corynebacterium
Eggerthella
Enterobacter
Enterococcus
Erysipelatoclostridium
Escherichia
Eubacterium
Flavonifractor
Gemella
Gordonibacter
Haemophilus
Intestinibacter
Klebsiella
Lachnoclostridium
Lactobacillus
Other
Parabacteroides
Phascolarctobacterium
Rothia
Staphylococcus
Streptococcus
Veillonella

Figure 7

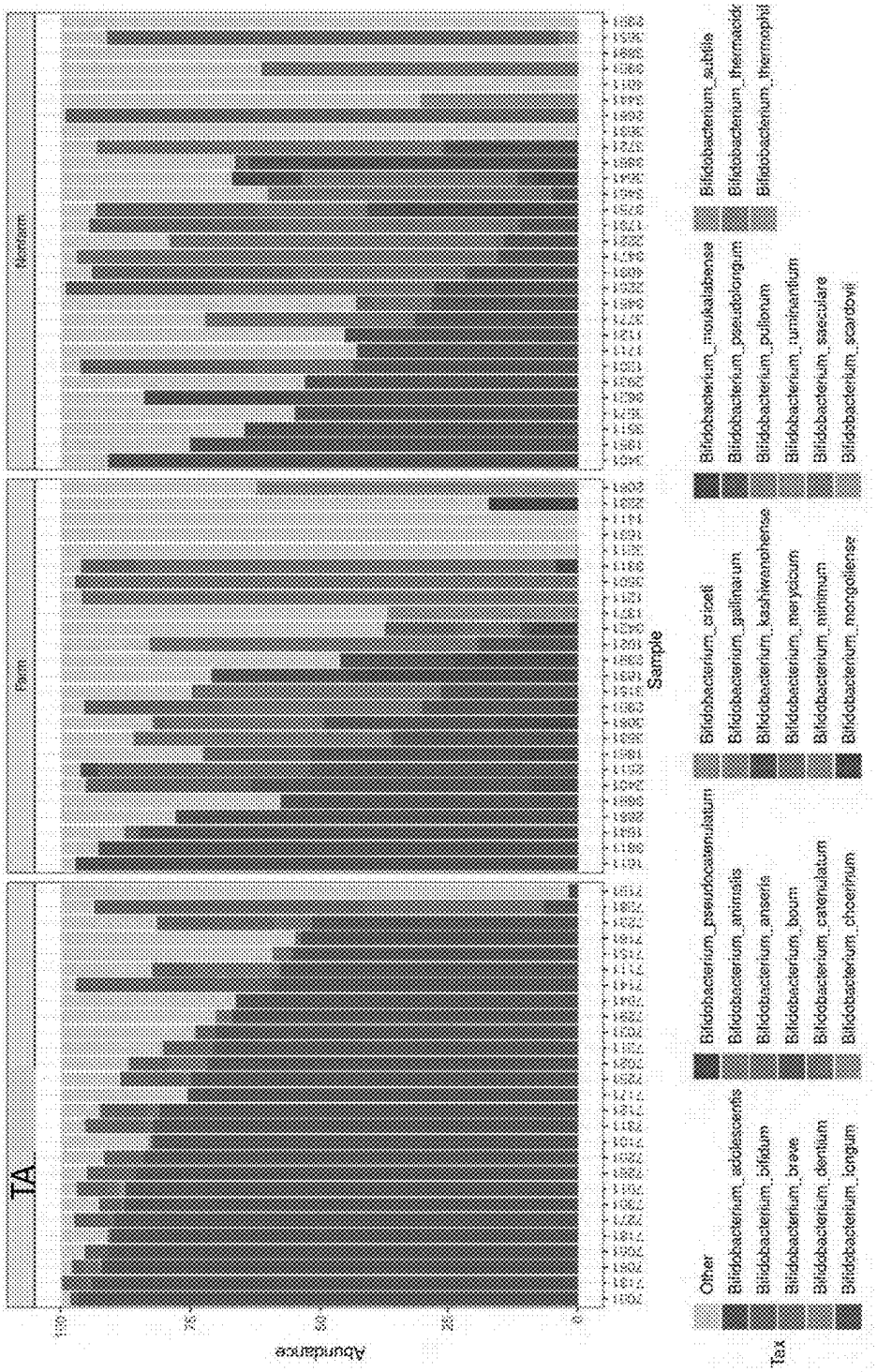

TA
Term
Newborn
Abundance
Sample
Tax
Other
Bifidobacterium_adolescentis
Bifidobacterium_bifidum
Bifidobacterium_breve
Bifidobacterium_dentium
Bifidobacterium_longum
Bifidobacterium_pseudocatenulatum
Bifidobacterium_animalis
Bifidobacterium_animalis
Bifidobacterium_boum
Bifidobacterium_catenulatum
Bifidobacterium_choerinum
Bifidobacterium_cuniculi
Bifidobacterium_gallinarum
Bifidobacterium_kashiwanohense
Bifidobacterium_merycicum
Bifidobacterium_minimum
Bifidobacterium_mongoliense
Bifidobacterium_moukalabense
Bifidobacterium_pseudolongum
Bifidobacterium_pullorum
Bifidobacterium_ruminantium
Bifidobacterium_saeculare
Bifidobacterium_scardovii
Bifidobacterium_subtile
Bifidobacterium_thermacidc
Bifidobacterium_thermophil

Figure 8

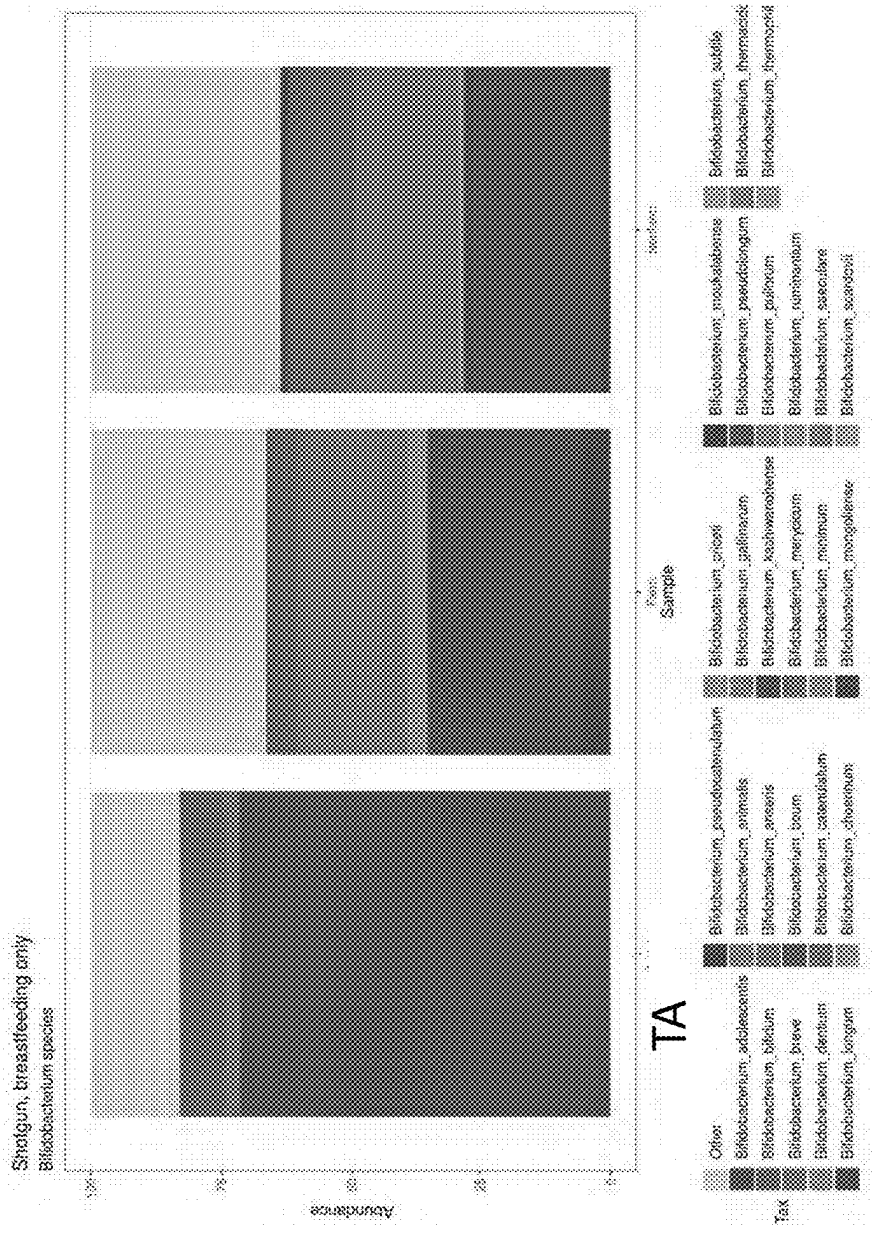

Shotgun, breastfeeding only
Bifidobacterium species
Abundance
Sample
TA
Tax
Other
Bifidobacterium_adolescentis
Bifidobacterium_bifidum
Bifidobacterium_breve
Bifidobacterium_dentium
Bifidobacterium_longum
Bifidobacterium_pseudocatenulatum
Bifidobacterium_animalis
Bifidobacterium_angulatum
Bifidobacterium_boum
Bifidobacterium_catenulatum
Bifidobacterium_choerinum
Bifidobacterium_gloriosum
Bifidobacterium_gallinarum
Bifidobacterium_kashiwanohense
Bifidobacterium_merycicum
Bifidobacterium_minimum
Bifidobacterium_mongoliense
Bifidobacterium_moukalabense
Bifidobacterium_pseudolongum
Bifidobacterium_pullorum
Bifidobacterium_ruminantium
Bifidobacterium_saeculare
Bifidobacterium_scardovii
Bifidobacterium_subtile
Bifidobacterium_thermacidi
Bifidobacterium_thermophil

Figure 9.

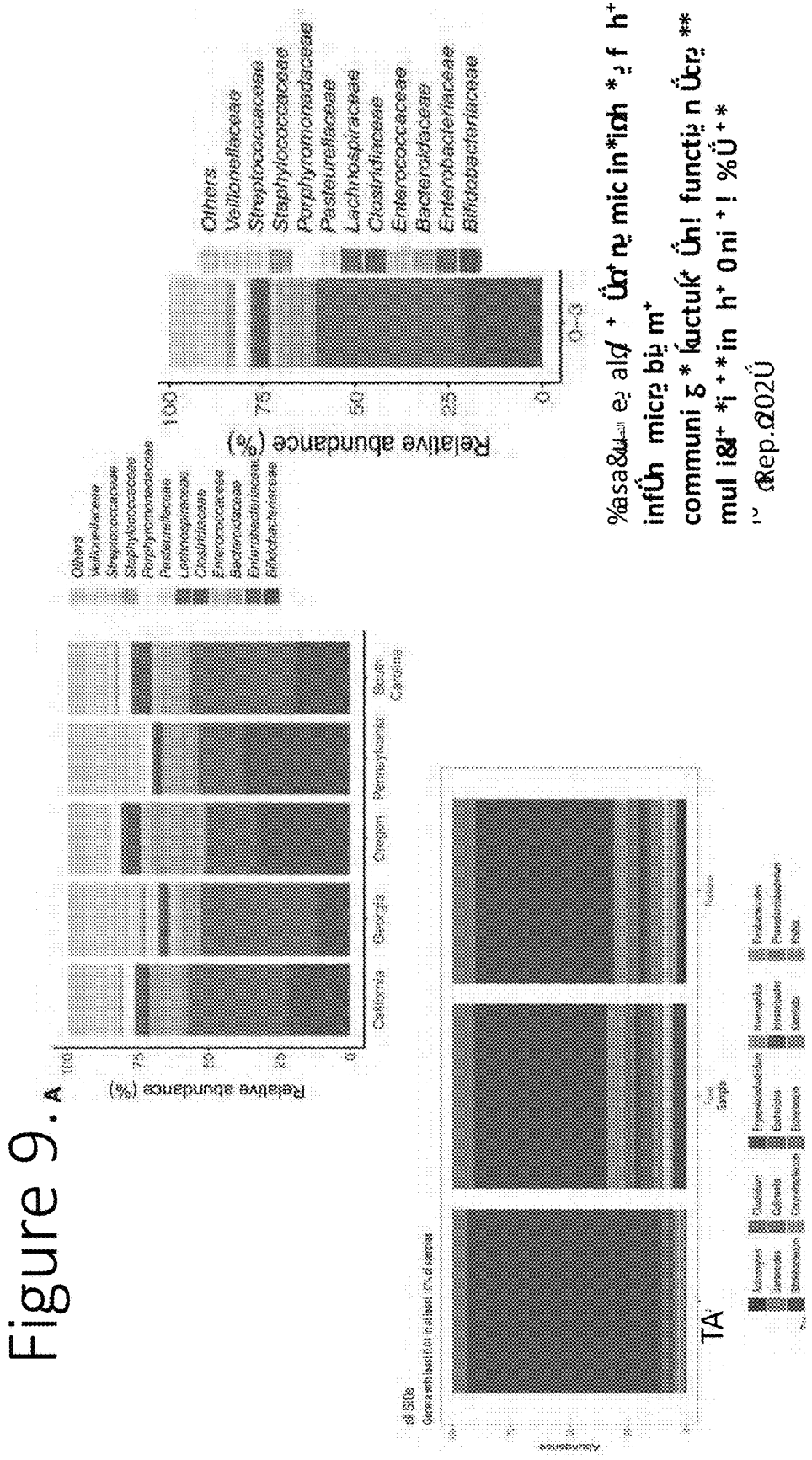
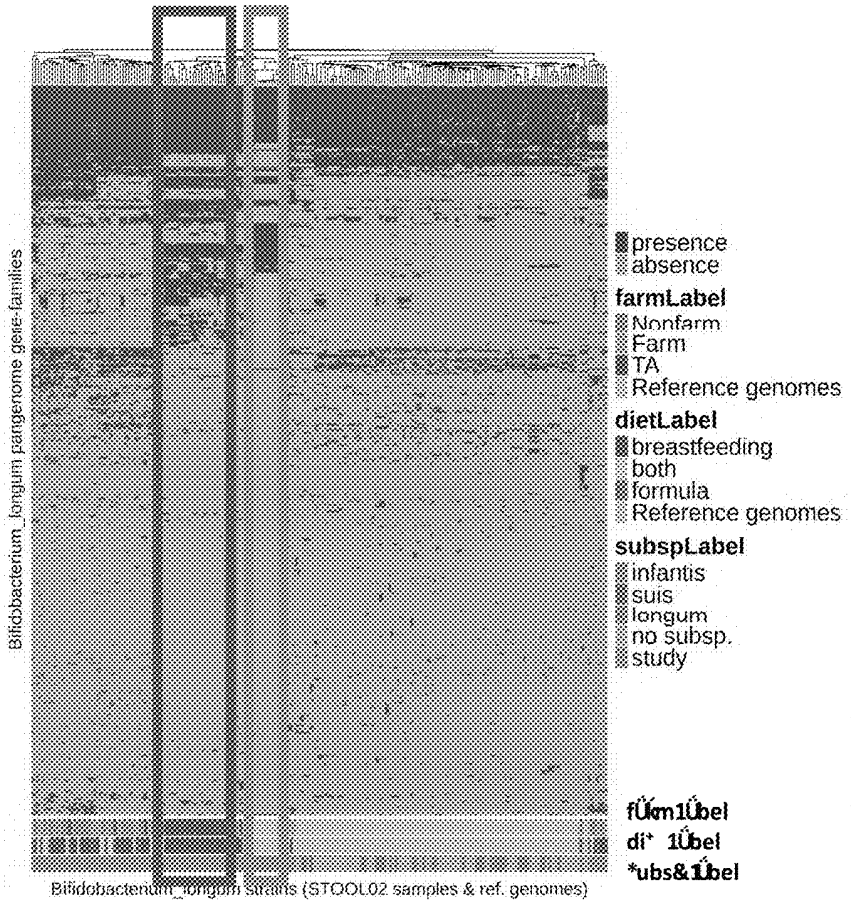
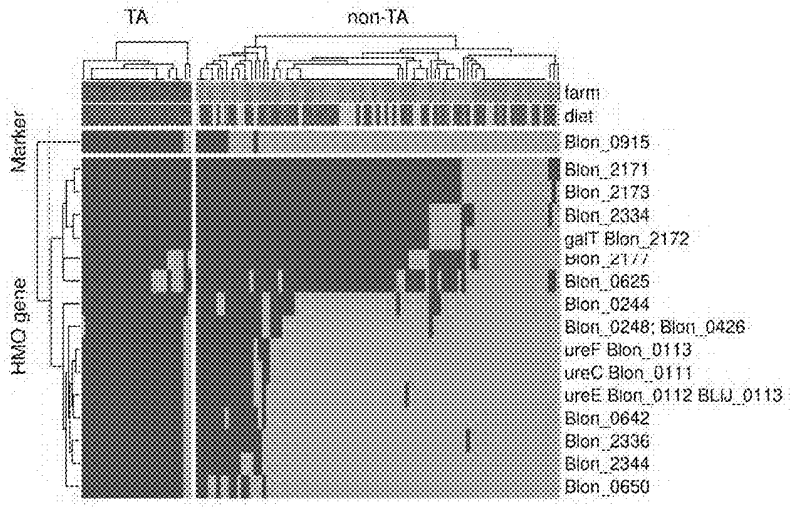

A
Relative abundance (%)
California
Georgia
Oregon
Pennsylvania
South Carolina
Others
Veillonellaceae
Streptococcaceae
Staphylococcaceae
Porphyromonadaceae
Pasteurellaceae
Lachnospiraceae
Clostridiaceae
Enterococcaceae
Bacteroidaceae
Enterobacteriaceae
Bifidobacteriaceae
Relative abundance (%)
0–3
TA
Abundance
Sample
Actinomyces
Bacteroides
Blautia
Collinsella
Corynebacterium
Dorea
Enterococcus
Eubacterium
Haemophilus
Immunobacter
Klebsiella
Pasteurella
Pseudomonadales
Rothia

Figure 10.

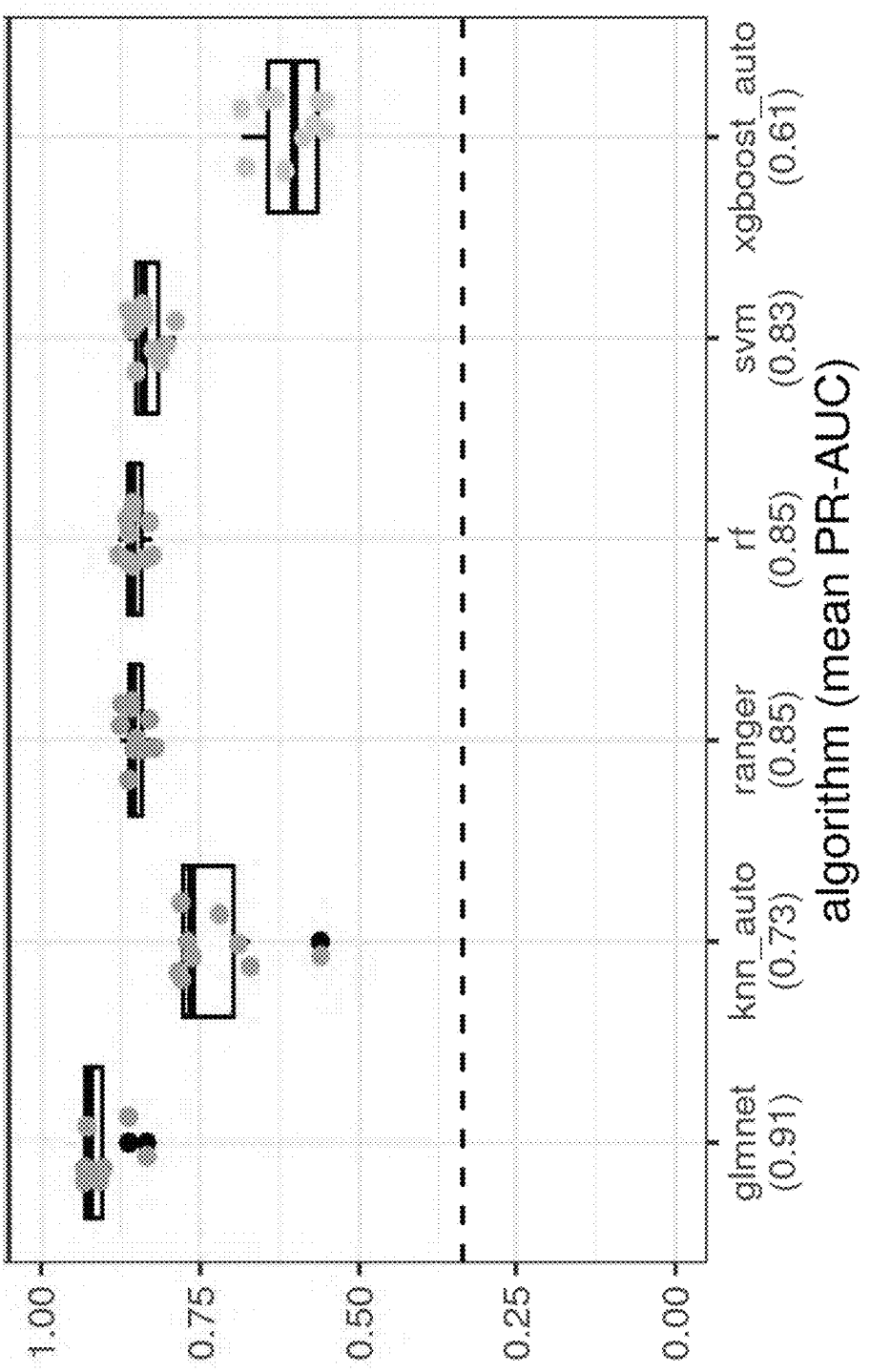




TAsamplesdha!eomoeo*infantis*(spe˘ f˘ Ω) umanΩ* l+Ω l $osa˘˘ ha eΩ) * , .Ω$enes

-) *, Ω˘apa˘ ˛ÕofΩnfan˛ s -/o%as˘ o e˛al 20Ű0Ω 1n e woo e˛ al 20Ű˙.
- 2nfan˛ s e˘ l n n$Ωn non-TAΩ˘ommun ˛ esΩDa! s e˛ al.0Ω 20Ű34%asa&u e˛al.0202Ű4Ω eppo e˛ al.0202Ű.

Figure 11


1.00
0.75
0.50
0.25
0.00
glmnet (0.91)
knn_auto (0.73)
ranger (0.85)
rf (0.85)
svm (0.83)
xgboost_auto (0.61)
algorithm (mean PR-AUC)

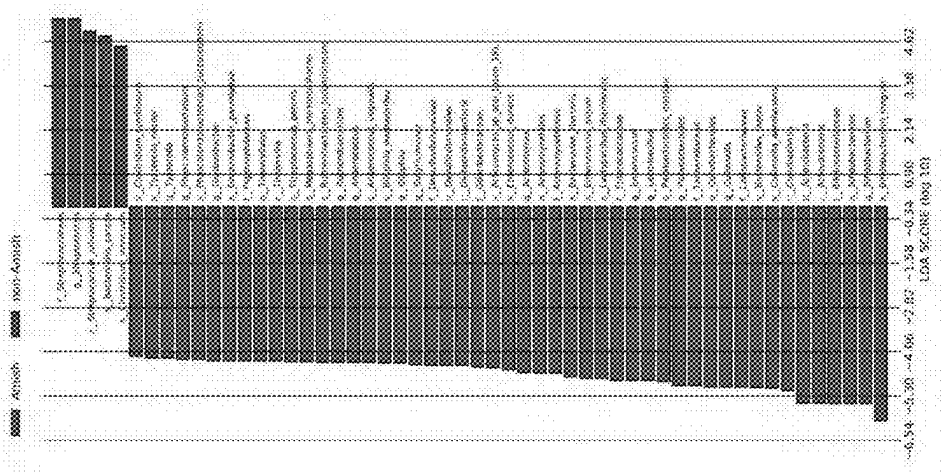

Arwin
non-Arwin
LDA SCORE (log 10)

Figure 12

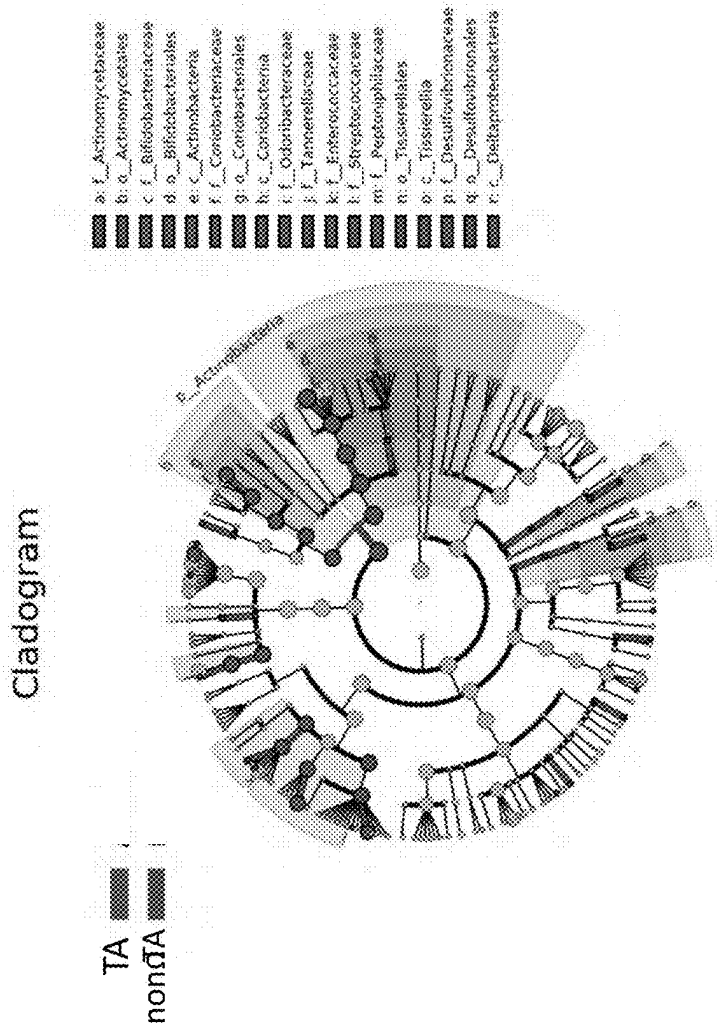
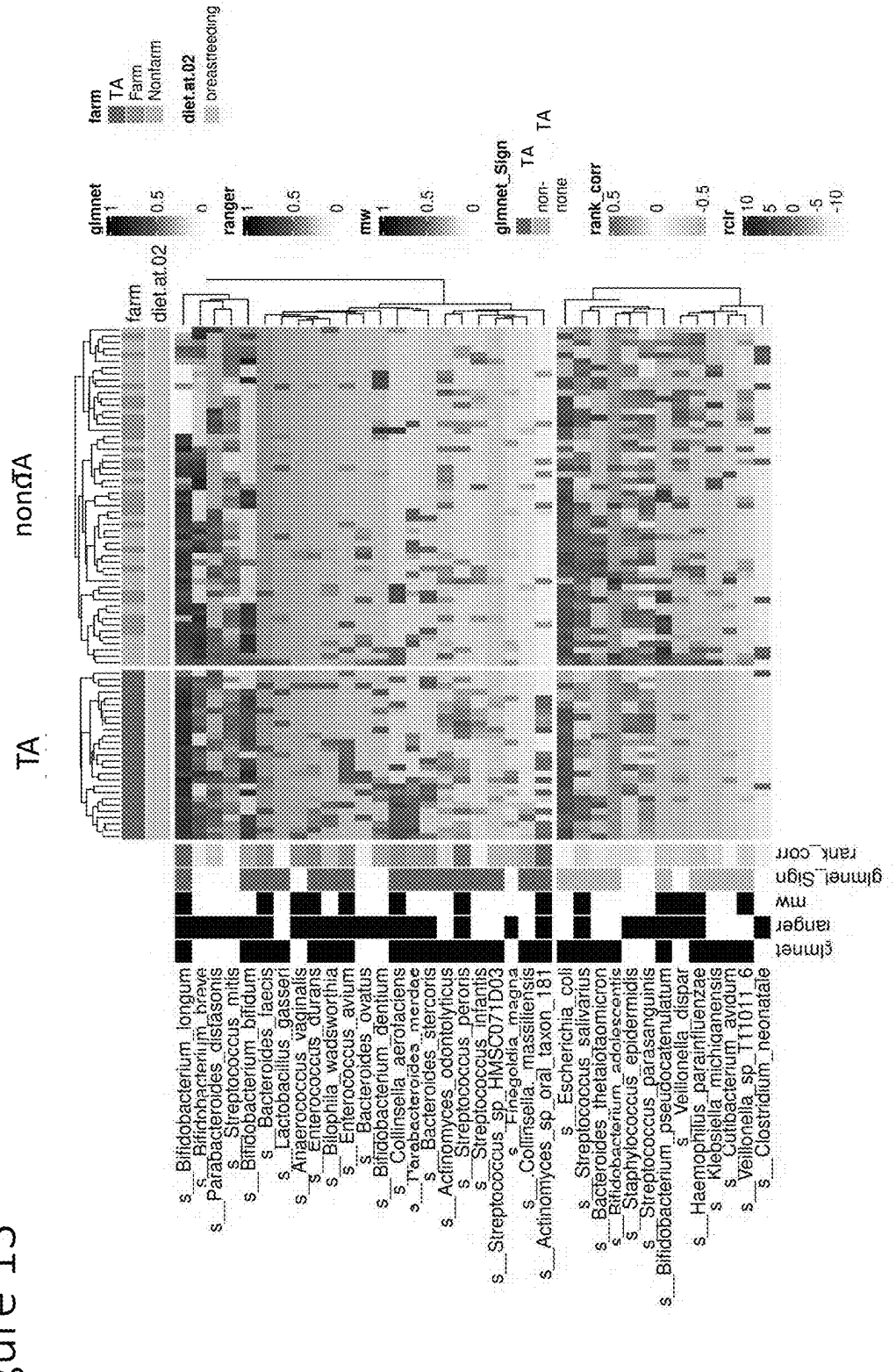

a: f_Actinomycetaceae
b: o_Actinomycetales
c: f_Bifidobacteriaceae
d: o_Bifidobacteriales
e: c_Actinobacteria
f: f_Coriobacteriaceae
g: o_Coriobacteriales
h: c_Coriobacteriia
i: f_Odoribacteraceae
j: f_Tannerellaceae
k: f_Enterococcaceae
l: f_Streptococcaceae
m: f_Peptostreptococcaceae
n: o_Tissierellales
o: c_Tissierellia
p: f_Desulfovibrionaceae
q: o_Desulfovibrionales
r: c_Deltaproteobacteria
Cladogram
TA
nonTA

Figure 13


TA
nonTA
farm
diet.at.02
glmnet
ranger
mw
glmnet_Sign
rank_corr
s__Bifidobacterium_longum
s__Bifidobacterium_breve
s__Parabacteroides_distasonis
s__Streptococcus_mitis
s__Bifidobacterium_bifidum
s__Bacteroides_faecis
s__Lactobacillus_gasseri
s__Anaerococcus_vaginalis
s__Enterococcus_durans
s__Blautia_wadsworthia
s__Enterococcus_avium
s__Bacteroides_ovatus
s__Collinsella_aerofaciens
s__Parabacteroides_merdae
s__Bacteroides_stercoris
s__Actinomyces_odontolyticus
s__Streptococcus_peroris
s__Streptococcus_infantis
s__Streptococcus_sp_HMSC071D03
s__Finegoldia_magna
s__Collinsella_massiliensis
s__Actinomyces_sp_oral_taxon_181
s__Escherichia_coli
s__Streptococcus_salivarius
s__Bacteroides_thetaiotaomicron
s__Bifidobacterium_adolescentis
s__Staphylococcus_epidermidis
s__Streptococcus_parasanguinis
s__Bifidobacterium_pseudocatenulatum
s__Veillonella_dispar
s__Haemophilus_parainfluenzae
s__Klebsiella_michiganensis
s__Cutibacterium_avidum
s__Veillonella_sp_T11011_6
s__Clostridium_neonatale

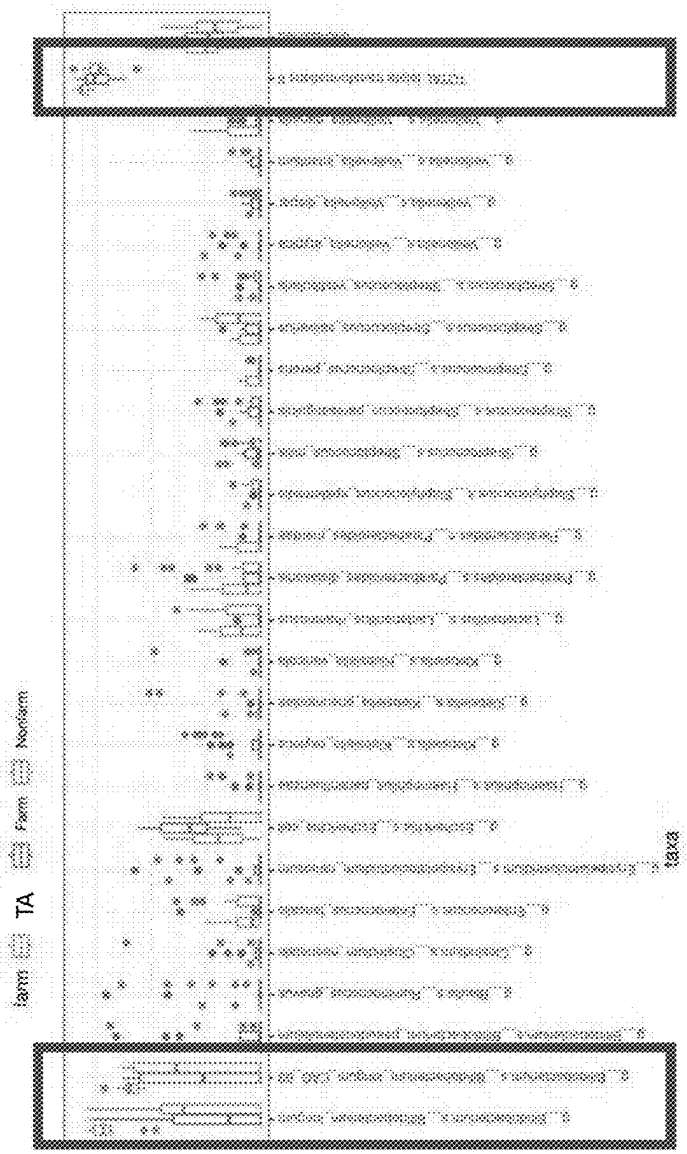

farm
TA
Farm
Nonfarm
taxa

Figure 14

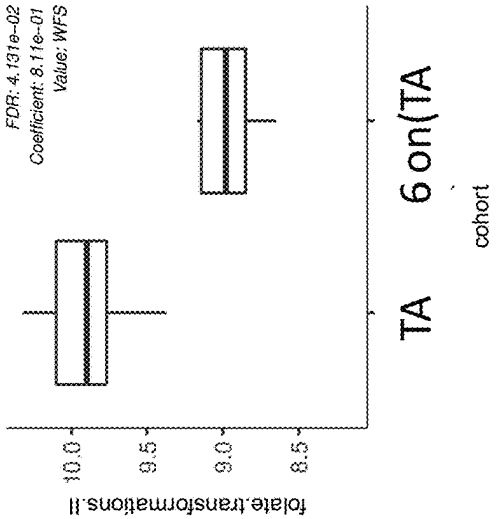

FDR: 4.131e-02
Coefficient: 8.11e-01
Value: WFS
folate.transformations.ll
10.0
9.5
9.0
8.5
TA
6 on(TA
cohort

Figure 15

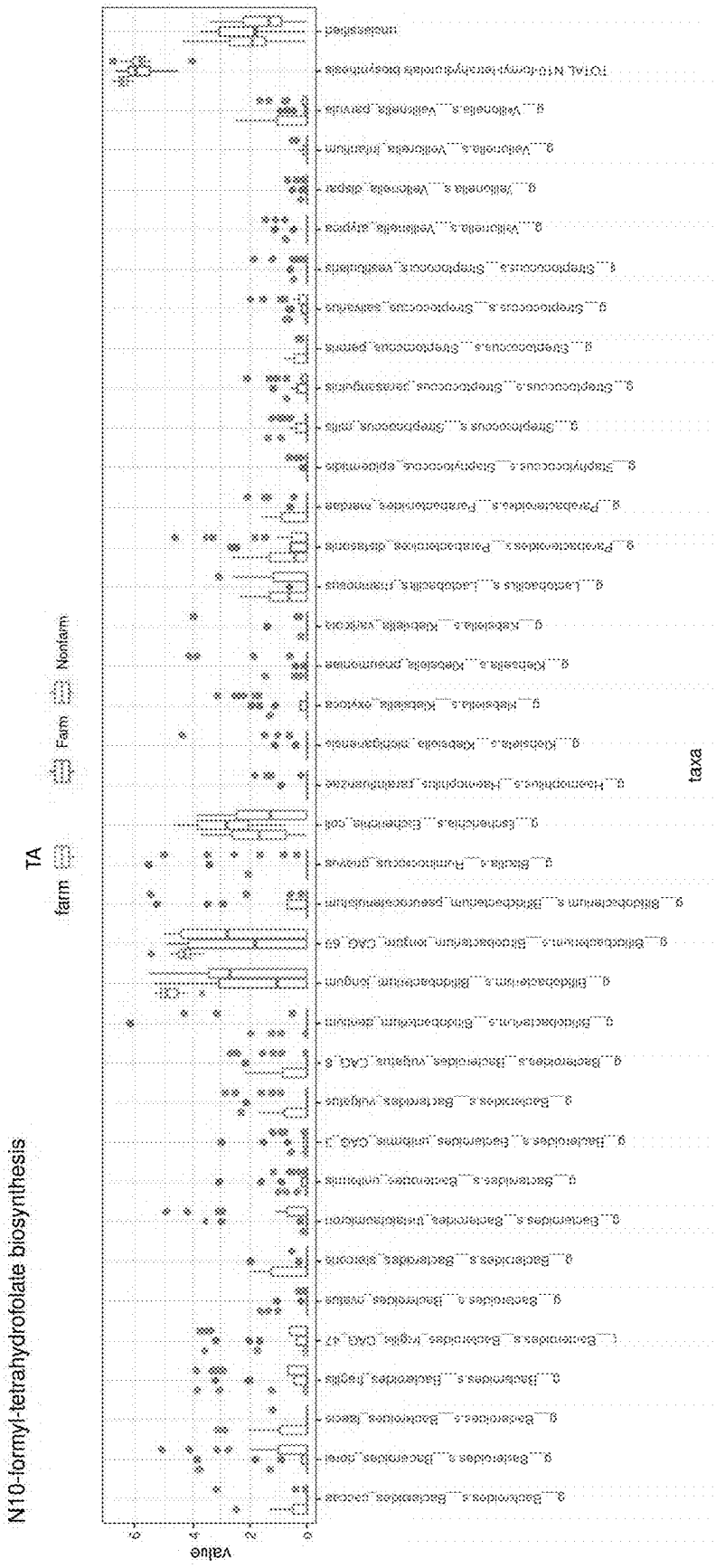
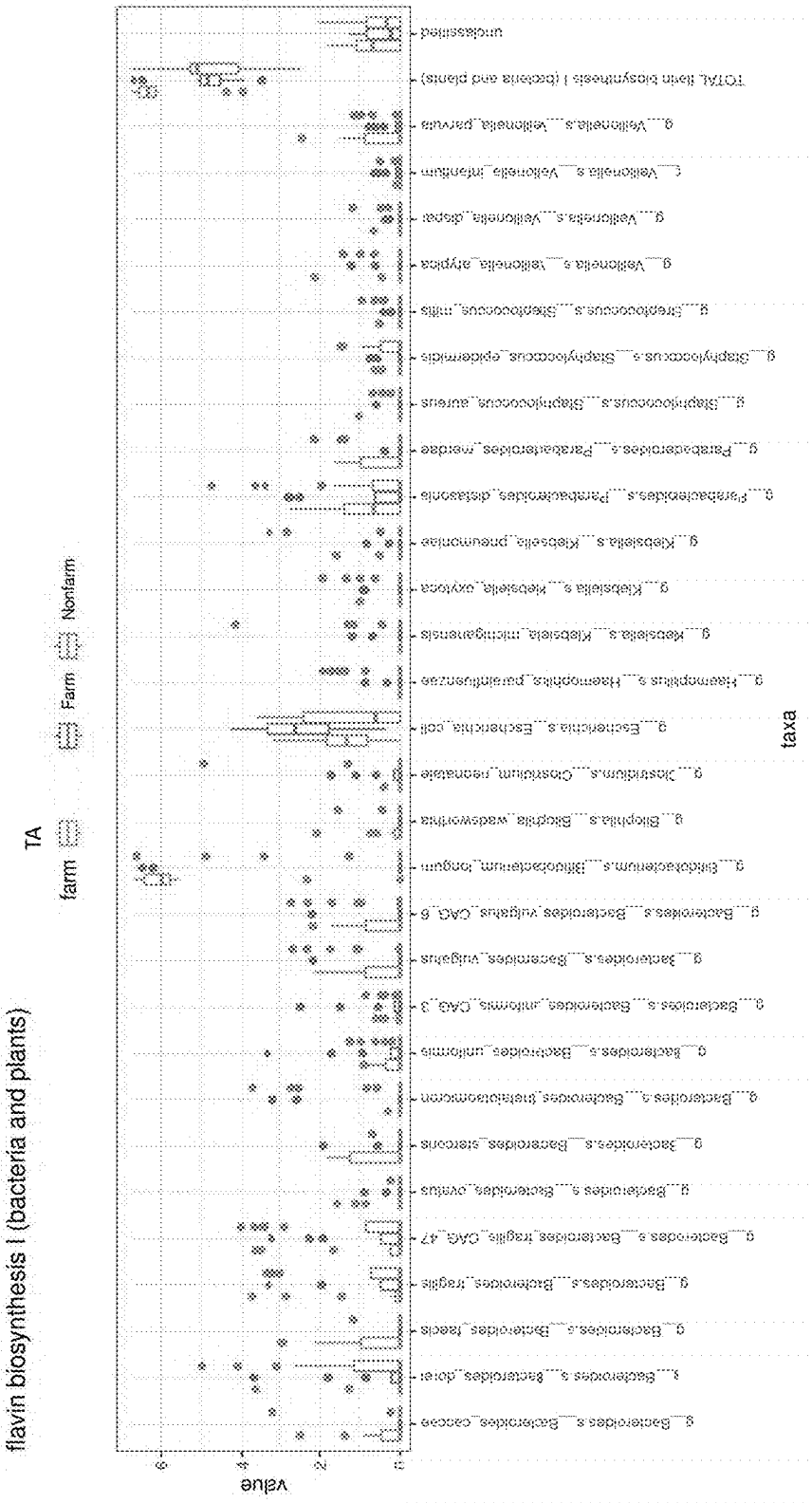

N10-formyl-tetrahydrofolate biosynthesis
TA
farm
Farm
Nonfarm
value
taxa
unclassified
TOTAL N10-formyltetrahydrofolate biosynthesis
g__Veillonella.s__Veillonella_parvula
g__Veillonella.s__Veillonella_ratti
g__Veillonella.s__Veillonella_dispar
g__Veillonella.s__Veillonella_atypica
g__Streptococcus.s__Streptococcus_vestibularis
g__Streptococcus.s__Streptococcus_salivarius
g__Streptococcus.s__Streptococcus_parasanguinis
g__Streptococcus.s__Streptococcus_mitis
g__Staphylococcus.s__Staphylococcus_epidermidis
g__Prevotella.s__Prevotella_melaninogenica
g__Prevotella.s__Prevotella_intermedia
g__Prevotella.s__Prevotella_denticola
g__Porphyromonas.s__Porphyromonas_gingivalis
g__Parabacteroides.s__Parabacteroides_merdae
g__Lactobacillus.s__Lactobacillus_rhamnosus
g__Klebsiella.s__Klebsiella_variicola
g__Klebsiella.s__Klebsiella_pneumoniae
g__Klebsiella.s__Klebsiella_oxytoca
g__Klebsiella.s__Klebsiella_michiganensis
g__Haemophilus.s__Haemophilus_parainfluenzae
g__Escherichia.s__Escherichia_coli
g__Bacteroides.s__Bacteroides_uniformis
g__Bacteroides.s__Bacteroides_thetaiotaomicron
g__Bacteroides.s__Bacteroides_stercoris
g__Bacteroides.s__Bacteroides_massiliensis_CAG_43
g__Bacteroides.s__Bacteroides_ovatus
g__Bacteroides.s__Bacteroides_eggerthii
g__Bacteroides.s__Bacteroides_caccae

Figure 16


flavin biosynthesis I (bacteria and plants)
farm
TA
Farm
Nonfarm
value
taxa
g__Bacteroides.s__Bacteroides_caccae
s__Bacteroides.s__Bacteroides_dorei
g__Bacteroides.s__Bacteroides_faecis
g__Bacteroides.s__Bacteroides_fragilis
g__Bacteroides.s__Bacteroides_fragilis_CAG_47
g__Bacteroides.s__Bacteroides_ovatus
g__Bacteroides.s__Bacteroides_sieraeis
g__Bacteroides.s__Bacteroides_thetaiotaomicron
g__Bacteroides.s__Bacteroides_uniformis
g__Bacteroides.s__Bacteroides_uniformis_CAG_3
g__Bacteroides.s__Bacteroides_vulgatus
g__Bacteroides.s__Bacteroides_vulgatus_CAG_6
g__Eubacterium.s__Ruminococcus_longum
g__Blautia.s__Blautia_wadsworthia
g__Clostridium.s__Clostridium_neonatale
g__Escherichia.s__Escherichia_coli
g__Haemophilus.s__Haemophilus_parainfluenzae
g__Klebsiella.s__Klebsiella_michiganensis
g__Klebsiella.s__Klebsiella_oxytoca
g__Klebsiella.s__Klebsiella_pneumoniae
g__Parabacteroides.s__Parabacteroides_distasonis
g__Parabacteroides.s__Parabacteroides_merdae
g__Staphylococcus.s__Staphylococcus_aureus
g__Staphylococcus.s__Staphylococcus_epidermidis
g__Streptococcus.s__Streptococcus_mitis
g__Veillonella.s__Veillonella_atypica
g__Veillonella.s__Veillonella_dispar
g__Veillonella.s__Veillonella_montpellierensis
g__Veillonella.s__Veillonella_parvula
TOTAL (fatty biosynthesis I (bacteria and plants))
unclassified

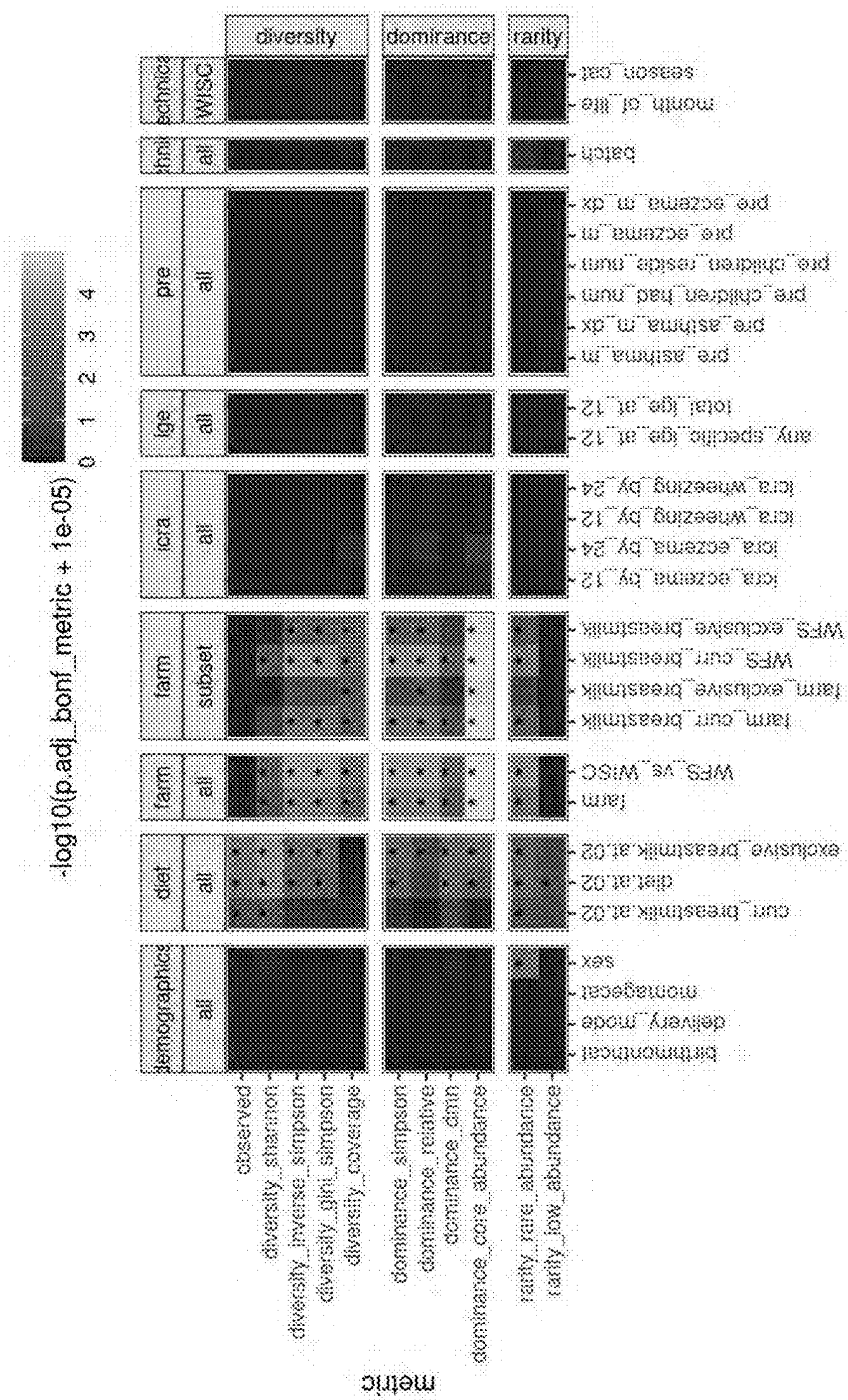

-log10(p.adj_bonf_metric + 1e-05)
0
1
2
3
4
diversity
dominance
rarity
demographics
diet
farm
farm
icra
ige
pre
all
subset
WISC
season_cat
month_of_life
batch
pre_eczema_m_dx
pre_eczema_m
pre_children_reside_num
pre_children_had_num
pre_asthma_m_dx
pre_asthma_m
total_ige_at_12
any_specific_ige_at_12
icra_wheezing_by_24
icra_wheezing_by_12
icra_eczema_by_24
icra_eczema_by_12
WFS_exclusive_breastmilk
WFS_curr_breastmilk
farm_exclusive_breastmilk
farm_curr_breastmilk
WFS_vs_WISC
farm
exclusive_breastmilk.at.02
diet.at.02
curr_breastmilk.at.02
sex
momagecat
delivery_mode
birthmonthcat
observed
diversity_shannon
diversity_inverse_simpson
diversity_gini_simpson
diversity_coverage
dominance_simpson
dominance_relative
dominance_dmn
dominance_core_abundance
rarity_rare_abundance
rarity_low_abundance
metric

Figure 17

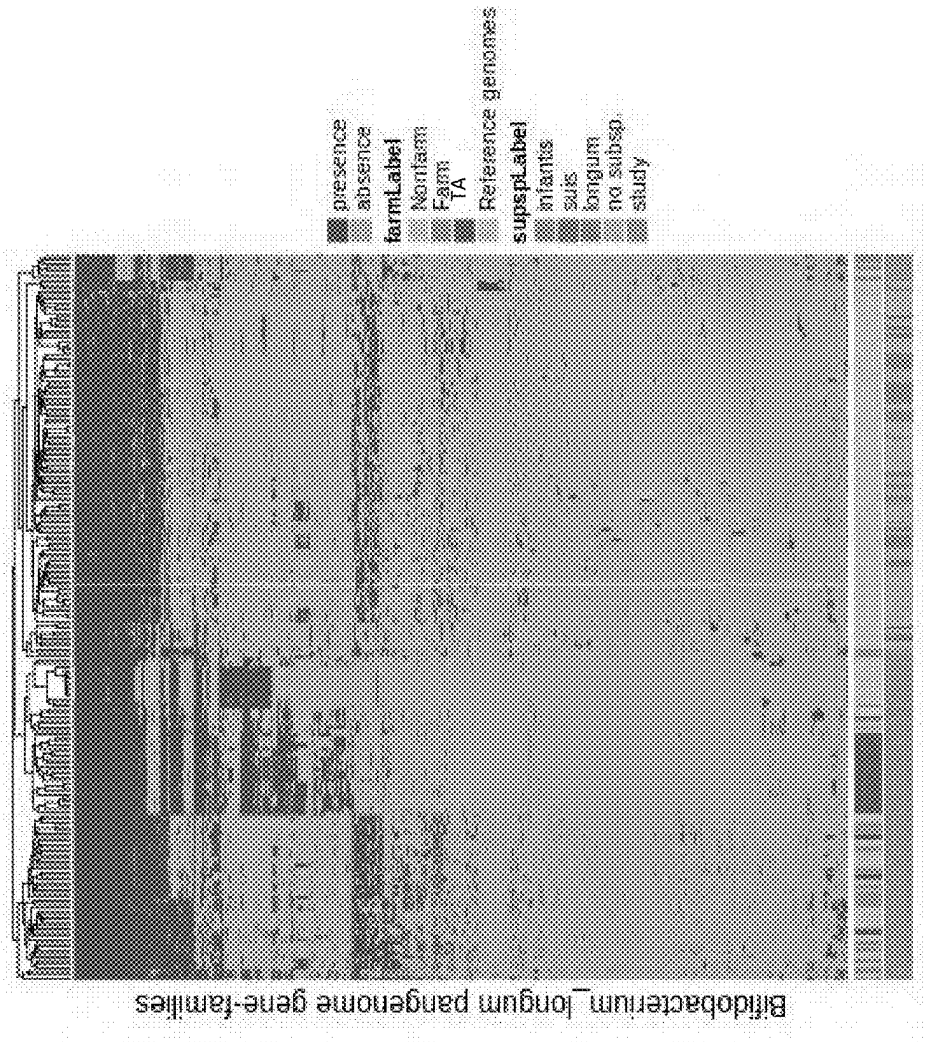

presence
absence
farmLabel
Nonfarm
Farm
TA
Reference genomes
suspLabel
infantis
suis
longum
no subsp.
study
Bifidobacterium_longum pangenome gene-families

Figure 18

Figure 19

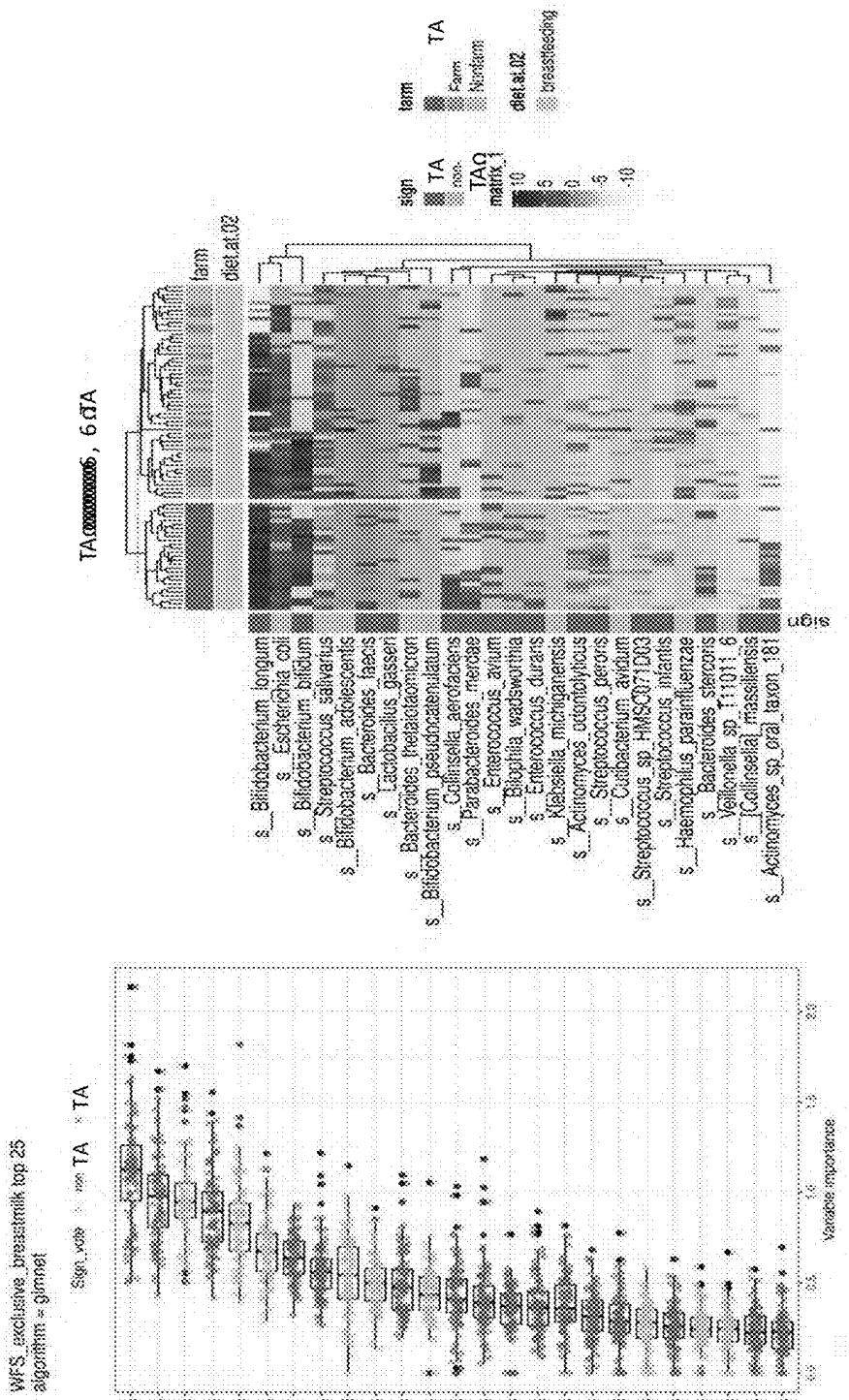

WFS_exclusive_breastmilk top 25
algorithm = glmnet
Sign_vote
TA
Variable importance
farm
diet.at.02
sign
TAQ matrix_1
Farm
Nonfarm
breastfeeding

Figure 20

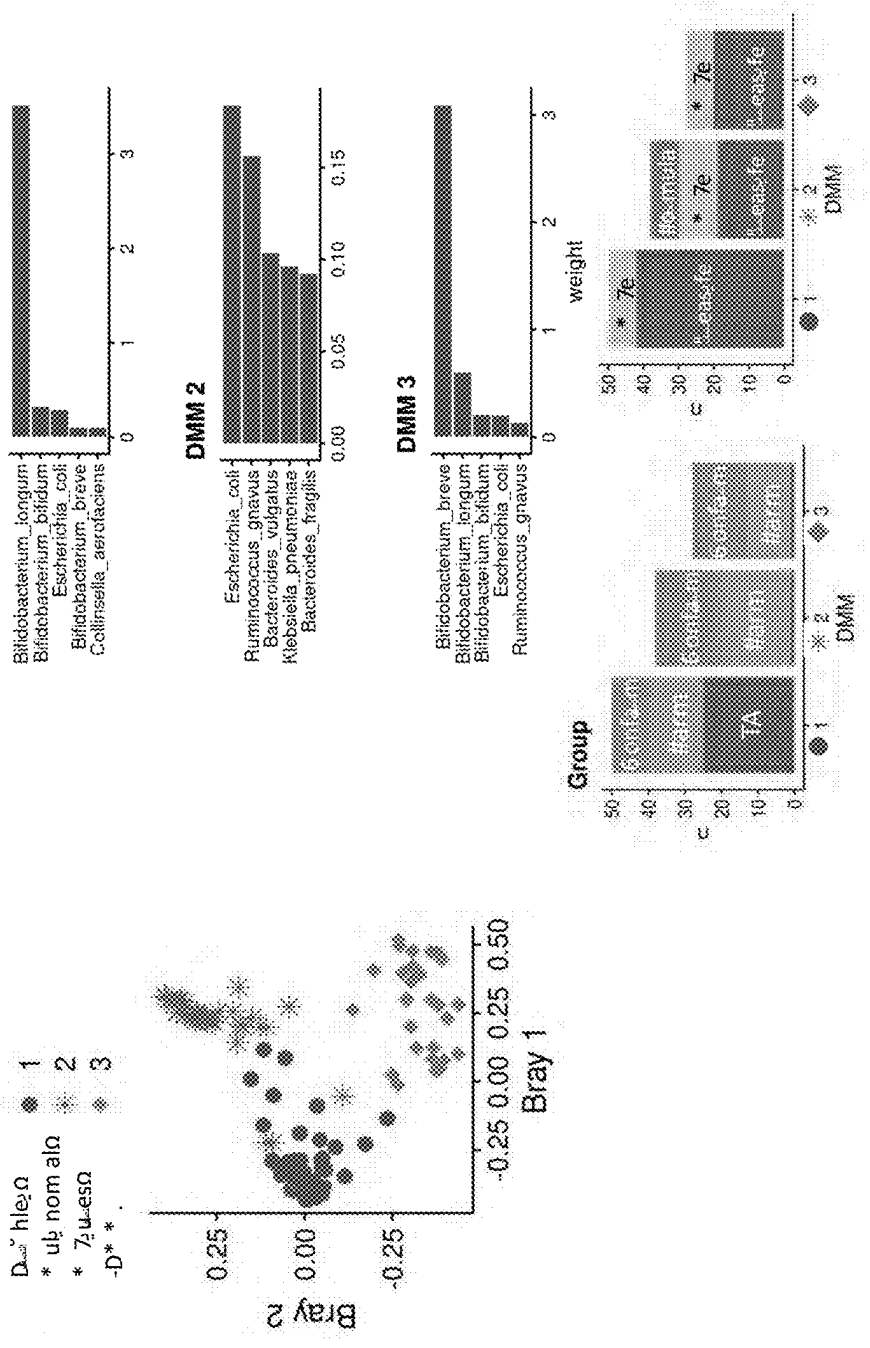

DMM 1
Bifidobacterium_longum
Bifidobacterium_bifidum
Escherichia_coli
Bifidobacterium_breve
Collinsella_aerofaciens
DMM 2
Escherichia_coli
Ruminococcus_gnavus
Bacteroides_vulgatus
Klebsiella_pneumoniae
Bacteroides_fragilis
DMM 3
Bifidobacterium_breve
Bifidobacterium_longum
Bifidobacterium_bifidum
Escherichia_coli
Ruminococcus_gnavus
weight
Group
CONTROL
FMT-01
FMT-02
TA
DMM
Bray 1
Bray 2

Figure 21

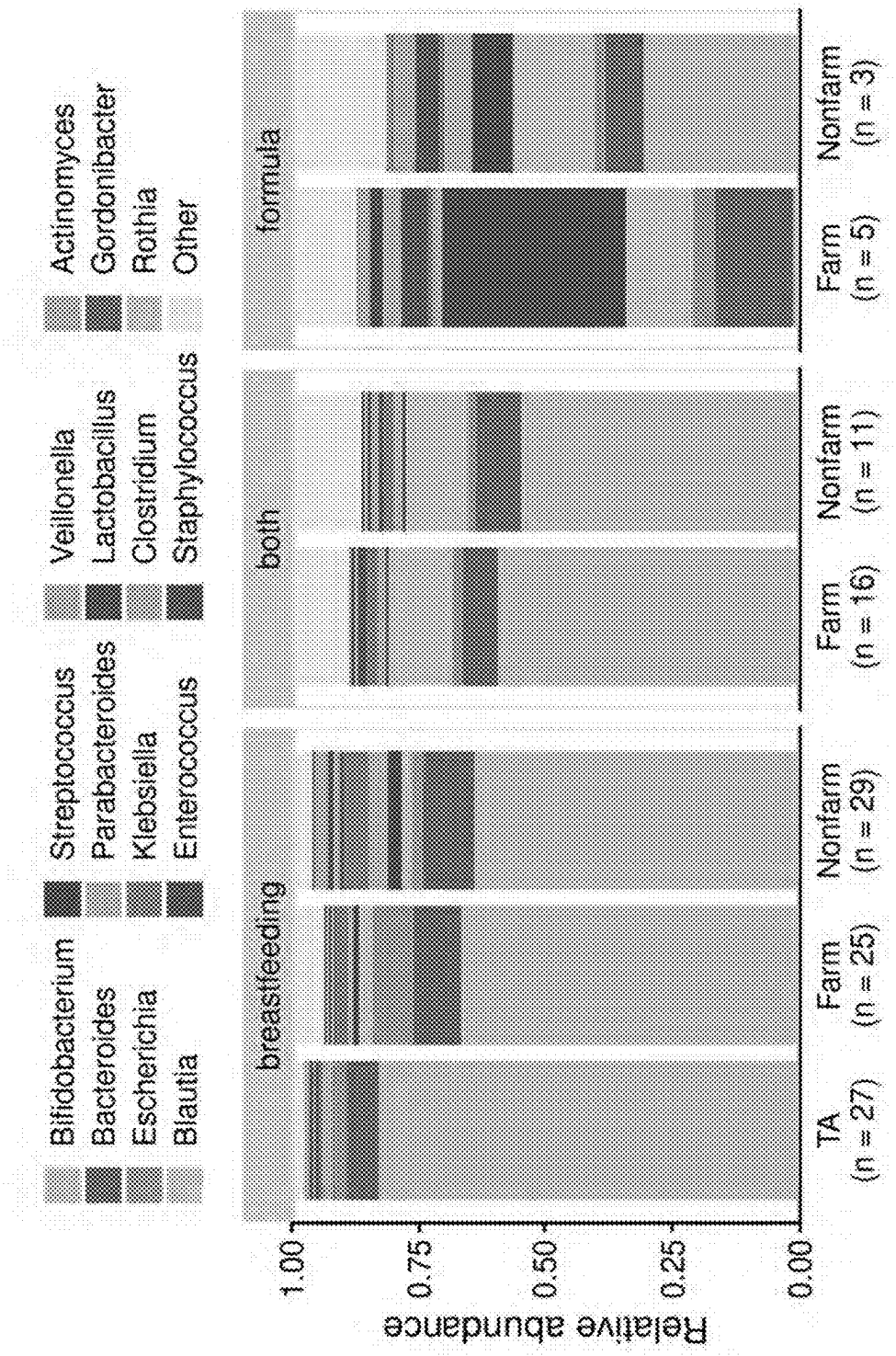
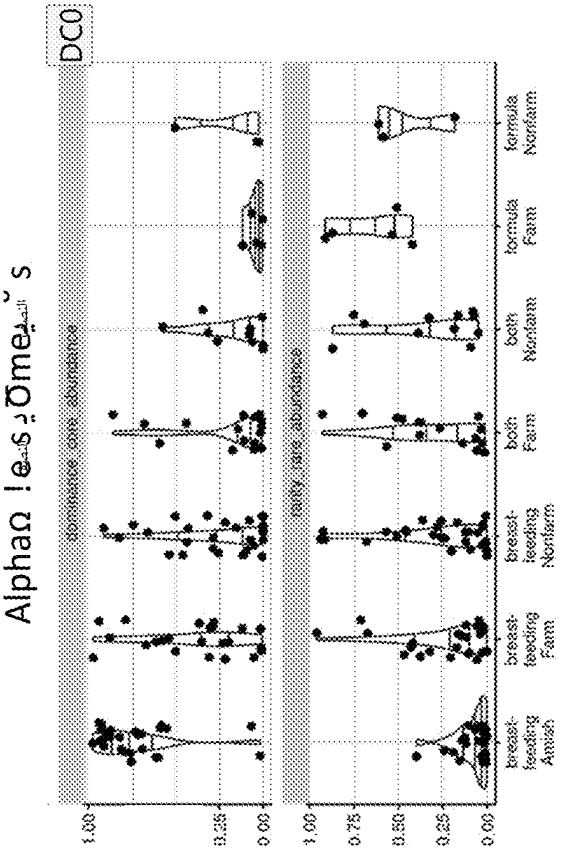

Bifidobacterium
Bacteroides
Escherichia
Blautia
Streptococcus
Parabacteroides
Klebsiella
Enterococcus
Veillonella
Lactobacillus
Clostridium
Staphylococcus
Actinomyces
Gordonibacter
Rothia
Other
breastfeeding
both
formula
Relative abundance
1.00
0.75
0.50
0.25
0.00
TA (n = 27)
Farm (n = 25)
Nonfarm (n = 29)
Farm (n = 16)
Nonfarm (n = 11)
Farm (n = 5)
Nonfarm (n = 3)

Figure 22


DC0
dominance core abundance
rare core abundance
1.00
0.75
0.50
0.25
0.00
breast-feeding Amish
breast-feeding Farm
breast-feeding Nonfarm
both Farm
both Nonfarm
formula Farm
formula Nonfarm

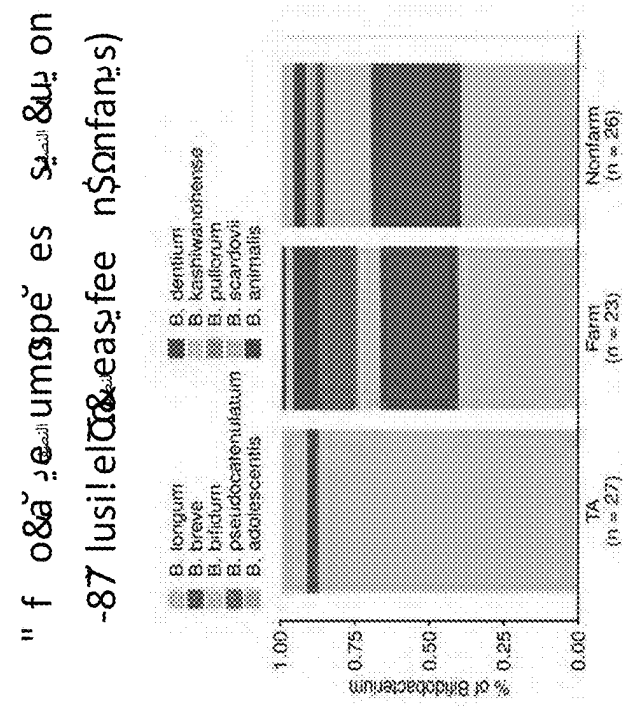
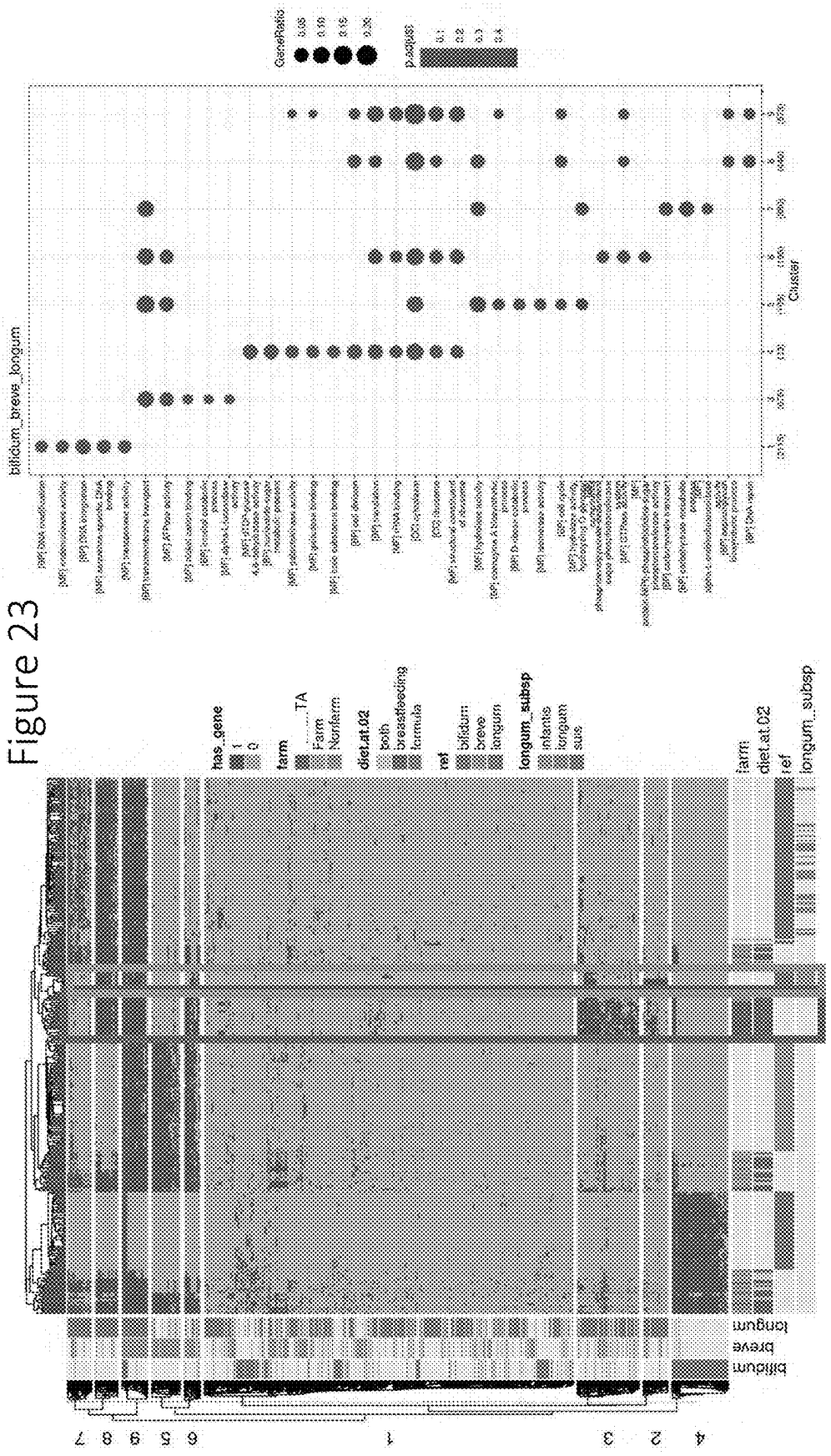

B. longum
B. breve
B. bifidum
B. pseudocatenulatum
B. adolescentis
B. dentium
B. kashiwanohense
B. pullorum
B. scardovii
B. animalis
% of Bifidobacterium
1.00
0.75
0.50
0.25
0.00
TA (n = 27)
Farm (n = 23)
Nonfarm (n = 26)

Figure 23


GeneRatio
p.adjust
bifidum_breve_longum
Cluster
has_gene
farm
diet.at.02
ref
longum_subsp
bifidum
breve
longum

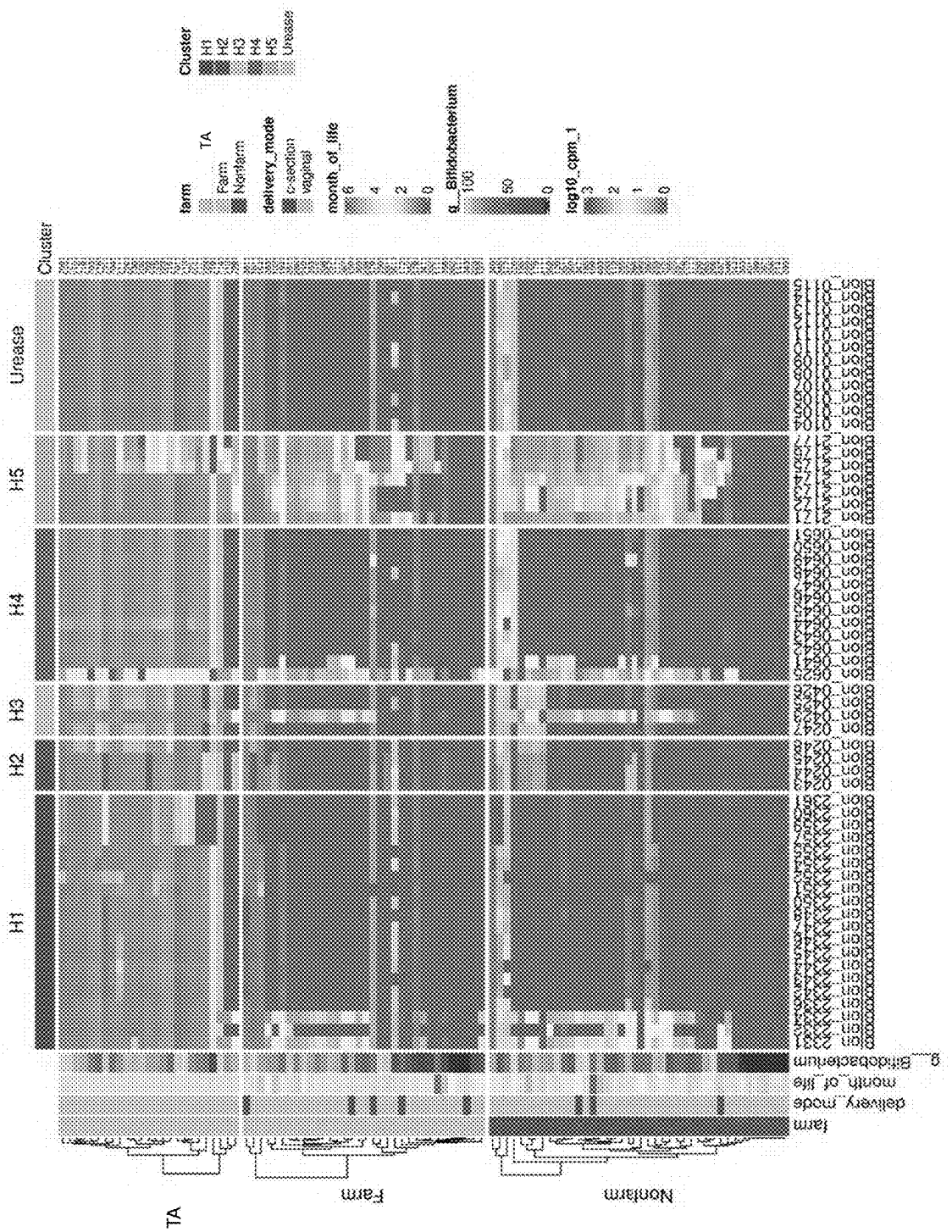
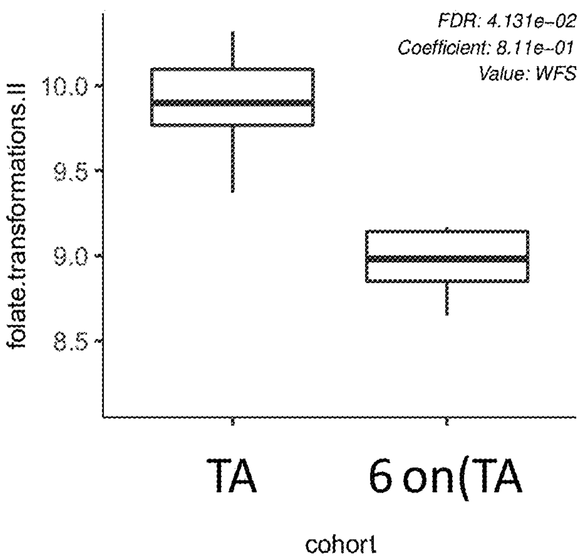

Cluster
H1
H2
H3
H4
H5
Urease
farm
Farm
Nonfarm
TA
delivery_mode
c-section
vagina
month_of_life
6
4
2
0
g__Bifidobacterium
100
50
0
log10_cpm_1
3
2
1
0
TA
Farm
Nonfarm
farm
delivery_mode
month_of_life
g__Bifidobacterium

Figure 24

Figure 25



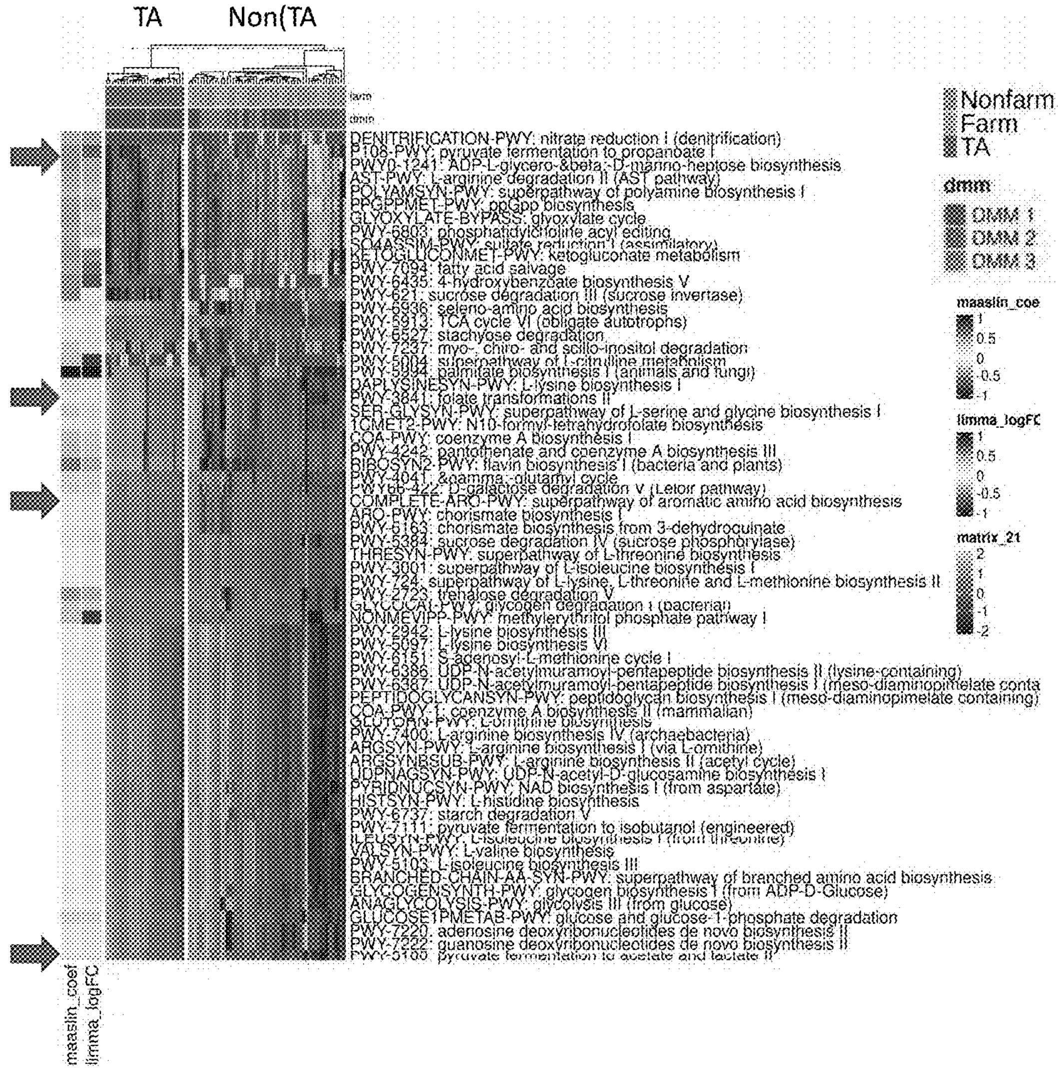
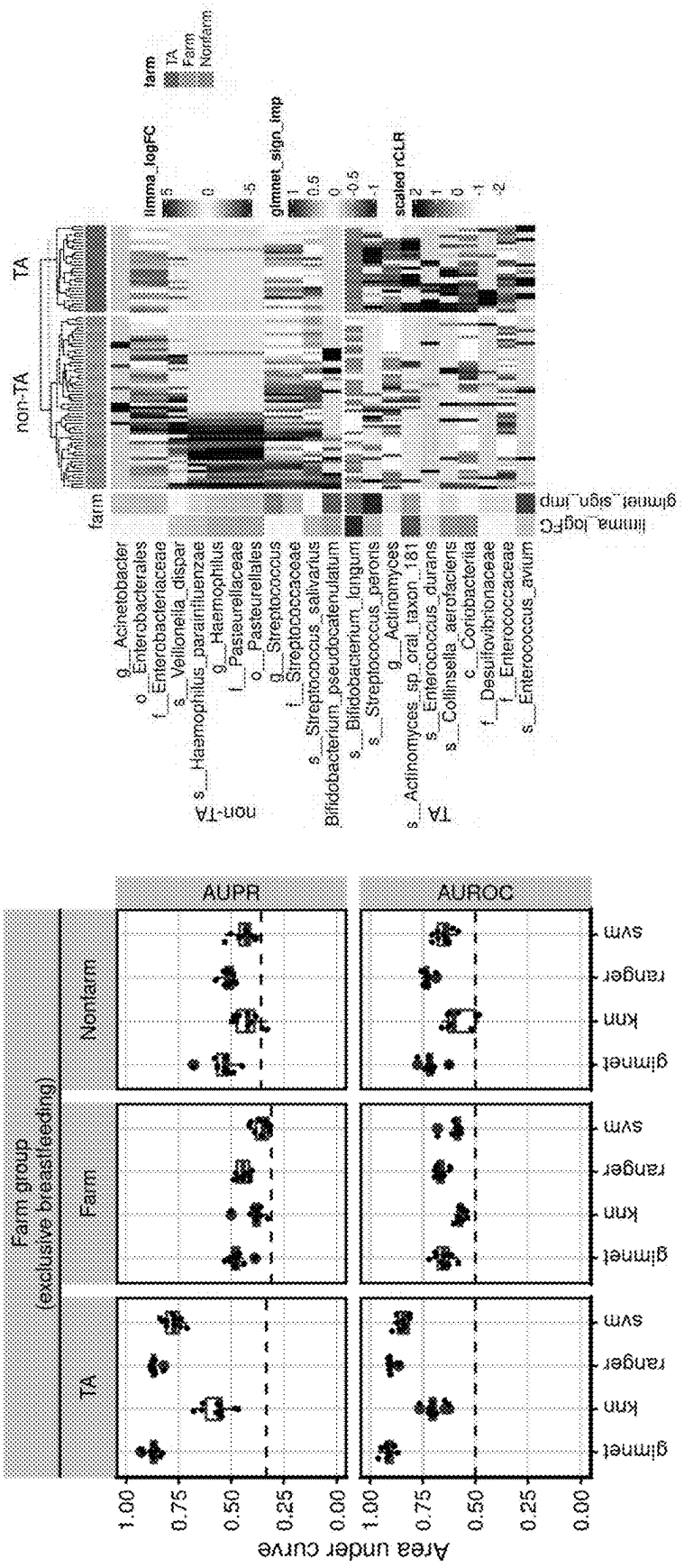


Figure 26


Area under curve
1.00
0.75
0.50
0.25
0.00
AUPR
AUROC
Farm group
(exclusive breastfeeding)
TA
Farm
Nonfarm
gimmet
knn
ranger
svm
non-TA
TA
farm
limma_logFC
gimmet_sign_imp
g__Acinetobacter
o__Enterobacterales
f__Enterobacteriaceae
s__Veillonella_dispar
s__Haemophilus_parainfluenzae
g__Haemophilus
f__Pasteurellaceae
o__Pasteurellales
g__Streptococcus
f__Streptococcaceae
s__Streptococcus_salivarius
Bifidobacterium_pseudocatenulatum
s__Bifidobacterium_longum
s__Streptococcus_peroris
g__Actinomyces
s__Actinomyces_sp_oral_taxon_181
s__Enterococcus_durans
s__Collinsella_aerofaciens
c__Coriobacteriia
f__Desulfovibrionaceae
f__Enterococcaceae
s__Enterococcus_avium
limma_logFC
5
0
-5
gimmet_sign_imp
1
0.5
0
-0.5
-1
scaled rCLR
2
1
0
-1
-2
farm
TA
Farm
Nonfarm

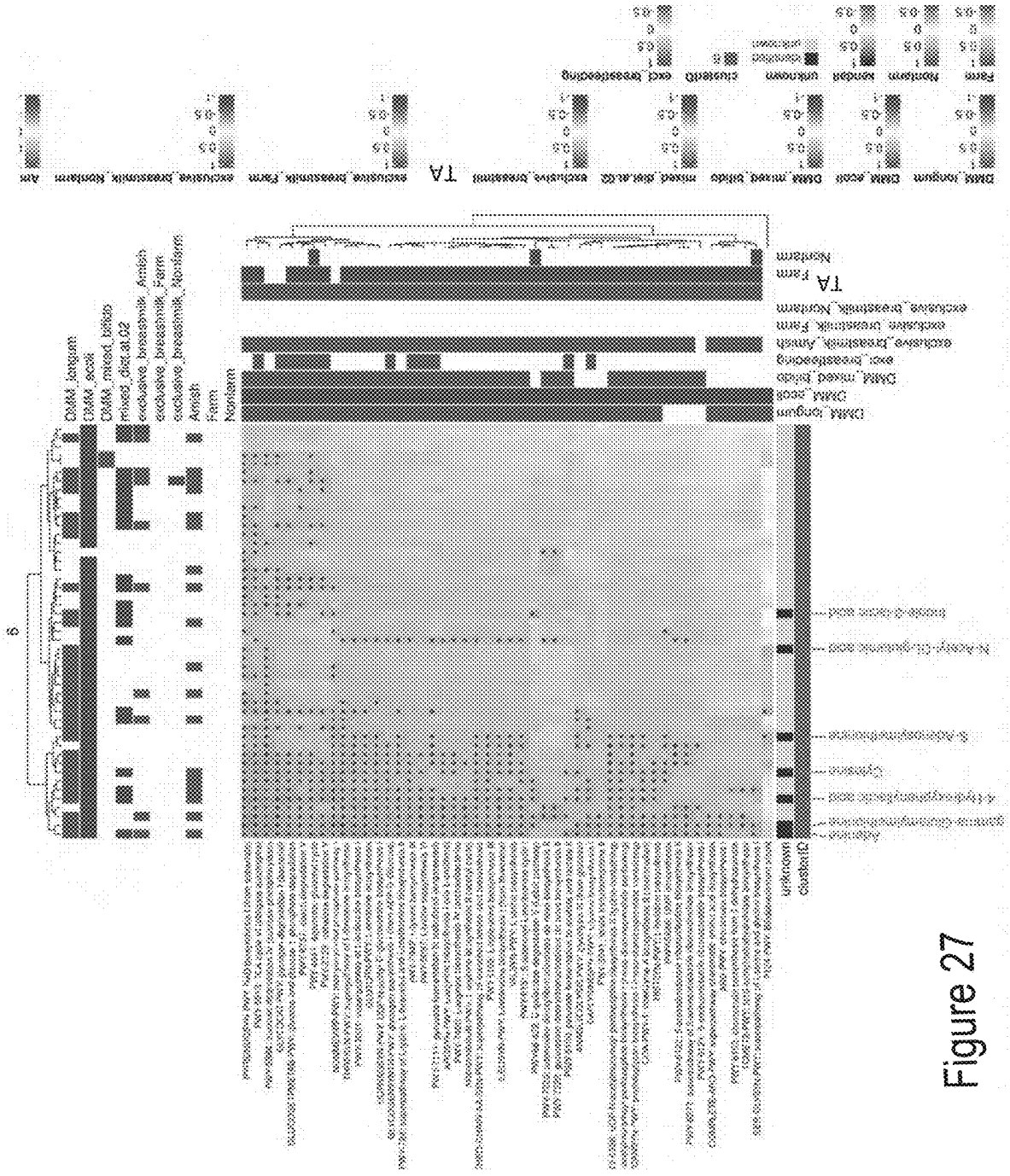


Figure 27

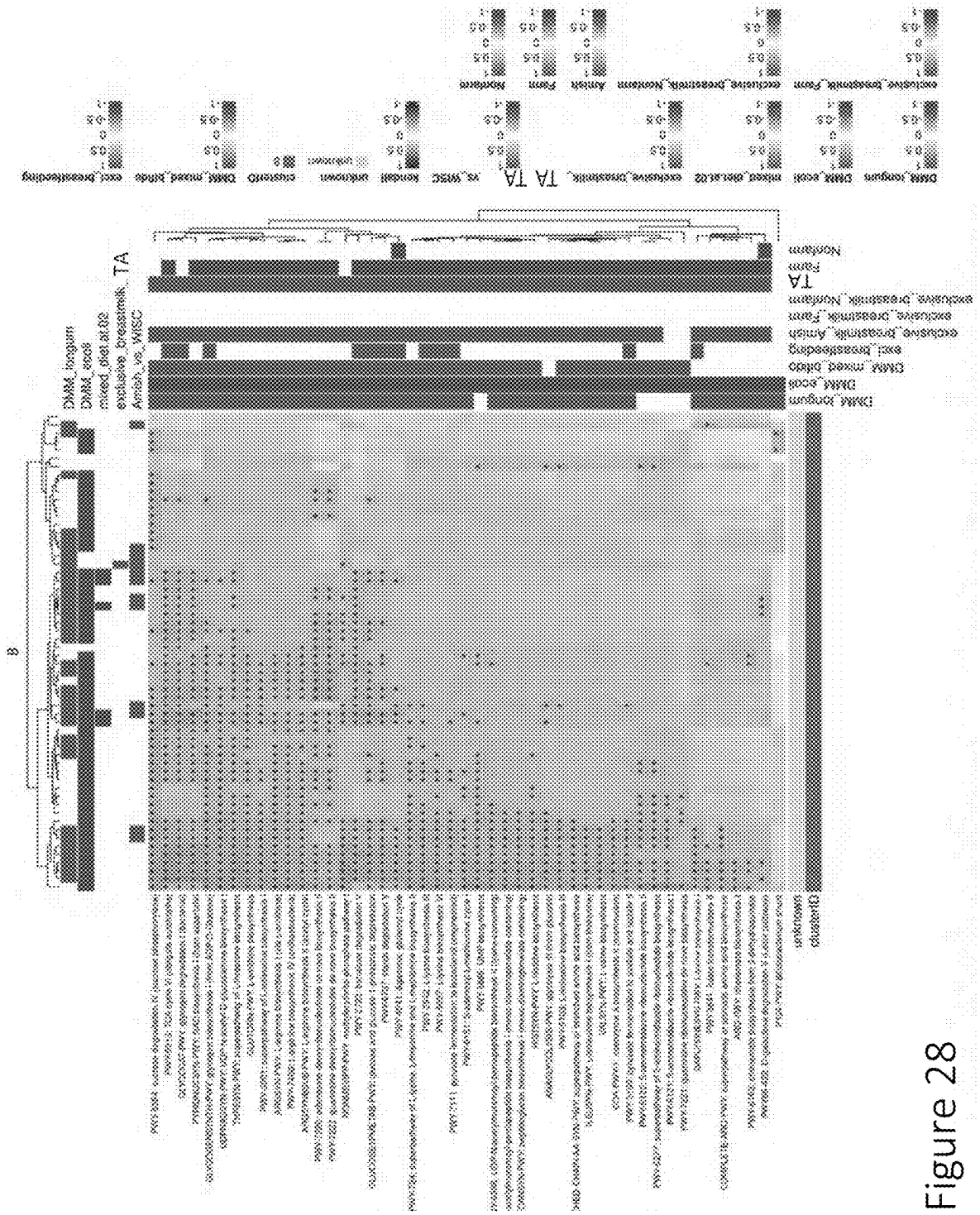

B
DMM_longum
DMM_scot
mixed_diet_ai.02
exclusive_breastmilk
Amish_vs_WISC
TA
DMM_longum
DMM_scot
DMM_mixed_bibo
excl_breastfeeding
exclusive_breastmilk_Amish
exclusive_breastmilk_Fijm
exclusive_breastmilk_Nonfijm
TA
Fijm
Nonfijm
clusterID
sampleID

Figure 28

Figure 29

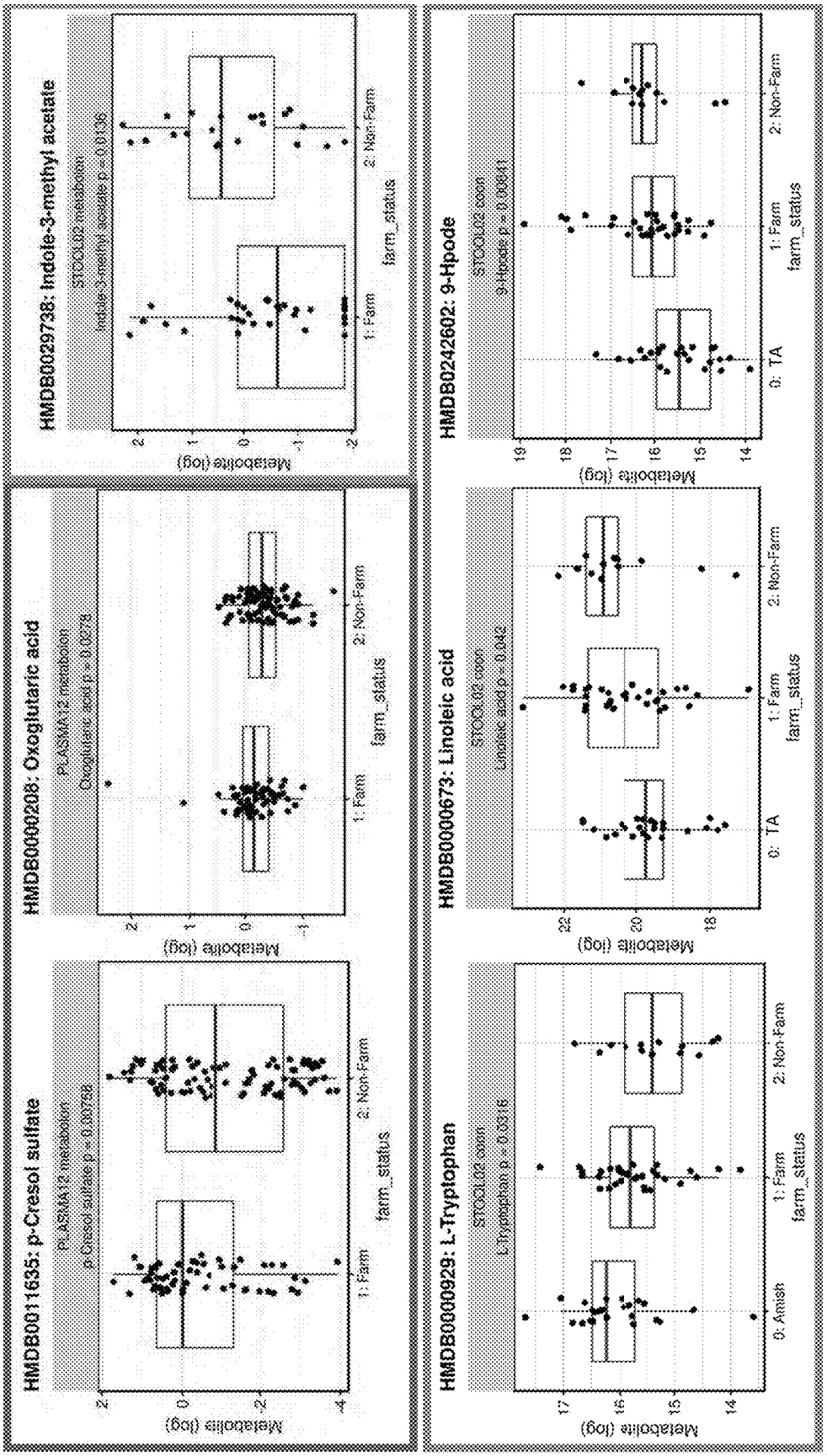

HMDB0011635: p-Cresol sulfate
HMDB0000208: Oxoglutaric acid
HMDB0029738: Indole-3-methyl acetate
HMDB0000929: L-Tryptophan
HMDB0000673: Linoleic acid
HMDB0242602: 9-Hpode
Metabolite (log)
farm_status
0: Amish
0: TA
1: Farm
2: Non-Farm

Figure 30

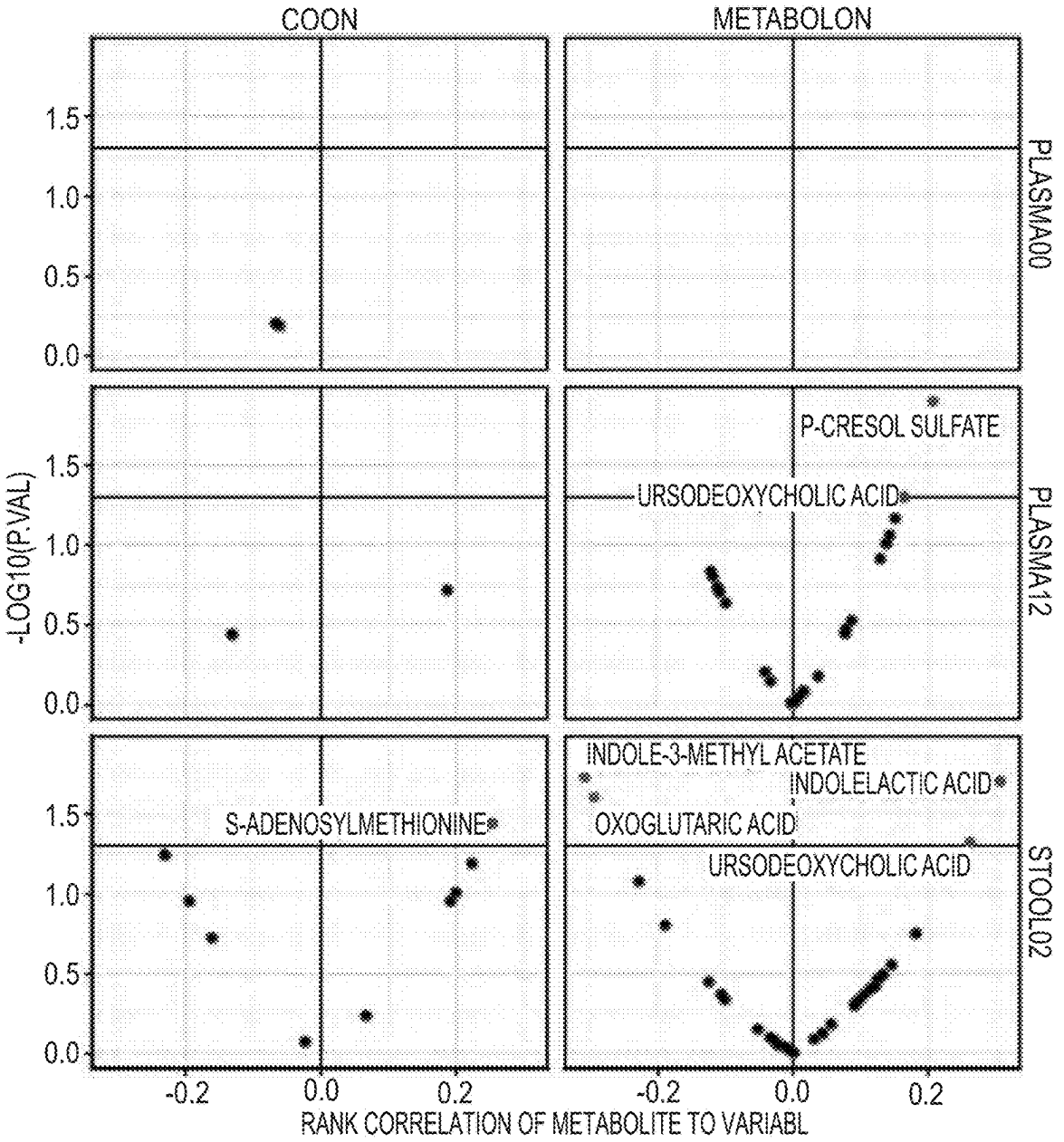
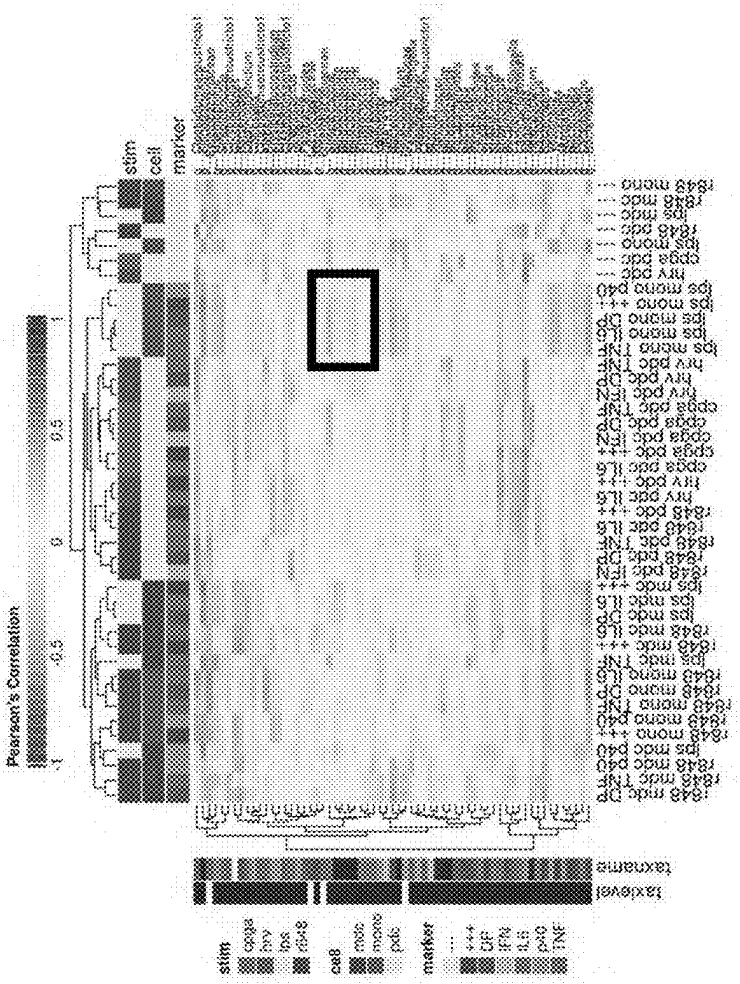

COON
METABOLON
PLASMA00
PLASMA12
STOOL02
-LOG10(P.VAL)
1.5
1.0
0.5
0.0
P-CRESOL SULFATE
URSODEOXYCHOLIC ACID
INDOLE-3-METHYL ACETATE
INDOLELACTIC ACID
OXOGLUTARIC ACID
URSODEOXYCHOLIC ACID
S-ADENOSYLMETHIONINE
-0.2
0.0
0.2
RANK CORRELATION OF METABOLITE TO VARIABL

Figure 31


Pearson's Correlation
1
0.5
0
-0.5
-1
stim
cell
marker
taxname
taxlevel
stim
cpgb
hiv
lps
rb48
cell
mdc
mono
pdc
marker
++
DP
IFN
IL6
p40
TNF

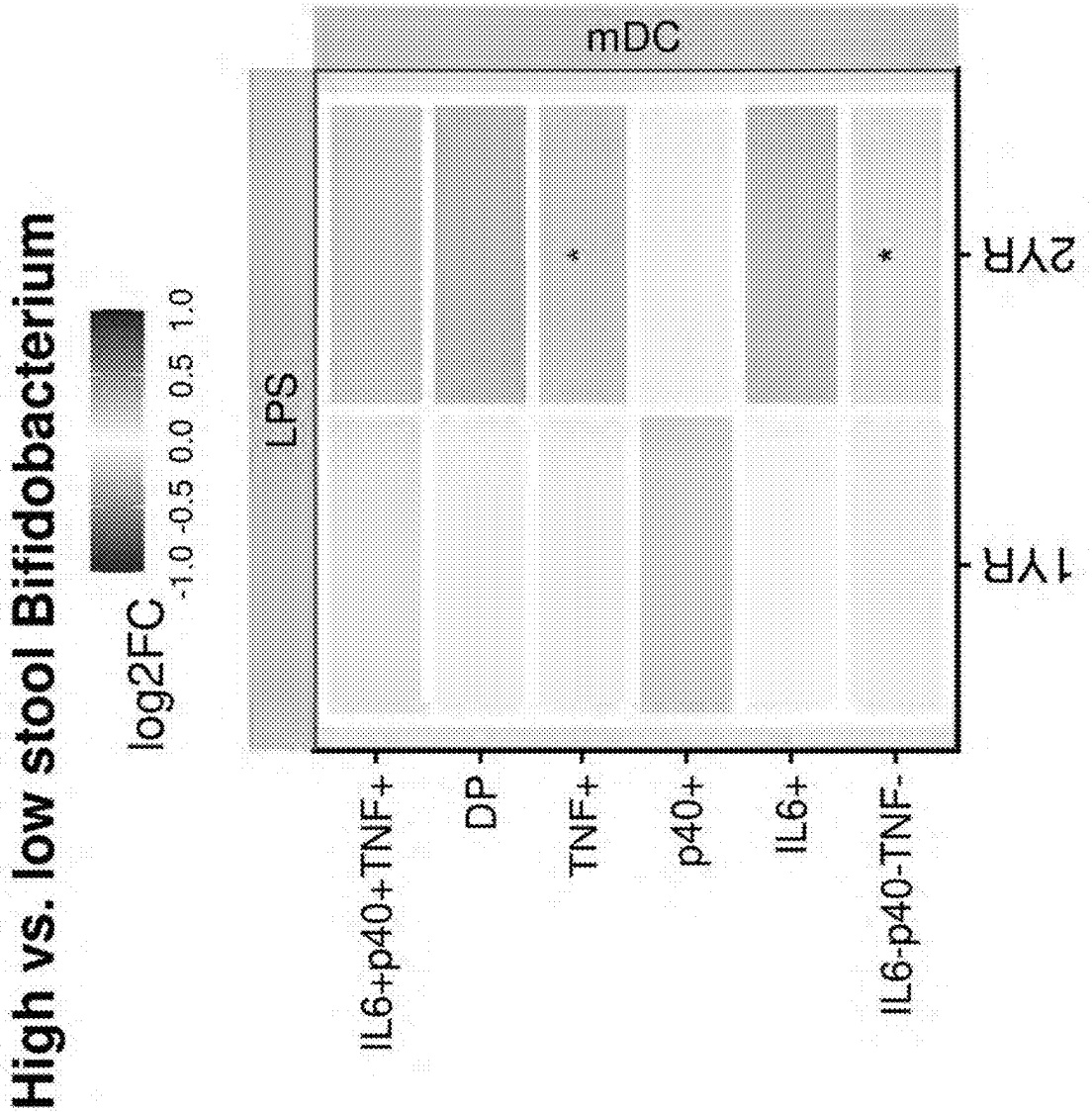

High vs. low stool Bifidobacterium
log2FC
-1.0 -0.5 0.0 0.5 1.0
LPS
mDC
1YR
2YR
IL6+p40+TNF+
DP
TNF+
p40+
IL6+
IL6-p40-TNF-
*
*

Figure 32

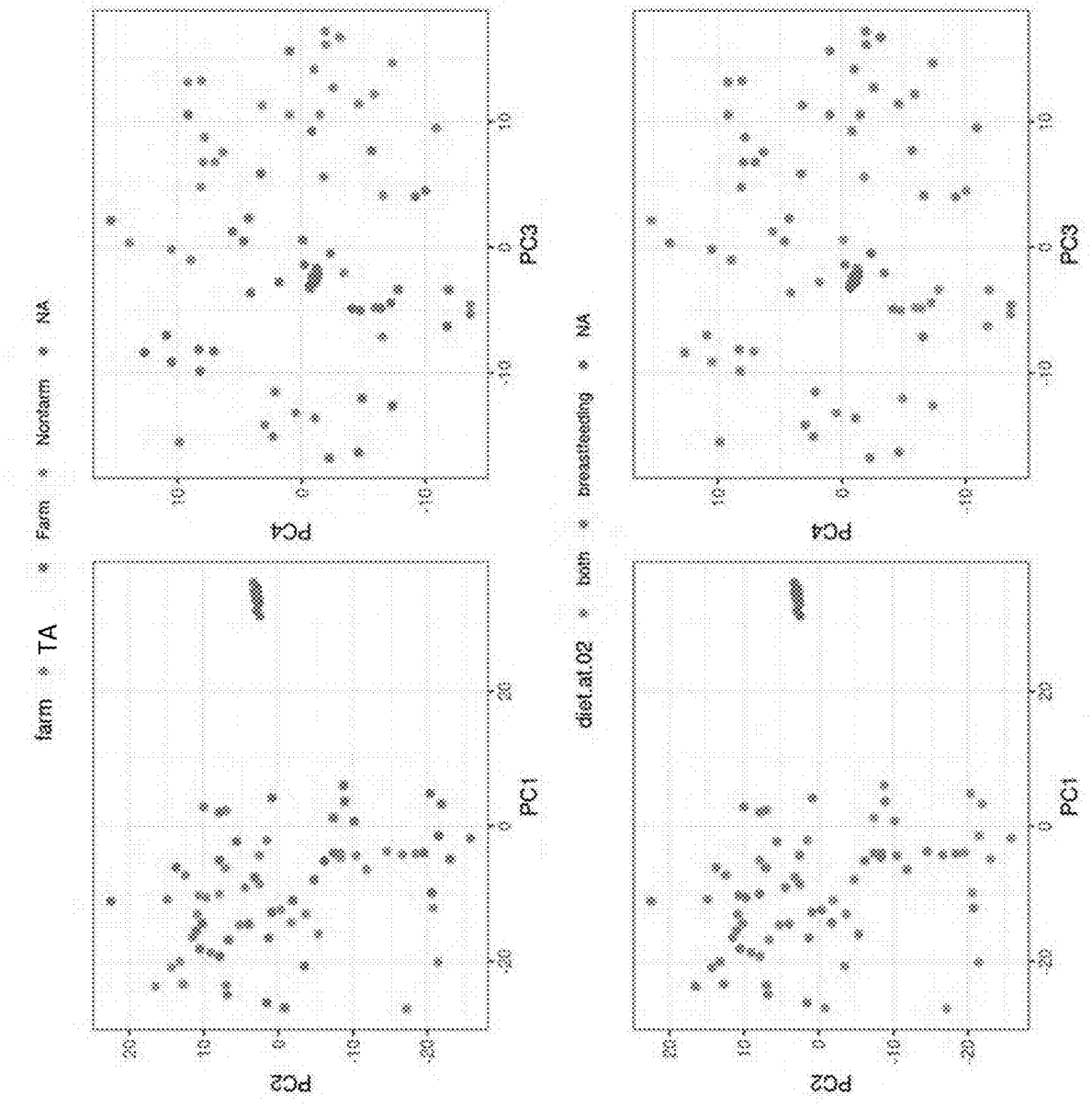
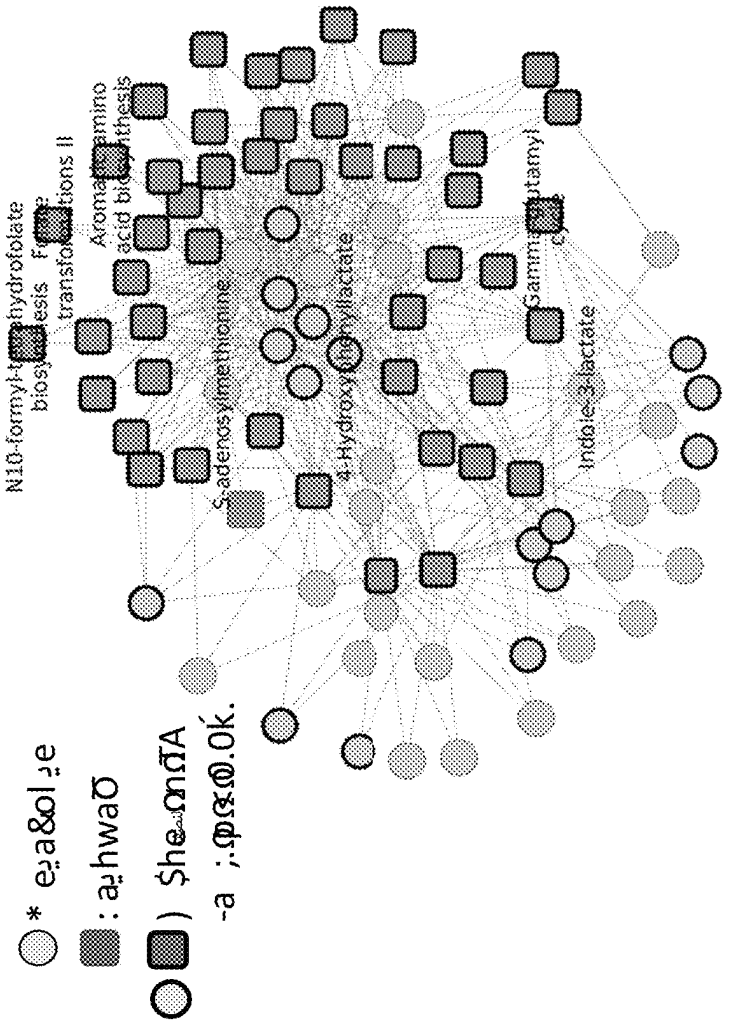

farm
TA
Farm
Nonfarm
NA
PC1
PC2
PC3
PC4
diet.at.02
both
breastfeeding
NA
PC1
PC2
PC3
PC4

Figure 33

Figure 34


N10-formyl-tetrahydrofolate biosynthesis
Aromatic amino acid biosynthesis
transformations II
S-adenosylmethionine
4-Hydroxyphenyllactate
Gamma-glutamyl
Indole-3-lactate

KEY PREDOMINANT SPECIES OF GUT BACTERIA COLONIZING FARM-EXPOSED INFANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of the filing date of U.S. application No. 63/248,940, filed on Sep. 27, 2021, the disclosure of which is incorporated by reference herein.

BACKGROUND

[0002] Allergic diseases, including asthma, are initiated in early life by the development of sensitization to environmental allergens. Once allergic sensitization is established, treatment is primarily focused on management of symptoms. Children living in farming households have lower prevalence of these diseases (Jatzlauk et al. 2017; Stein et al. 2016; Haahtela et al. 2015; Tantoco et al. 2018; Alfvén et al. 2006; Ege et al. 2011; Riedler et al. 2001; Von Ehrenstein et al.

[0003] 2000), and even lower allergic disease prevalence is commensurate with longer term and earlier life exposures (Riedler et al. 2001), especially in more traditional agrarian communities, such as the TA (Tantoco et al. 2018; Stein et al. 2016). The reasons for this health disparity are not secondary to genetic differences, but in large part are attributable to environmental pressures that are believed to be modifiable, if the protective environmental exposures and their interaction with the developing immune system can be accurately defined (Stein et al. 2016; von Mutius and Vercelli 2010).

SUMMARY

[0004] There is a growing understanding that microbes in the environment interact with the immune cells in our bodies in many ways including through our gut and that there is a small window in the first few years of life for intervention. That is, diverse bacteria in early life may be necessary for the development of a normal immune system and there are increasing numbers of the population who live in industrialized settings who have gut microbiomes that are lacking protective species as beginning at birth. For example, differences in lifestyle, which includes diet, between non-farm, farm and Amish children, the latter of which are referred to herein as traditional agrarian (TA) (FIG. **1**) lead to different levels of animal and thus microbial exposures experienced by these children growing up on farm or non-farm environments. As disclosed herein, the level of farm exposure correlates with the degree of protection, e.g., people following traditional agrarian lifestyles experience lower rates of asthma compared to more industrialized farming communities. Thus, microbial exposures in early life may lay the foundation for the development of a healthy immune system.

[0005] The depletion of good microbes or the presence of bad microbes in the gut at certain times, and in particular microbes established in early life, can influence development of necrotizing enterocolitis and irritable bowel disease (IBD), which can in turn increase the prevalence for the development of early onset colon cancer. Herein it is shown that diet and the environment in which an individual lives (lifestyle) influences the relative abundance of good and bad microorganisms that thrive in the gut. The presence of *Bifidobacterium* species in the maternal vagina and infant gut is an evolutionary trait that selects for these organisms to be primary colonizers of the newborn intestinal tract. Their ability to utilize human milk oligosaccharides and the fact that human milk IgA antibodies bind to *Bifidobacterium,* fosters their establishment as core health-promoting organisms throughout life. A reduction in their abundance in infants has been associated with the prevalence of obesity, diabetes, metabolic disorder, cancer and other causes of mortality later in life.

[0006] In particular, the Wisconsin Infant Study Cohort (WISC) birth cohort aimed at characterizing the impact of early life farming exposures on immune development, respiratory health, and allergic diseases (Seroogy et al., 2019). Study participants were recruited for three arms: TA, modern dairy farming, and rural non-farming study group. As disclosed herein, stool samples collected from study infants at 2 months of age underwent shotgun metagenomic sequencing to perform a comparative analysis between the study arms. The findings showed a significant increase in *Bifidobacteria* in the Wisconsin Farm Study group (WFS, composed of

[0007] TA infants) as compared to the other study groups (modern, non-TA dairy farming, and rural, non-TA and non-farming). Specifically, the microbiota from the WFS infant stool samples were characterized by a striking dominance of *Bifidobacterium longum.* While *Bifidobacterium* species were high in breastfeeding children across all three groups (e.g., compared to formula fed infants), the relative abundance of *Bifidobacterium longum* was higher in the WFS group, while other *Bifidobacterium* species (*breve, bifidum*) were found in higher abundance in the non-TA study groups. Furthermore, the WFS microbiota harbored unique gene families, including several that are specific to previously annotated strains of *Bifidobacterium longum* subsp. *infantis.* As shown in FIG. **7**, between newborn to 6 months of age, infants with microbiomes comprising predominantly of *Bifidobacterium* species (as associated with agrarian lifestyle, which is associated with better health), whereas those with higher composition of diverse microbiomes especially of microbes other than *Bifidobacterium* (e.g. see formula panel where there are high *Streptococcus, Staphylococcus, E. coli,* and other non-*Bifidobacterium* and non-*Lactobacillus*), during this time period, are associated with WISC farm and non-farm, which have increased likelihood of developing immune-related diseases such as allergies. Specifically, *Bifidobacterium longum* subsp. *infantis* in particular, provides decreased risk of allergies.(viz-a-viz TA vs WISC farm and non-farm allergy prevalence). In general, a higher abundance of *Bifidobacterium* species is beneficial, e.g., 80% vs 25%, because these microbes may provide beneficial metabolites and/or prevent colonization by pathogenic bacteria or other microbes (e.g., *E. coli, Streptococcus,* and/or *Staphylococcus* or fungi), while a lower *Bifidobacterium* percentage may not confer these benefits, e.g., there may be a higher percentage of other microbes (e.g., higher non-*Bifidobacterium* diversity) including but not limited to pathogenic bacteria such as *E. coli, Streptococcus,* and *Staphylococcus,* which leads to increased risk of allergies and other diseases. For example, the over-abundance of specifically *B. longum* subsp. *infantis* (from 60% up to 90% in TA infants) and/or a total percentage of *B. longum* subsp. *infantis, B. bifidum* and *B. breve* as >60% may promote and/or may be indicative of immune health.

[0008] Thus, bacteria and molecules that enhance the prevalence and/or activity of certain bacteria in the gut microbiome, e.g., human milk oligosaccharides, antibodies, e.g., from breastmilk, as well as other molecules, such as mucin binding proteins or peptides, e.g., produced by *B. longum* or *B. infantis,* oligosaccharides, or glycans, molecules that increase mucin production, or exosomes produced by bacteria including *Bifidobacterium,* e.g., *B. longum,* or any molecules that reduce leaky gut or enhance bifidobacterial adhesion and survival in the GI tract, thereby enhancing growth and higher abundance of *Bifidobacterium,* may be employed in compositions, e.g., as a prebiotic (substance or food ingredient that promotes the growth of beneficial microbes in the gut) or probiotic (culture of a specific microbe or combination of microbes) supplement that can be included with or added to formula or ingested prepregnancy by women who are trying to become pregnant, by expectant (pregnant) or breastfeeding mothers to promote and/or increase the *Bifidobacterium longum infantis* abundance in maternal or infant gut microbiome, thereby promoting healthy development including protections from immune-related diseases such as allergies or other diseases. Comparative functional analysis of *Bifidobacterium* also identified that *Bifidobacterium* can produce more indole-3-lactic acid, folic acid and riboflavin (vitamin B2) among other metabolites. Some of these metabolites, including indole-3-lactic acid have shown immunoregulatory effects, including suppression of TH2 and TH17 cytokines and induction of interferon beta. In one embodiment, the composition is a liquid comprising an amount of *Bifidobacterium longum infantis* such as a strain obtained from a WFS infant, optionally with one or more prebiotic and/or probiotics, or immunoglobulin A (IgA) antibodies, that select for prevalence of *Bifidobacterium longum infanti,* and/or that enhance the activity, e.g., colonization or enzyme activity, of *Bifidobacterium longum infantis* in the gut of a newborn or child. In one embodiment, the composition is ingested by prepregnancy by women who are trying to become pregnant, by expectant (pregnant) or a lactating mother (e.g., human) or exclusively breastfed infant, or by an infant via formula. In one embodiment, the composition comprises microbes with the functional capacities of *Bifidobacterium longum infantis,* e.g., to metabolize human milk oligosaccharides by transforming the microbes with genes that encode human milk oligosaccharide metabolizing enzymes and/or other genes that promote health, e.g., genes for biosynthesis of folic acid, riboflavin, p-Cresol sulfate, tryptophan and/or other metabolites in the tryptophan pathway.

[0009] The disclosure provides a method to detect immune health status and potentially for providing as prebiotic or probiotic for treatment of metabolism, immune or neurodegenerative related diseases (e.g., autism) in a human infant (e.g., up to 6 to 9 months or 1 year of age) or child (including toddlers from 1 to 3 years of age and adolescents up to 18 years of age). The method includes providing a physiological sample, e.g., a stool sample, from a human infant or child and determining in the sample i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of one, two or more of Blon_0915, Blon_2171, Blon_2173, Blon_2334, galT Blon_2172, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650 or one, two or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650. In one embodiment, a relative abundance of *Bacteroides* of >10%, of *Bifidobacterium* of <60% or of *Blautia* of >10% is indicative of an infant or child at increased risk of allergies or other diseases, e.g., IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bifidobacterium* of <60% is indicative of an infant or child at increased risk of allergies or other diseases, e.g., IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of >10%, of *Bifidobacterium* of <60% and of *Blautia* of >10% is indicative of an infant or child at increased risk of allergies, e.g., IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% or of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies or other diseases, e.g., IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bifidobacterium* of >60 is indicative of an infant or child at decreased risk of allergies or other diseases, e.g., IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% and of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies or other diseases, e.g., IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater or 10% or less, *Bifidobacterium breve* of 2% or greater or 25% or less, *Bifidobacterium longum* of 25% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater or 10% or less, *Bifidobacterium breve* of 2% or greater or 25% or less, *Bifidobacterium longum* of 25% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater or 10% or less, *Bifidobacterium breve* of 2% or greater or 25% or less, *Bifidobacterium longum* of 25% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater, *Bifidobacterium breve* of 20% or less, *Bifidobacterium longum* of 50% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater, *Bifidobacterium breve* of 20% or less, *Bifidobacterium longum* of 50% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of less than 5%, *Bifidobacterium breve* of greater than 20%, *Bifidobacterium longum* of less than 50%, or of *Bifidobacterium pseudocatenulatum* of greater than 2% is indicative of impaired immune health in the infant or child. In one embodiment, an increase in the relative abundance of expression of one or more of Blon_0915, Blon_2171, Blon_2173, Blon_2334, galT Blon_2172, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_

0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650 is indicative of immune health in the infant or child.mIn one embodiment, the sample is from a newborn. In one embodiment, the sample is from a newborn to 3 month old. In one embodiment, the sample is from a 3 month old to a 6 month old. In one embodiment, the sample is from an infant treated with a drug. In one embodiment, the drug is an antibiotic. In one embodiment, the prebiotic and/or probiotic is administered before the antibiotic, e.g., 1, 2, 3, 4 5 or 6 hours or more, apart. In one embodiment, the infant or child has necrotizing enterocolitis. In one embodiment, the method includes administering to the infant or child a prebiotic or a probiotic. In one embodiment, the prebiotic or probiotic comprises one or more bacteria, one or more antibodies, or one or more molecules that enhance the relative abundance of *Bifidobacterium longum.* In one embodiment, the relative abundance of *Bifidobacterium longum infantis* is enhanced. In one embodiment, the abundance is enhanced to greater than 60%, 70%, 80% or 90%. In one embodiment, the sample is analyzed using a nucleic acid amplification reaction. In one embodiment, the sample is analyzed using genome sequencing. In some embodiments, the sample is analyzed using bioluminescence or antibodies with fluorophores, or tags such as a nucleic acid barcode or magnetic beads.

[0010] In one embodiment, a relative abundance of *Bacteroides* of >8%, of *Bifidobacterium* of <65% or of *Blautia* of >2% is indicative of an infant or child at increased risk of allergies. In one embodiment, a relative abundance of *Bacteroides* of >10%, of *Bifidobacterium* of <60% and of *Blautia* of >10% is indicative of an infant or child at increased risk of allergies, BD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of >8%, of *Bifidobacterium* of <65% and of *Blautia* of >2% is indicative of an infant or child at increased risk of allergies, I BD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% or of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies or *Bacteroides* of <10%, of *Bifidobacterium* of >65% or of *Blautia* of <2% is indicative of an infant or child at decreased risk of allergies, I BD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% and of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies, I BD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >65% or of *Blautia* of <2% is indicative of an infant or child at decreased risk of allergies, IBD, type 2 diabetes, or obesity. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% to 10%, *Bifidobacterium breve* of 2% to 25%, *Bifidobacterium longum* of 25% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 10% or less, *Bifidobacterium breve* of 25% or less, *Bifidobacterium longum* of 25% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child or of *Bifidobacterium breve* of 15% or less, *Bifidobacterium longum* of 65% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 3% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 10% or less, *Bifidobacterium breve* of 25% or less, *Bifidobacterium longum* of 25% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child or of *Bifidobacterium breve* of 15% or less, *Bifidobacterium longum* of 65% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 3% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater, *Bifidobacterium breve* of 20% or less, *Bifidobacterium longum* of 50% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater, *Bifidobacterium breve* of 20% or less, *Bifidobacterium longum* of 50% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of less than 5%, *Bifidobacterium breve* of greater than 20%, *Bifidobacterium longum* of less than 50%, or of *Bifidobacterium pseudocatenulatum* of greater than 2% is indicative of impaired immune health in the infant or child or of *Bifidobacterium breve* of greater than 15%, *Bifidobacterium longum* of less than 30%, or of *Bifidobacterium pseudocatenulatum* of greater than 3% is indicative of impaired immune health in the infant or child.

[0011] In one embodiment, a method to identify a human infant or child at higher risk of developing allergies is provided. The method includes providing a stool sample from a human infant or child; and determining in the sample i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of one, two or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF, Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650.

[0012] Other organisms that may be detected include but are not limited to *Parabacteroides merdae* or *Bacteroides stercoris* (associated with WFS; glmnet features and others), *Bacteroides thetaiotaomicron* (associated with WISC), *Parabacteroides* and *Bacteroides* identified by screening for (adult) gut microbes that could attenuate epithelial cell line IL-8 response to LPS https://www.ncbi.nlm.nih.gov/pmc/articles.PMC7230855/ ((Hiippala et al., 2020) or *Collinsella aerofaciens* (higher in WFS) (*Collinsella* species were previously associated with higher *Bifidobacterium* in infant gut (Milani et al. 2017)), and those of higher abundance in WISC (based on glmnet features), e.g., *Veillonella* or *Cutibacterium.*

[0013] Also provided are products for consumption, e.g., a composition comprising one or more agents such as a prebiotic(s) and/or probiotic(s), for example, to promote infant health and/or long term immune health, thereby decreasing the incidence of aberrant immune responses that are observed in autoimmune diseases such as allergies, inflammatory bowel disease (IBD), type 2 diabetes, metabolic disease, such as obesity, and neurodegenerative diseases such as ADHD, autism and the like. The compositions may be useful to stimulate an anti-inflammatory state in a pregnant female, infant, toddler or child, e.g., under the age of 5 years old. An anti-inflammatory state may also be useful

to prevent or inhibit cancer. In one embodiment, the composition may include one or more B vitamins, one or more short chain fatty acids, linoleic acid, linolenic acid, tryptophan, one or more tryptophan metabolites such as p-cresol, oxoglutaric acid, indole-3-methylacetate, or one or more hydroxyoctadecadienoic acids, or combinations thereof, or isolated bacteria such as *Bifidobacteria* (e.g., *B. infantis, B. longum, B. breve,* and/or *B. bifidum,* or a combination thereof), or bacteria genetically modified to overexpress human breast milk oligosaccharide metabolizing enzymes, or modified with, for example, galT, ureF, ureC and/or ureE genes, e.g., from *Bifidobacterium longum* subsp. *infantis.* (*B. infantis*), *B. longum, B. breve* and/or *B. bifidum*), that may be used as probiotics along with breastmilk or sugars present in breastmilk such as 2-fucosylactose, sialylated lactose, lacto-N-biose, galacto-N-biose, and the like. Furthermore, some of the exopolypeptides and metabolites produced by these *Bifidobacteria* microbes modulate immune responses and neural growth, e.g., *Bifidobacteria*-specific surface exopolysaccharide (EPS), which may provide a protective biofilm against pathogens, an indole such as indolelactic acid: products of tryptophan degradation, which promote anti-inflammation and immune tolerance in gut epithelial cells and immune cells via aryl hydrocarbon receptor (AHR) signaling pathway , gamma-aminobutyrate (GABA), and acetate, a short chain fatty acid (SCFA) which stimulates 5-hydroxytryptamine (serotonin, important neurotransmitter) production by gut enterochromaffin cells. Acetate is produced by *Bifidobacteria.* In one embodiment, the composition is breast milk formula (baby or infant formula) (e.g., powder or liquid) supplemented with the agents, e.g., prebiotic(s) and/or probiotic(s) disclosed herein. For example, molecules that are more prevalent in TA and/or farm 2-month-old infant stool compared to WISC (see FIGS. **27** and **29**) may be employed in the compositions, molecules including but not limited to, folic acid, riboflavin, aromatic amino acids (tryptophan, tyrosine, phenylalanine), adenine, 4-hydroxyphenyllactic acid (4-OH-PLA), pnenyllactic acid (PLA), and indole-3-lactic acid (ILA) (aryllactic acids) which are ligands for hydroxycarboxylic acid receptors, which play important roles in maintaining energy and immune homeostasis (Ahmed et al Front. Endocrinol. 2011 doi: 10.3389/fendo.2011.00051), gamma-glutamylmethionine, cysteine, a sulfur containing amino acid, which exerts functions through metabolites such as S-adenosylmethionine (SAM), N-acyl-DL-glutamic acid and the like. FIG. **29** shows that indole-3-methyl-acetate was found to be lower in 2-month-old infant stool metabolites from farm compared to non-farm infants and 1-year old infant plasma metabolites measured identified oxoglutaric acid, and p-cresol sulfate as significantly higher in farm infants compared to non-farm infants. Exopolypeptides from *Bifidobacterium breve* have been shown to prevent maturation of dendritic cells and activation of antigen specific CD4+ T cells responses to *B. breve* in mice, suggesting it may be important for immune evasion of adaptive immunity and contribute to host-microbe mutualism

[0014] Further provided are methods of using the compositions, e.g., to prevent, inhibit or treat an inflammatory, metabolic, gastrointestinal, or neurodegenerative conditions in a mammal in need thereof, e.g., to enhance an anti-inflammatory response to one or more antigens in a mammal, or to prevent, inhibit or treat one or more symptoms in a mammal having or at risk of an allergic disease, e.g., asthma, eczema, or other autoimmune diseases, or metabolic, gastrointestinal, or neurodegenerative diseases.

BRIEF DESCRIPTION OF FIGURES

[0015] FIG. **1**A. Early life environment is associated with decreased prevalence of allergic diseases. TA children have a lower prevalence of allergic disease. For example, TA children have a 10 times lower eczema prevalence. Children who moved to farms after the age of 5 did not seem to gain protective effects experienced by those who lived on farms from birth.

[0016] FIG. **1**B. Early life farm exposures protect against development of allergic diseases and asthma. Marshfield Epidemiological Study Area (MESA). Adapted from Ludka-Gaulke et al. JACI 2018.

[0017] FIG. **1**C. Eczema prevalence is 10X lower in WI TA children and early life farm exposures protective. The Wisconsin Plain Community Project survey (Tantoco et al., *Ann Allergy Asthma Immunol* 2018, n=2781 children). Wisconsin Infant Study Cohort (Seroogy et al., *Respir Res* 2019, Steiman et al., *J Allergy Clin Immunol* 2020).

[0018] FIG. **2**A. Wisconsin Infant Study Cohort (WISC) and Wisconsin Farm Study (WFS). The Wisconsin Infant Study Cohort (WISC) and Wisconsin Farm Study are prospective birth cohort studies that aim to identify molecular contributors of farm exposures on development of asthma and childhood respiratory illness.

[0019] FIG. **2**B. Metagenomics sequencing.

[0020] FIG. **2**C. 116 metagenomics profiles of stool from two-month-old infants.

[0021] FIG. **3**. Infant gut microbiome is associated with diet and farm exposure. Association between subject characteristics and alpha diversity metrics. Tests are either Kruskal-Wallis (three categorical outcomes: diet.at.02, farm) or Mann-Whitney (binary outcomes: WFS_vs_WISC, curr breastmilk.at.02, exclusive_breastmilk.at.02). *$p<0.05$ after correcting for multiple hypothesis tests by Benjamini-Hochberg 25 procedure. "Farm" subsets are results of performing farm group comparisons restricted to "currently breastfeeding" or "exclusively breastfeeding" participants only.

[0022] FIG. **4**. Association between subject characteristics and beta diversity metrics. *PERMANOVA $p<0.05$ after 6 correcting for multiple hypothesis tests by Benjamini-Hochberg procedure. "Farm" subsets are results of performing farm group comparisons restricted to "currently breastfeeding" or "exclusively breastfeeding" participants only.

[0023] FIG. **5**. Relative abundance of top genera by child diet at sample collection (all infants). Breastfeeding: exclusive breastfeeding; formula: exclusively formula feeding; both: both breastfeeding and formula feeding. Genera were included with relative abundance of at least 1% in at least 10% of study samples.

[0024] FIG. **6**. Average relative abundance of top genera (all infants). Genera were included with relative abundance of at least 1% in at least 10% of study samples.

[0025] FIG. **7**. Relative abundance of *Bifidobacterium* species in microbiota of study participants. Grey ("Other"): non-*Bifidobacterium* species.

[0026] FIG. **8**. Relative abundance of *Bifidobacterium* species, aggregated by farm group. Grey ("Other"): non-*Bifidobacterium* species.

[0027] FIG. **9**. Comparison of infant microbiome structure across US.

[0028] FIG. **10**. Comparison of *Bifidobacterium longum* gene family representation in study samples and reference genomes. A pangenome analysis was conducted to survey which *Bifidobacterium* genes that were present in each sample and to compare them to reference genomes. Each row in this heatmap represents a UniRef90 gene family that was found in at least one publicly available reference genome for *Bifidobacterium longum*. Each column is either a study sample or a reference genome. Red indicates presence and orange indicates absence of the gene. The first bottom annotation indicates reference genomes in light blue, TA by dark blue, farm by green, and nonfarm by orange. A diet annotation in included with exclusive breastfeeding in blue. For reference genomes, subspecies annotation, if available, are shown in the bottom row annotation. Subspecies *infantis* are shown in pink, suis in green, and *longum* in blue. Using hierarchical clustering to compare the gene family representation in the study samples with reference genomes, this heatmap shows that TA samples clustered next to known *infantis* strains (boxes).

[0029] FIG. **11**. Machine learning performance on discriminating TA from non-TA, exclusively breastfeeding infants only. Value is area under the precision-recall curve (PR-AUC).

[0030] FIG. **12**. Cladogram of differentially abundant microbial taxa as assessed by LEfSE. Selected with $p<0.05$ and LDA score >2.

[0031] FIG. **13**. Union of top 25 features selected by each of elastic net (glmnet) and random forest (ranger). Top 25 features ranked by median importance from 100 models. Color indicates the sign of the coefficient (positive for TA, negative for non-TA). Values are centered log-ratio relative abundances.

[0032] FIG. **14**. *Bifidobacterium longum* subsp. *infantis* functional capacity to produce folic acid. MaAslin2 software was used to perform linear hypothesis tests on each pathway, to determine whether the pathway was differentially abundant between TA and non-TA. *Bifidobacterium* is protective, provides nutrients, and metabolites necessary for growth and development, whereas most other bacteria cause inflammation. The total folate transformations pathway is shown here with contributions broken down by taxon. Red boxplots represent TA, green for farm, and blue for non-farm. Most of the contribution is from different strains of B. longum.

[0033] FIG. **15**. *Bifidobacterium longum* subsp. *infantis* functional capacity to produce tetrahydrofolate. TA and WISC infants have differential functional capacity to produce tetrahydrofolate.

[0034] FIG. **16**. *Bifidobacterium longum* subsp. *infantis* functional capacity to produce flavin. TA and WISC infants have differential functional capacity to produce flavin.

[0035] FIG. **17**. Association between subject characteristics and alpha diversity metrics. Tests are either Kruskal-Wallis (categorical outcomes) or Mann-Whitney (binary outcomes). *$p<0.05$ after correcting for multiple hypothesis tests by Benjamini-Hochberg procedure. "Farm" subsets are results of performing farm group comparisons restricted to "currently breastfeeding" or "exclusively breastfeeding" participants only.

[0036] FIG. **18**. Comparison of *Bifidobacterium longum* gene family representation in study samples and reference genomes. Each row is a UniRef90 gene family that was found in at least one publicly available reference genome for *Bifidobacterium longum*. Each column is either a study sample or a reference genome, as indicated in the second from bottom column annotation. For reference genomes, subspecies annotation (if available) is given in the bottom column annotation.

[0037] FIG. **19**. Top **25** features selected by elastic net (glmnet). Top: top 25 features ranked by median importance from 100 models. Color indicates the sign of the coefficient (positive for TA, negative for non-TA). Bottom: same features, visualized as heatmap. Values are centered log-ratio relative abundances.

[0038] FIG. **20**. Microbial community structure varies with farm group and diet. Beta diversity from species level features was computed using the Bray distance, and the samples were clustered with Dirichlet Multinomial Mixtures to identify latent structure. The Beta diversity plot on the left is annotated by the DMM cluster assignment, with cluster 1 in red, cluster 2 in blue and cluster 3 in green. The plot in the middle uses the same coordinates but is labeled by farm group. TA in blue squares, Farm in green triangles, Nonfarm in orange circles. The plot on the right is annotated by the infant's diet at the time the sample was collected. Exclusively breastfed infants are blue stars, exclusively formula-fed infants in red circles, and those with mixed diet of formula and breastfeeding in yellow diamonds.

[0039] FIG. **21**. Breastfeeding infant gut microbiome is dominated by *Bifidobacterium*. The stacked plots show the relative abundance of top genera aggregated by farm group and diet. The bars on the left show the exclusively breastfeeding infants, in the middle those with mixed diet, and on the right the exclusively formula-fed.

[0040] FIG. **22**. Non-TA infants have more diverse microbiomes at species level.

[0041] FIG. **23**. Pangenome files were obtained from the PanPhlan authors for *B. longum, B. breve,* and *B. bifidum.*

[0042] FIG. **24**. WISC/WFS HMO profiles. Values are log 10(CPM+1). Top annotation is gene cluster, which is based on the organization of the genes on the B *infantis* genome. Most of these genes are highly prevalent among TA samples and not among WISC samples. LoCascio reports that H5 is found commonly in other *B. longum* strains, so it is not surprising to see that it is prevalent in Farm and Nonfarm as well.

[0043] FIG. **25**. Differential functional capacities.

[0044] FIG. **26**. Machine learning models trained on stool metagenomics profiles can distinguish TA from non-TA.

[0045] FIG. **27**. Correlated module of 2mo stool microbial pathway capacity and measured metabolites that are associated with *Bifidobacterium longum*-dominated microbiome and TA status. Partial correlations between microbial pathways and stool metabolites. *adjusted $p<0.05$.

[0046] FIG. **28**. Correlated module of 2mo stool microbial pathway capacity and measured lipids that are associated with *Bifidobacterium longum*-dominated microbiome and TA status. Partial correlations between microbial pathways and stool.

[0047] FIG. **29**. Farm status vs. selected tryptophan pathway metabolites (MW or

[0048] KW test). Top row is Metabolon data from PLASMA12 (blue) and STOOLO2 (orange). Bottom Row is STOOL02.

[0049] FIG. **30**. Farm score vs tryptophan metabolites. Farm score is a function of number and frequency of farm animal exposures. Datasets: PLASMA00: data includes WFS and WISC; PLASMA12: either WFS and WISC or

Metabolon (WISC only); and STOOL02: either WFS and WISC or Metabolon (WISC only). Rank (spearman) correlation of metabolite level to farm score (based on maternal and child farm exposures). No adjustment by sex or diet. Y axis is uncorrected $p<0.05$ Red indicates a positive correlation of metabolite to farm exposure; blue indicates a negative correlation of metabolite to farm exposure.

[0050] FIG. **31**. Microbiome-immune partial least squares (PLS) regression

[0051] FIG. **32**. Mixed effects model.

[0052] FIG. **33**. Principal components analysis (PCA) on STOOL02 metabolomics, lipidomics. Control samples in gray.

[0053] FIG. **34**. Microbe-metabolomics module in network form (edges for significant partial Kendall correlations). This map shows connections between pathway (squares) and metabolites (circles). The ones with a wider outline around the circles and squares indicate they are higher in TA.

DETAILED DESCRIPTION

[0054] One of the health-promoting attributes of human breast milk is to provide substrates for the developing gut microbiome. The loss of *Bifidobacterium* species from the infant gut microbiome, particularly *Bifidobacterium longum infantis,* in the first 3 months of life has been associated with a variety of negative health consequences including increased risk for allergic and other diseases. A recent report profiling infant gut microbial composition in the United States showed an overall low abundance (<50% on average) of *Bifidobacterium* genus in infants during the first 3 months of life. Thus, there is a need to identify dietary interventions to safely improve the altered infant gut microbiome. Human milk oligosaccharides (HMOs) present in human breast milk are one known substrate for promoting *Bifidobacterium* species, e.g., utilization of host derived glycans.

[0055] Studies have been steadily converging on the hypothesis that a major environmental contributor to immune development actually comes from within: the gut microbiome. Within the first few months of life, before the introduction of solid food, a microbiome dominated by only a few crucial taxa, including genus *Bifidobacterium,* has been associated with protection against asthma and other diseases later in life (Fujimura et al. 2016; Stokholm et al. 2018; Arrieta et al. 2015). However, which particular *Bifidobacterium* species and the composition of each species that contribute to lower prevalence has not been fully characterized. *Bifidobacterium longum* subspecies efficiently metabolize human milk oligosaccharides (HMOs); in particular, subsp. *infantis* has a contingent of unique genes for HMO metabolism compared to other subspecies (LoCascio et al. 2010) and have the capacity, e.g., genes to produce aromatic amino acids, aryllactic acids, sulfur amino acids, exopolysaccharides, and the like. Cohort studies have identified greater prevalence of *infantis* in traditional farming communities compared to communities that follow Western lifestyles (Seppo et al. 2021; Davis et al. 2017).

[0056] As disclosed herein below, Wisconsin TA (n=2, 879) have a low rate (2.4%) of allergic diseases. Metagenomic sequencing was used to study the gut microbiomes of Wisconsin farm, non-farm and TA infants. Surprisingly, the predominant strain comprising —60-90% of the bacterial composition of the TA children's gut consists of one species: *Bifidobacterium longum infantis.* This bacteria co-evolved and so may have enhanced properties for breaking down human milk oligosaccharides, regulating metabolism, immune cells, neural, gastrointestinal and other cells in a human infant and other properties such as anti-viral properties.

[0057] In particular, gene profiling and metabolic potential bacterial colonies present in the gut microbiome of the infants were analyzed. Strains of bacteria isolated from the gut microbiome in the first two months of life in infants were isolated, e.g., strains of *Bifidobacterium* from infants having an increased prevalence of those strains. Those strains may be useful in a product to enhance immune health or prevent or lower the incidence of allergies or other diseases, e.g., the product may be used in newborns, children, adults, prepregnancy, and during pregnancy (expectant moms). For example, newborn stool may be analyzed to profile the microbiome through, for example, gene sequencing or nucleic acid amplification of specific genes, to characterize the potential immune health of the child and/or to identify deficiencies in the microbiome.

[0058] The postnatal, early-life developmental window is a critical time for establishing host-microbe interactions as the colonization by appropriate gastrointestinal microbes lay the foundation for the future health and well-being of the infant. Colonization by pioneer microbes shortly after birth, and the maintenance of this population, shapes the microbial community which in turn impacts numerous host physiological processes which can lead to a variety of negative consequences for host health including a predisposition to allergic disease or other diseases.

Compositions, Routes of Administration, Dosages and Dosage Forms

[0059] Provided herein are compositions that include but are not limited to one or more agents such as B vitamins, short chain fatty acids, linoleic caid, linolenic acid, tryptophan, tryptophan metabolites, and other metabolites such as folate or folic acid, aromatic amino acids (tryptophan, tyrosine, phenylalanine), tryptophan catabolites, aryllactic acids (4-OH-PLA, indole-3-lactic acid), GABA, SAM, sulfur amino acids (cysteine), exopolysaccharides. p-cresol, oxoglutaric acid, indole-3-methylacetate, or hydroxyoctadecadienoic acids, or combinations thereof, or isolated bacteria such as *Bifidobacteria* (e.g., *B. infantis, B. longum, B. breve* and/or *B. bifidum,* or a combination thereof), or bacteria genetically modified to overexpress breast milk oligosaccharide metabolizing enzymes, or are modified with galT, ureF, ureC or ureE genes, e.g., from *Bifidobacterium longum* subsp. *infantis.* The compositions may include one or more pharmaceutically or neutraceutically acceptable carriers. The compositions can be prepared using any methods known in the art, e.g., added to an existing mixture or formulated as part of a mixture. For example, such compositions can be prepared using acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980); incorporated herein by reference), and in the form of powder or lyophilized formulations or aqueous solutions.

[0060] Mixtures of one or more of the agents described herein may be prepared in water suitably mixed with one or more excipients, carriers, or diluents. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. The forms include aqueous solutions or dispersions and sterile powders for the extem-

poraneous preparation of sterile solutions or dispersions (e.g., U.S. Pat. No. 5,466,468). In any case, the formulation may be sterile and may be fluid. Formulations may be stable under the conditions of manufacture and storage. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), methylcellulose, suitable mixtures thereof, and/or vegetable oils. In many cases, the composition may include isotonic agents, for example, sugars or sodium chloride. In some embodiments, the composition includes methylcellulose. In some embodiments, the composition includes a surfactant (e.g., a poloxamer such as PLURONIC®).

[0061] For example, a solution containing a composition described herein may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. In these solutions, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. Some variation in dosage may occur depending on the condition of the subject being treated. Moreover, for human administration, preparations may meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biologics standards.

[0062] Administration of the compositions may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, and other factors known to skilled practitioners. The administration of the composition(s) may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Any route of administration may be employed, e.g., oral, or local administration. In one embodiment, the composition is formulated for oral administration. In one embodiment, oral administration is achieved after suspension of a powder composition into a suitable liquid oral vehicle.

[0063] The formulations may, where appropriate, be conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to the art. Such methods may include the step of bringing into association the active agent with carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

[0064] The amount of composition(s) administered to achieve a particular outcome may vary depending on various factors including, but not limited to, the formulation, the condition, patient specific parameters, e.g., height, weight and age, and the like.

[0065] Compositions may conveniently be provided in the form of formulations suitable for administration. A suitable administration format may best be determined by a medical practitioner for each patient individually, according to standard procedures. Suitable pharmaceutically acceptable carriers (excipients) and their formulation are described in standard formulations treatises, e.g., Remington's Pharmaceuticals Sciences. By "pharmaceutically acceptable" it is meant a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

[0066] Compositions may be formulated in solution at neutral pH, for example, about pH 6.5 to about pH 8.5, or from about pH 7 to 8, with an excipient to bring the solution to about isotonicity, for example, 4.5% mannitol or 0.9% sodium chloride, pH buffered with art-known buffer solutions, such as sodium phosphate, that are generally regarded as safe, together with an accepted preservative such as metacresol 0.1% to 0.75%, or from 0.15% to 0.4% metacresol. Obtaining a desired isotonicity can be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is useful for buffers containing sodium ions. If desired, solutions of the above compositions can also be prepared to enhance shelf life and stability. Useful compositions can be prepared by mixing the ingredients following generally accepted procedures. For example, the selected components can be mixed to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water and/or a buffer to control pH or an additional solute to control tonicity.

[0067] Formulations can be prepared by procedures known in the art using well known and readily available ingredients. For example, the composition can be formulated with one or more common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. The compositions can also be formulated as elixirs or solutions appropriate for parenteral administration.

[0068] The formulations can also take the form of an aqueous or anhydrous solution, e.g., a lyophilized formulation, or dispersion, or alternatively the form of an emulsion or suspension.

[0069] The active ingredients may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

[0070] These formulations can contain pharmaceutically or neutraceutically acceptable vehicles and adjuvants which are well known in the prior art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint.

Exemplary Compositions and Uses

[0071] In one embodiment, a composition having one or more of the disclosed agents is/are provided in powdered or aqueous form in premeasured amounts, e.g., in pouches, for addition to baby formula, breast milk or milk derived from human cells. In one embodiment, a composition having one or more of the disclosed agents is provided in other foods such as snack bars, cookies, gels, or baby food, e.g., solid or semi-solid food. In one embodiment, a baby formula composition having two or more of the disclosed agents is provided in powdered or liquid form, e.g., in individual containers. In one embodiment, the premeasured doses are in a form of pre-dosed, e.g., single use, daily packets, packages, pouches, measured powder supplements, gels, infant formula or other foods.

[0072] "Infant" means a human subject ranging in age from birth to not more than one year and includes infants from 0 to 12 months of age.

[0073] "Child" means a subject ranging in age from 12 months to about 13 years. In some embodiments, a child is a subject between the ages of 1 and 5 years old.

[0074] "Infant formula" or "baby formula" means a composition that satisfies at least a portion of the nutrient

requirements of an infant. In the United States, the content of an infant formula is dictated by the federal regulations set forth at 21

[0075] C.F.R. Sections 100, 106, and 107. The term "infant formula" also includes starter infant formula and follow-on formula.

[0076] The term "starter infant formula" means an infant formula for use during the first four to six months of the life of the infant.

[0077] The term "follow-on formula" means an infant formula intended to use by an infant aged from four months or six months to 12 months of age.

[0078] In one embodiment, the composition may include a plurality of prebiotics. In certain embodiments, the composition includes prebiotics which can exert additional health benefits, which may include, but are not limited to, selective stimulation of the growth and/or activity of one or a limited number of beneficial gut bacteria, stimulation of the growth and/or activity of ingested probiotic microorganisms, selective reduction in gut pathogens, and favorable influence on gut short chain fatty acid profile. Such prebiotics may be naturally-occurring, synthetic, or developed through the genetic manipulation of organisms and/or plants. Prebiotics include but are not limited to oligosaccharides, polysaccharides, and other prebiotics that contain fructose, xylose, soya, galactose, glucose and mannose. Exemplary prebiotics include but are not limited to lactulose, lactosucrose, raffinose, gluco-oligosaccharide, inulin, fructo-oligosaccharide (FOS), isomalto-oligosaccharide, soybean oligosaccharides, lactosucrose, xylo-oligosaccharide (XOS), chito-oligosaccharide, manno-oligosaccharide, aribino-oligosaccharide, siallyl-oligosaccharide, fuco-oligosaccharide, or gentio-oligosaccharides.

[0079] In an embodiment, the total amount of prebiotics present in the composition may be from about 1.0 g/L to about 10.0 g/L of the composition. In one embodiment, the total amount of prebiotics present in the composition may be from about 2.0 g/L and about 8.0 g/L of the composition. In some embodiments, the total amount of prebiotics present in the composition may be from about 0.01 g/100 Kcal to about 1.5 g/100 Kcal. In certain embodiments, the total amount of prebiotics present in the composition may be from about 0.15 g/100 Kcal to about 1.5 g/100 Kcal.

[0080] The composition(s) may also comprise a carbohydrate source. Carbohydrate sources can be any used in the art, e.g., lactose, glucose, fructose, corn syrup solids, maltodextrins, sucrose, starch, rice syrup solids, and the like. The amount of carbohydrate in the composition typically can vary from between about 5 g and about 25 g/100 Kcal. In some embodiments, the amount of carbohydrate is between about 6 g and about 22 g/100 Kcal. In other embodiments, the amount of carbohydrate is between about 12 g and about 14 g/100 Kcal. In some embodiments, corn syrup solids are preferred. Moreover, hydrolyzed, partially hydrolyzed, and/or extensively hydrolyzed carbohydrates may be desirable for inclusion in the composition due to their easy digestibility.

[0081] Non-limiting examples of carbohydrate materials suitable for use herein include hydrolyzed or intact, naturally or chemically modified, starches sourced from corn, tapioca, rice or potato, in waxy or non-waxy forms. Non-limiting examples of suitable carbohydrates include various hydrolyzed starches characterized as hydrolyzed cornstarch, maltodextrin, maltose, corn syrup, dextrose, corn syrup solids, glucose, and various other glucose polymers and combinations thereof. Non-limiting examples of other suitable carbohydrates include those often referred to as sucrose, lactose, fructose, high fructose corn syrup, indigestible oligosaccharides such as fructooligosaccharides and combinations thereof.

[0082] In some embodiments, the composition described herein comprises a fat or lipid source. In certain embodiments, appropriate fat sources include, but are not limited to, animal sources, e.g., milk fat, butter, butter fat, egg yolk lipid; marine sources, such as fish oils, marine oils, single cell oils; vegetable and plant oils, such as corn oil, canola oil, sunflower oil, soybean oil, palm olein oil, coconut oil, high oleic sunflower oil, evening primrose oil, rapeseed oil, olive oil, flaxseed (linseed) oil, cottonseed oil, high oleic safflower oil, palm stearin, palm kernel oil, wheat germ oil; medium chain triglyceride oils and emulsions and esters of fatty acids; and any combinations thereof. In some embodiment the composition comprises between about 1 g/100 Kcal to about 10 g/100 Kcal of a fat or lipid source. In some embodiments, the composition comprises between about 2 g/100 Kcal to about 7 g/100 Kcal of a fat source. In other embodiments the fat source may be present in an amount from about 2.5 g/100 Kcal to about 6 g/100 Kcal. In still other embodiments, the fat source may be present in the composition in an amount from about 3 g/100 Kcal to about 4 g/100 Kcal.

[0083] In some embodiments, the fat or lipid source comprises from about 10% to about 35% palm oil per the total amount of fat or lipid. In some embodiments, the fat or lipid source comprises from about 15% to about 30% palm oil per the total amount of fat or lipid. Yet in other embodiments, the fat or lipid source may comprise from about 18% to about 25% palm oil per the total amount of fat or lipid.

[0084] In certain embodiments, the fat or lipid source may be formulated to include from about 2% to about 16% soybean oil based on the total amount of fat or lipid. In some embodiments, the fat or lipid source may be formulated to include from about 4% to about 12% soybean oil based on the total amount of fat or lipid. In some embodiments, the fat or lipid source may be formulated to include from about 6% to about 10% soybean oil based on the total amount of fat or lipid.

[0085] In certain embodiments, the fat or lipid source may be formulated to include from about 2% to about 16% coconut oil based on the total amount of fat or lipid. In some embodiments, the fat or lipid source may be formulated to include from about 4% to about 12% coconut oil based on the total amount of fat or lipid. In some embodiments, the fat or lipid source may be formulated to include from about 6% to about 10% coconut oil based on the total amount of fat or lipid.

[0086] In certain embodiments, the fat or lipid source may be formulated to include from about 2% to about 16% sunflower oil based on the total amount of fat or lipid.

[0087] In some embodiments, the fat or lipid source may be formulated to include from about 4% to about 12% sunflower oil based on the total amount of fat or lipid. In some embodiments, the fat or lipid source may be formulated to include from about 6% to about 10% sunflower oil based on the total amount of fat or lipid.

[0088] In some embodiments, the oils, e.g., sunflower oil, soybean oil, sunflower oil, palm oil, etc. are meant to cover fortified versions of such oils known in the art. For example,

in certain embodiments, the use of sunflower oil may include high oleic sunflower oil. In other examples, the use of such oils may be fortified with certain fatty acids, as known in the art, and may be used in the fat or lipid source disclosed herein.

[0089] In some embodiments the composition may also include a source of long chain polyunsaturated fatty acids (LCPUFAs). In one embodiment the amount of LCPUFA in the composition is advantageously at least about 5 mg/100 Kcal, and may vary from about 5 mg/100 Kcal to about 100 mg/100 Kcal, more preferably from about 10 mg/100 Kcal to about 50 mg/100 Kcal. Non-limiting examples of LCPUFAs include, but are not limited to, docosahexanoic acid (DHA) arachidonic acid (ARA), linoleic (18:2 n-6), .gamma.-linolenic (18:3 n-6), dihomo-gamma-linolenic (20:3 n-6) acids in the n-6 pathway, .alpha.-linolenic (18:3 n-3), stearidonic (18:4 n-3), eicosatetraenoic (20:4 n-3), eicosapentaenoic (20:5 n-3), and docosapentaenoic (22:6 n-3).

[0090] In some embodiments, the LCPUFA included in the composition may comprise DHA. In one embodiment the amount of DHA in the composition is advantageously at least about 17 mg/100 Kcal, and may vary from about 5 mg/100 Kcal to about 75 mg/100 Kcal, more preferably from about 10 mg/100 Kcal to about 50 mg/100 Kcal.

[0091] In another embodiment, if the composition is an infant formula, the composition may be supplemented with both docosahexanoic acid (DHA) and arachidonic acid (ARA). In this embodiment, the weight ratio of ARA:DHA may be between about 1:3 and about 9:1. In a particular embodiment, the ratio of ARA:DHA is from about 1:2 to about 4:1. The DHA and ARA can be in natural form, provided that the remainder of the LCPUFA source does not result in any substantial deleterious effect on the infant. Alternatively, the DHA and ARA can be used in refined form.

[0092] The disclosed composition described herein can, in some embodiments, also comprise a source of beta-glucan. Glucans are polysaccharides, specifically polymers of glucose, which are naturally occurring and may be found in cell walls of bacteria, yeast, fungi, and plants. Beta glucans (.beta.-glucans) are themselves a diverse subset of glucose polymers, which are made up of chains of glucose monomers linked together via beta-type glycosidic bonds to form complex carbohydrates. Beta-1,3-glucans are carbohydrate polymers purified from, for example, yeast, mushroom, bacteria, algae, or cereals. The chemical structure of beta-1,3-glucan depends on the source of the beta-1,3-glucan. Moreover, various physiochemical parameters, such as solubility, primary structure, molecular weight, and branching, play a role in biological activities of beta-1,3-glucans.

[0093] Beta-1,3-glucans are naturally occurring polysaccharides, with or without beta-1,6-glucose side chains that are found in the cell walls of a variety of plants, yeasts, fungi and bacteria. Beta-1,3;1,6-glucans are those containing glucose units with (1,3) links having side chains attached at the (1,6) position(s). Beta-1,3;1,6 glucans are a heterogeneous group of glucose polymers that share structural commonalities, including a backbone of straight chain glucose units linked by a beta-1,3 bond with beta-1,6-linked glucose branches extending from this backbone. While this is the basic structure for the presently described class of .beta.-glucans, some variations may exist. For example, certain yeast beta-glucans have additional regions of beta(1,3) branching extending from the beta(1,6) branches, which add further complexity to their respective structures.

[0094] Beta-glucans derived from baker's yeast, Saccharomyces cerevisiae, are made up of chains of D-glucose molecules connected at the 1 and 3 positions, having side chains of glucose attached at the 1 and 6 positions. Yeast-derived .beta.-glucan is an insoluble, fiber-like, complex sugar having the general structure of a linear chain of glucose units with a beta-1,3 backbone interspersed with beta-1,6 side chains that are generally 6-8 glucose units in length. More specifically, beta-glucan derived from baker's yeast is poly-(1,6)-beta-D-glucopyranosyl-(1,3)-beta-D-glucopyranose.

[0095] In some embodiments, the beta-glucan is beta-1,3;1,6-glucan. In some embodiments, the beta-1,3;1,6-glucan is derived from baker's yeast. The composition may comprise whole glucan particle beta.-glucan, particulate .beta.-glucan, PGG-glucan (poly-1,6-.beta.-D-glucopyranosyl-1,3-.beta.-D-glucopyranose) or any mixture thereof. In some embodiments, the amount of .beta.-glucan in the composition is between about 3 mg and about 17 mg per 100 Kcal. In another embodiment the amount of .beta.-glucan is between about 6 mg and about 17 mg per 100 Kcal.

[0096] One or more vitamins and/or minerals may also be added in to the composition in amounts sufficient to supply the daily nutritional requirements of a subject. It is to be understood by one of ordinary skill in the art that vitamin and mineral requirements will vary, for example, based on the age of the child. For instance, an infant may have different vitamin and mineral requirements than a child between the ages of one and thirteen years. Thus, the embodiments are not intended to limit the composition to a particular age group but, rather, to provide a range of acceptable vitamin and mineral components.

[0097] In embodiments providing a composition for a child, the composition may optionally include, but is not limited to, one or more of the following vitamins or derivations thereof: vitamin B1 (thiamin, thiamin pyrophosphate, TPP, thiamin triphosphate, TTP, thiamin hydrochloride, thiamin mononitrate), vitamin B2 (riboflavin, flavin mononucleotide, FMN, flavin adenine dinucleotide, FAD, lactoflavin, ovoflavin), vitamin B3 (niacin, nicotinic acid, nicotinamide, niacinamide, nicotinamide adenine dinucleotide, NAD, nicotinic acid mononucleotide, NicMN, pyridine-3-carboxylic acid), vitamin B.sub.3-precursor tryptophan, vitamin B6 (pyridoxine, pyridoxal, pyridoxamine, pyridoxine hydrochloride), pantothenic acid (pantothenate, panthenol), folate (folic acid, folacin, pteroylglutamic acid), vitamin B12 (cobalamin, methylcobalamin, deoxyadenosylcobalamin, cyanocobalamin, hydroxycobalamin, adenosylcobalamin), biotin, vitamin C (ascorbic acid), vitamin A (retinol, retinyl acetate, retinyl palmitate, retinyl esters with other long-chain fatty acids, retinal, retinoic acid, retinol esters), vitamin D (calciferol, cholecalciferol, vitamin D.sub.3, 1,25,-dihydroxyvitamin D), vitamin E (alpha-tocopherol, alpha-tocopherol acetate, .alpha.-tocopherol succinate, .alpha.-tocopherol nicotinate, .alpha.-tocopherol), vitamin K (vitamin K1, phylloquinone, naphthoquinone, vitamin K2, menaquinone-7, vitamin K3, menaquinone-4, menadione, menaquinone-8, menaquinone-8H, menaquinone-9, menaquinone-9H, menaquinone-10, menaquinone-11, menaquinone-12, menaquinone-13), choline, inositol, beta-carotene and any combinations thereof.

[0098] In embodiments providing a children's product, such as a growing-up milk, the composition may optionally include, but is not limited to, one or more of the following minerals or derivations thereof: boron, calcium, calcium acetate, calcium gluconate, calcium chloride, calcium lactate, calcium phosphate, calcium sulfate, chloride, chromium, chromium chloride, chromium picolonate, copper, copper sulfate, copper gluconate, cupric sulfate, fluoride, iron, carbonyl iron, ferric iron, ferrous fumarate, ferric orthophosphate, iron trituration, polysaccharide iron, iodide, iodine, magnesium, magnesium carbonate, magnesium hydroxide, magnesium oxide, magnesium stearate, magnesium sulfate, manganese, molybdenum, phosphorus, potassium, potassium phosphate, potassium iodide, potassium chloride, potassium acetate, selenium, sulfur, sodium, docusate sodium, sodium chloride, sodium selenate, sodium molybdate, zinc, zinc oxide, zinc sulfate and mixtures thereof. Non-limiting exemplary derivatives of mineral compounds include salts, alkaline salts, esters and chelates of any mineral compound.

[0099] The minerals can be added in the form of salts such as calcium phosphate, calcium glycerol phosphate, sodium citrate, potassium chloride, potassium phosphate, magnesium phosphate, ferrous sulfate, zinc sulfate, cupric sulfate, manganese sulfate, and sodium selenite. Additional vitamins and minerals can be added as known within the art.

[0100] The compositions may optionally include one or more of the following flavoring agents, including, but not limited to, flavored extracts, volatile oils, cocoa or chocolate flavorings, peanut butter flavoring, cookie crumbs, vanilla or any commercially available flavoring. Examples of useful flavorings include, but are not limited to, pure anise extract, imitation banana extract, imitation cherry extract, chocolate extract, pure lemon extract, pure orange extract, pure peppermint extract, honey, imitation pineapple extract, imitation rum extract, imitation strawberry extract, or vanilla extract; or volatile oils, such as balm oil, bay oil, bergamot oil, cedarwood oil, cherry oil, cinnamon oil, clove oil, or peppermint oil; peanut butter, chocolate flavoring, vanilla cookie crumb, butterscotch, toffee, and mixtures thereof. The amounts of flavoring agent can vary greatly depending upon the flavoring agent used. The type and amount of flavoring agent can be selected as is known in the art.

[0101] The compositions may optionally include one or more emulsifiers that may be added for stability of the final product. Examples of suitable emulsifiers include, but are not limited to, lecithin (e.g., from egg or soy), alpha lactalbumin and/or mono- and di-glycerides, and mixtures thereof. Other emulsifiers are readily apparent to the skilled artisan and selection of suitable emulsifier(s) will depend, in part, upon the formulation and final product. Indeed, the incorporation of a blend of intact protein, protein hydrolysates, and amino acids into a composition, such as an infant formula, may require the presence of at least on emulsifier to ensure that the blend of intact protein, hydrolysates, and amino acids do not separate from the fat or proteins contained within the infant formula during shelf-storage or preparation.

[0102] In some embodiments, the composition may be formulated to include from about 0.5 wt % to about 1 wt % of emulsifier based on the total dry weight of the composition. In other embodiments, the composition may be formulated to include from about 0.7 wt % to about 1 wt % of emulsifier based on the total dry weight of the composition.

[0103] In some embodiments where the composition is a ready-to-use liquid composition, the composition may be formulated to include from about 200 mg/L to about 600 mg/L of emulsifier. Still, in certain embodiments, the composition may include from about 300 mg/L to about 500 mg/L of emulsifier. In other embodiments, the composition may include from about 400 mg/L to about 500 mg/L of emulsifier.

[0104] The compositions may optionally include one or more preservatives that may also be added to extend product shelf life. Suitable preservatives include, but are not limited to, potassium sorbate, sodium sorbate, potassium benzoate, sodium benzoate, potassium citrate, calcium disodium EDTA, and mixtures thereof. The incorporation of a preservative in the composition including a blend of intact protein, protein hydrolysates, and/or amino acids ensures that the composition has a suitable shelf-life such that, once reconstituted for administration, the composition delivers nutrients that are bioavailable and/or provide health and nutrition benefits for the target subject.

[0105] In some embodiments the composition may be formulated to include from about 0.1 wt % to about 1.0 wt % of a preservative based on the total dry weight of the composition. In other embodiments, the composition may be formulated to include from about 0.4 wt % to about 0.7 wt % of a preservative based on the total dry weight of the composition.

[0106] In some embodiments where the composition is a ready-to-use liquid composition, the composition may be formulated to include from about 0.5 g/L to about 5 g/L of preservative. Still, in certain embodiments, the composition may include from about 1 g/L to about 3 g/L of preservative.

[0107] The composition may optionally include one or more stabilizers. Suitable stabilizers for use in practicing the composition of the present disclosure include, but are not limited to, gum arabic, gum ghatti, gum karaya, gum tragacanth, agar, furcellaran, guar gum, gellan gum, locust bean gum, pectin, low methoxyl pectin, gelatin, microcrystalline cellulose, CMC (sodium carboxymethylcellulose), methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, DATEM (diacetyl tartaric acid esters of mono- and diglycerides), dextran, carrageenans, and mixtures thereof. Indeed, incorporating a suitable stabilizer in the composition including intact protein, protein hydrolysates, and/or amino acids ensures that the composition has a suitable shelf-life such that, once reconstituted for administration, the composition delivers nutrients that are bioavailable and/or provide health and nutrition benefits for the target subject.

[0108] In some embodiments where the composition is a ready-to-use liquid composition, the composition may be formulated to include from about 50 mg/L to about 150 mg/L of stabilizer. Still, in certain embodiments, the composition may include from about 80 mg/L to about 120 mg/L of stabilizer.

[0109] In an embodiment, the children's composition may contain between about 10 and about 50% of the maximum dietary recommendation for any given country, or between about 10 and about 50% of the average dietary recommendation for a group of countries, per serving of vitamins A, C, and E, zinc, iron, iodine, selenium, and choline. In another embodiment, the children's composition may supply about 10-30% of the maximum dietary recommendation for any given country, or about 10-30% of the average dietary recommendation for a group of countries, per serving of

B-vitamins. In yet another embodiment, the levels of vitamin D, calcium, magnesium, phosphorus, and potassium in the children's nutritional product may correspond with the average levels found in milk. In other embodiments, other nutrients in the children's composition may be present at about 20% of the maximum dietary recommendation for any given country, or about 20% of the average dietary recommendation for a group of countries, per serving.

[0110] In some embodiments the composition is an infant formula. Infant formulas are fortified compositions for an infant. The content of an infant formula is dictated by federal regulations, which define macronutrient, vitamin, mineral, and other ingredient levels in an effort to simulate the nutritional and other properties of human breast milk. Infant formulas are designed to support overall health and development in a pediatric human subject, such as an infant or a child.

[0111] In some embodiments, the composition of the present disclosure is a growing-up milk. Growing-up milks are fortified milk-based beverages intended for children over 1 year of age (typically from 1-3 years of age, from 4-6 years of age or from 1-6 years of age). Growing-up milks are designed with the intent to serve as a complement to a diverse diet to provide additional insurance that a child achieves continual, daily intake of all essential vitamins and minerals, macronutrients plus additional functional dietary components, such as non-essential nutrients that have purported health-promoting properties.

[0112] The exact composition of a growing-up milk or other composition according to the present disclosure can vary from market-to-market, depending on local regulations and dietary intake information of the population of interest. In some embodiments, compositions according to the disclosure consist of a milk protein source, such as whole or skim milk, plus added sugar and sweeteners to achieve desired sensory properties, and added vitamins and minerals. The fat composition includes an enriched lipid fraction derived from milk. Total protein can be targeted to match that of human milk, cow milk or a lower value. Total carbohydrate is usually targeted to provide as little added sugar, such as sucrose or fructose, as possible to achieve an acceptable taste. Typically, Vitamin A, calcium and Vitamin D are added at levels to match the nutrient contribution of regional cow milk. Otherwise, in some embodiments, vitamins and minerals can be added at levels that provide approximately 20% of the dietary reference intake (DRI) or 20% of the Daily Value (DV) per serving. Moreover, nutrient values can vary between markets depending on the identified nutritional needs of the intended population, raw material contributions and regional regulations.

[0113] The disclosed composition(s) may be provided in any form known in the art, such as a powder, a gel, a suspension, a paste, a solid, a liquid, a liquid concentrate, a reconstitutable powdered milk substitute or a ready-to-use product. The composition may, in certain embodiments, comprise a nutritional supplement, children's nutritional product, infant formula, human milk fortifier, growing-up milk or any other composition designed for an infant or a pediatric subject. Compositions of the present disclosure include, for example, orally-ingestible, health-promoting substances including, for example, foods, beverages, tablets, capsules and powders. Moreover, the composition of the present disclosure may be standardized to a specific caloric content, it may be provided as a ready-to-use product, or it may be provided in a concentrated form.

[0114] The compositions may be provided in a suitable container system. For example, non-limiting examples of suitable container systems include plastic containers, metal containers, foil pouches, plastic pouches, multi-layered pouches, and combinations thereof. In certain embodiments, the composition may be a powdered composition that is contained within a plastic container. In certain other embodiments, the composition may be contained within a plastic pouch located inside a plastic container.

Exemplary Embodiments

[0115] In one embodiment, a method to detect immune health status in a human infant or child is provided. The method includes providing a stool sample from a human infant or child; and determining in the sample i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of one, two or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF, Blon_0113, ureC Blon_0111, ureE Blon_0112, BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650 or one, two or more of H1 (Blon_2331-2361), H2 (Blon_0243-Blon_0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), or Urease (Blon_0104-Blon_0115). In one embodiment, the child is less than about 5 years old. In one embodiment, a relative abundance of *Bacteroides* of >10%, of *Bifidobacterium* of <60% or of *Blautia* of >10% is indicative of an infant or child at increased risk of allergies or other diseases or a relative abundance of *Bacteroides* of >8%, of *Bifidobacterium* of <65% or of *Blautia* of >2% is indicative of an infant or child at increased risk of allergies or other diseases. In one embodiment, a relative abundance of *Bacteroides* of >10%, of *Bifidobacterium* of <60% and of *Blautia* of >10% is indicative of an infant or child at increased risk of allergies or other diseases or a relative abundance of *Bacteroides* of >8%, of *Bifidobacterium* of <65% and of *Blautia* of >2% is indicative of an infant or child at increased risk of allergies or other diseases. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% or of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies or other diseases or *Bacteroides* of <10%, of *Bifidobacterium* of >65% or of *Blautia* of <2% is indicative of an infant or child at decreased risk of allergies or other diseases. In one embodiment, a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% and of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies or other diseases or *Bacteroides* of <10%, of

[0116] *Bifidobacterium* of >65% or of *Blautia* of <2% is indicative of an infant or child at decreased risk of allergies or other diseases. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% to 10%, *Bifidobacterium breve* of 2% to 25%, *Bifidobacterium longum* of 25% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 10% or less, *Bifidobacterium breve* of 25% or less, *Bifidobacterium longum* of 25% or greater, or of

Bifidobacterium pseudocatenulatum of less than 2% is indicative of immune health in the infant or child or of *Bifidobacterium breve* of 15% or less, *Bifidobacterium longum* of 65% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 3% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 10% or less, *Bifidobacterium breve* of 25% or less, *Bifidobacterium longum* of 25% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child or of *Bifidobacterium breve* of 15% or less, *Bifidobacterium longum* of 65% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 3% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater, *Bifidobacterium breve* of 20% or less, *Bifidobacterium longum* of 50% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of 5% or greater, *Bifidobacterium breve* of 20% or less, *Bifidobacterium longum* of 50% or greater, and of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child. In one embodiment, a relative abundance of *Bifidobacterium bifidum* of less than 5%, *Bifidobacterium breve* of greater than 20%, *Bifidobacterium longum* of less than 50%, or of *Bifidobacterium pseudocatenulatum* of greater than 2% is indicative of impaired immune health in the infant or child or of *Bifidobacterium breve of greater than* 15%, *Bifidobacterium longum* of less than 30%, or of *Bifidobacterium pseudocatenulatum* of greater than 3% is indicative of impaired immune health in the infant or child. In one embodiment, an increase in the relative abundance of expression of two or more of Blon_0915, Blon_2171, Blon_2173, Blon_2334, galT Blon_2172, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650, or of two or more of H1 (Blon_2331-2361), H2 (Blon_0243-Blon_0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), and Urease (Blon_0104-Blon_0115) is indicative of immune health in the infant or child. In one embodiment, the sample is from a newborn. In one embodiment, the sample is from a newborn up to a 3 month old infant. In one embodiment, the sample is from a 3 month old up to a 9 month old infant. In one embodiment, the sample is from an infant or child treated with a drug. In one embodiment, the drug is an antibiotic. In one embodiment, the infant or child has necrotizing enterocolitis. In one embodiment, the method further comprising administering to the mother of the infant or child, or a pregnant mother, a composition optionally comprising one or more prebiotics or one or more probiotics. In one embodiment, the prebiotic or probiotic comprises one or more bacteria, one or more antibodies, or one or more molecules that enhance the relative abundance of *Bifidobacterium longum*. In one embodiment, the relative abundance of *Bifidobacterium longum infantis* is enhanced. In one embodiment, the relative abundance of *Bifidobacterium longum infantis* is greater than 60%, 70%, 80% or 90% after taking the composition. In one embodiment, the sample is analyzed using a nucleic acid amplification reaction. In one embodiment, the sample is analyzed using genome sequencing.

[0117] Further provided is a method to identify a human infant or child at higher risk of developing allergies as an adolescent or adult, comprising: providing a stool sample from a human infant or child; and determining in the sample i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of two or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650.

[0118] In one embodiment, a kit is provided comprising a plurality of probes or primers to determine i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia* in a physiological sample, ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum* in a physiological sample, or iii) the relative abundance or expression of two or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650 in a physiological sample.

[0119] Also provided is a method to detect immune health status in a human infant or child, comprising: providing a stool sample from a human infant or child; and determining in the sample i) the relative abundance of *Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including one or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of one or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0426, ureF, Blon_0113, ureC Blon_0111, ureE Blon_0112, BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650. In one embodiment, the relative abundance of *Bifidobacterium* is >60%. In one embodiment, the relative abundance of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum* is >60%. In one embodiment, the relative abundance of *Bifidobacterium Bifidobacterium longum* is >60%.

[0120] The invention will be described by the following non-limiting examples.

EXAMPLE 1

[0121] There are circumstances that might prevent a mother from breastfeeding or an infant or child may require the use of antibiotics, leading to a reduced prevalence of key bacterial species in an infant or child's gut. In the first months of birth, the loss of *Bifidobacterium* species, particularly *Bifidobacterium longum infantis,* or gain of other bacteria during this window of opportunity, may significantly alter the 'natural' progression of the microbial community that may lead to a variety of negative consequences for host health including a predisposition to autoimmune, metabolic, and neurobehavioral diseases (such as IBD, allergies, childhood obesity, ADHD, and autism). A recent report profiling children's gut microbiomes in the United States clearly show an overall low abundance of *Bifidobacterium* genus in infants 0-3 months of age. There is an unmet need

to provide alternatives to infant formula for better nutrition that promote health and well-being.

[0122] It is highly likely that human breast milk HMOs are not the sole promoters of a healthy gut microbiome. The Wisconsin Infant Study Cohort (WISC) birth cohort (U19 AI104317, MPI Gern/Seroogy) consists of three distinct study arms (animal farming study group, rural non-farming study group, and TA study group) aimed at characterizing the impact of early life farming exposures on immune development, respiratory health, and allergic diseases Stool sample collected from study infants at 2 months of age underwent shotgun metagenomic sequencing. As disclosed herein, there is an increased abundance (80%) of several *Bifidobacteria* species in the TA infant study group compared to the non-TA infants (50%). Specifically, the predominant strain comprising ~75% of the bacterial composition of TA infant's gut microbial community consists of one species: *Bifidobacterium longum infantis,* whereas the non-TA infants comprise ~30% of *Bifidobacterium longum* subsp. *longum.* Importantly, this is controlled for breast feeding. The difference in abundance amongst breastfed infants is and strongly suggests that differences in breast milk components are impacting the predominance of *Bifidobacterium longum infantis.*

Materials and Methods

[0123] Recruitment. Study participants for the WISC Farm and Nonfarm study arms were recruited from families receiving prenatal care at the Marshfield Clinic (various locations across Wisconsin), and for the WFS arm, the LaFarge Birthing Center (LaFarge, Wis.).

[0124] Stool sample selection. Stool was collected from study participants at approximately 2 months of age. The allowed collection window spanned 1.5 to 6 months of age, with most samples falling close to the two month date. DNA from stool samples had been previously extracted and frozen. To select samples for shotgun metagenomics sequencing, we included all children in the WFS study arm for whom at least 100 ng DNA was available (n=27). To select Farm and Nonfarm samples with matching attributes, samples from infants with vaginal deliveries, who were exclusively breastfed at the time of sample collection, and who enrolled in the study close to the same time as the TA participants, were analyzed. A total of 46 Farm and 43 Nonfarm samples were analyzed.

Metagenomics and Sample Preparation

[0125] Sample preparation for sequencing. DNA was extracted from stool using a modified cetyltrimethylammonium bromide (CTAB)-buffer-based protocol

[0126] (DeAngelis et al. 2009), as described previously by Fujimura et al (Fujimura et al. 2016). Metagenomic shotgun library preparation and sequencing were performed at the DNA Sequencing Facility at the University of Wisconsin-Madison on the Illumina NovaSeq 6000 platform using a paired-end sequencing approach with a targeted read length of 150 bp.

Primary Processing of Metagenomics and MS Data

[0127] Basic processing of metagenomics sequencing data. Initial processing, taxonomic classification, and functional profiling of metagenomics samples was performed using bioBakery3 utilities (Beghini et al. 2021). KneadData was applied for automated quality control, which included quality trimming and removal of reads that map to the human genome (hg38). MetaPhlan v3 (Segata et al. 2012; Beghini et al. 2021) was applied for taxonomic classification and computation of relative abundance matrices.

Downstream Analysis

[0128] *Bifidobacterium longum* gene family detection. To inspect *Bifidobacterium longum* gene presence in the samples, PanPhlan (Beghini et al. 2021) was used to evaluate the presence/absence of UniRef90 gene families identified in a *Bifidobacterium longum* pangenome that was computed by uniting several reference genomes. The pangenome was provided with the PanPhlan software.

[0129] Identifying differentially abundant microbes between TA and non-TA. We applied LEfSe (Segata et al. 2011), which uses Kruskal-Wallis sum-rank tests to evaluate whether a taxa is significantly different between study groups, followed by estimating the effect sizes of those differences using Linear Discriminant Analysis (LDA) with bootstrap resampling. Centered log ratio (CLR) transformation, per sample, was used to the relative abundance matrix prior to running LEfSE. Microbes were accepted at $p<0.05$ with LDA score of at least 2.

[0130] Functional analysis. HUMANn (Franzosa et al. 2018; Beghini et al. 2021) was used to estimate copies per million for UniRef90 gene families and MetaCyc Pathways. The first output of this approach is an estimated Copies per Million (CPM) for UniRef90 gene families within each sample. Each UniRef90 gene family is a cluster of genes from one or more taxa that were assigned based on a 90% sequence identity. Under each UniRef90 gene family, HUMANn also provides estimates of the CPMs for the taxa-specific genes within the family. MetaCyc pathway CPMs are estimated by aggregating the CPMs for gene families assigned to each MetaCyc pathway. Taxa-specific estimates are also provided for each pathway when possible.

[0131] The CPM was inspected and an *infantis* marker gene (Blon_0915) and genes involved in HMO metabolism (LoCascio et al. 2010) were identified. Of the 56 genes identified by LoCascio et al, 15 were found in the HUMANn gene families results file.

[0132] Identifying pathways associated with TA vs. Non-TA. Linear modeling, implemented in Maaslin2 (Mallick et al. 2021), was used to identify MetaCyc pathways that are differentially abundant at the community level between the TA and non-TA cohorts. The analysis was of infants who were exclusively breastfeeding at the time of sample collection. The statistical test was performed on the community-level total for each pathway, and accepted as significant those with p-value <0.01 after adjustment using the Benjamini-Hochberg procedure. For significant pathways, the taxa-specific distribution of the CPMs was visibly inspected to interpret the result. A stricter adjusted p-value threshold was used for this analysis compared to others (in other words, a threshold lower than 0.05) in order to prioritize a reasonable number of results for manual investigation.

[0133] Machine learning. The tidymodels (Kuhn and Wickham 2020) R libraries were used to build classifiers to discriminate TA from non-TA samples using the estimated microbial abundances. To reduce the potential of

learning dietary differences (formula vs. breastmilk) instead of farm exposure differences, the analysis was conducted on exclusively breastfeeding children.

[0134] Data preparation. W Features with near zero variance (defined as having less than 5% unique values, or a ratio of most-common value to second-most common level greater than 95/5) were removed. Relative abundances were converted using a modified mean-centered log ratio (computing the means using non-zero values) and ran the analysis with two versions of the features: features at all levels of the phylogeny (all_levels) as well as only species-level features (species). Results are shown for species-level predictions.

[0135] Modeling algorithms. For each of the following models, a tuning parameter grid of 20 parameters was generated in the default range for each parameter (defined in tidymodels model specifications).

[0136] random forest (randomForest and ranger)

[0137] elastic net (glmnet)

[0138] linear support vector machine (kernlab)

[0139] boosted gradient trees (xgboost)

[0140] k-nearest neighbor

[0141] For ranger and randomForest, we also ran with a set of default parameters: 1000 trees, mtry =sqrt(number of features) (number of random feature choices to consider at each split).

[0142] Model selection and evaluation. For each model, 10 repeats of nested cross-validation were run with 10 outer training/testing folds. For each outer fold, a five-fold cross-validation was used on the training set to estimate the performance of each parameter setting. The parameters selected were based on the area under the precision-recall curve (PR-AUC), and trained a single model for that training fold to make predictions on its paired testing fold. The predictions from all 10 folds were concatenated before computing evaluation metrics: PR-AUC and area under the receiver operating characteristic curve (ROC-AUC).

[0143] Variable importance. The 10 repeats of ten-fold cross-validation ultimately resulted in 100 trained models per algorithm. The top performing methods for variable interpretation were selected: glmnet and ranger, default parameters (which tied with randomForest, default parameters). The variable importance for each model was estimated and each feature summarized by the median importance across all 100 models (where an importance of 0 means that the feature was not used). For glmnet, the variable importance is the absolute value of the standardized coefficient. For ranger, the variable importance is the Gini Impurity, or the feature's mean improvement in the split criterion (decrease in node impurity) across the forest.

[0144] Miscellaneous libraries for computational analysis. In the course of this analysis, data structures and functions provided in the R libraries phyloseq (McMurdie and Holmes 2013), microbiome, ggplot2, tidyverse, were used.

Results

[0145] Shotgun metagenomics sequencing was used to profile the two-month-old gut microbiome of 116 infants, comprising 27 infants from TA families (referred to as WFS cohort from this point on), 46 from farming families (Farm cohort), and 43 from non-farming families (Nonfarm cohort). Compared to Farm and Nonfarm, the WFS families had a larger number of children living in the home, lower maternal age, and a higher rate of male to female infants in the study (Table 1). Nearly all WFS mothers consumed unprocessed farm milk during pregnancy, while this was rare among the others.

TABLE 1

Study participant demographics and characteristics.

value	Amish	Farm	Nonfarm
batch			
stool__2021__07__08	0.44 (12/27)	0	0.09 (4/43)
stool__bmilk__2020__07__15	0.56 (15/27)	0.43 (20/46)	0.70 (30/43)
stool__bmilk__hsdust__2021__03__17	0	0.50 (23/46)	0.14 (6/43)
stool02__metagenomics__2019__09__04	0	0.07 (3/46)	0.07 (3/43)
birthmonthcat			
December-February	0.15 (4/27)	0.22 (10/46)	0.26 (11/43)
June-August	0.22 (6/27)	0.28 (13/46)	0.21 (9/43)
March-May	0.41 (11/27)	0.24 (11/46)	0.28 (12/43)
September-November	0.22 (6/27)	0.26 (12/46)	0.26 (11/43)
delivery__mode			
vaginal	1.00 (27/27)	0.85 (39/46)	0.93 (40/43)
c-section	0	0.15 (7/46)	0.07 (3/43)
momagecat			
>=40	0.08 (2/26)	0.02 (1/46)	0
18-24	0.27 (7/26)	0.09 (4/46)	0.05 (2/43)
24-30	0.38 (10/26)	0.39 (18/46)	0.37 (16/43)
30-34	0.12 (3/26)	0.28 (13/46)	0.42 (18/43)
34-39	0.15 (4/26)	0.22 (10/46)	0.16 (7/43)
season__cat			
December-February	0	0.26 (12/46)	0.19 (8/43)
June-August	0	0.33 (15/46)	0.16 (7/43)

TABLE 1-continued

Study participant demographics and characteristics.

value	Amish	Farm	Nonfarm
March-May	0	0.17 (8/46)	0.37 (16/43)
September-November	0	0.24 (11/46)	0.28 (12/43)
sex			
Female	0.33 (9/27)	0.41 (19/46)	0.56 (24/43)
Male	0.67 (18/27)	0.59 (27/46)	0.44 (19/43)
Total			
Count	27	46	43

The Infant Gut Microbiome is Associated with Diet and Farming Exposures

[0146] Alpha diversity metrics can provide a high-level description of the richness and distributional qualities of metagenomics samples. Various alpha diversity metrics were tested for association with sample variables including technical variables, demographics, family history of asthma and atopic dermatitis (eczema), and infant eczema, wheezing, and sensitization outcomes at one and two years (FIG. **17**). Diversity metrics were associated with infant diet and farming groups, but no others reached significance. Exclusive breastfeeding was associated with low species richness compared to children who were fed formula along with breastmilk or exclusively. Farm group was associated with dominance of core taxa. The sample-sample similarity structure also separated samples by study group and diet) based on PERMANOVA tests.

[0147] Visualization of highly prevalent genera and species (at least 1% relative abundance in at least 10% of study samples) quickly provided a simple explanation for the alpha diversity metrics that were associated with farm and diet: exclusively breastfeeding participants were characterized by high relative abundance of *Bifidobacterium* species, with *Bifidobacterium longum* particularly high in TA participants.

[0148] Next, the distribution of *Bifidobacterium* species among the exclusively breastfeeding participants was examined. Strikingly, the microbiota of TA participants were dominated by *Bifidobacterium longum* and to a lesser extent bifidum, while the non-TA participants displayed a more varied profile with high abundance of *longum, bifidum, breve,* and *pseudocatenulatum.*

Bifidobacterium longum Subsp. *infantis* Genes are Found in WFS Infants

[0149] A gene-level assessment of the genetic diversity of *Bifidobacterium longum* in the study samples (FIG. **19**) was conducted. PanPhlan (Scholz et al. 2016) was used to evaluate the presence or absence of *B. longum* genes (that is, UniRef90 clusters identified in *B. longum* reference genomes). The profiles of TA participants clustered together distinctly from the non-TA, and also clustered with the reference genomes that were labeled as *Bifidobacterium longum* subsp. *infantis.*

TABLE 2

HMO Cluster	UniRef90 Gene Family	Blon_gene	Protein_name
H1	UniRef90_B7GNN8	Blon_2336	Alpha-1,3/4-fucosidase, putative
H1	UniRef90_B7GNP6	Blon_2344	Extracellular solute-binding protein, family 1
H1	UniRef90_Q8G5N0	Blon_2334	Beta-galactosidase (EC 3.2.1.23) (Lactase)
H2	UniRef90_B7GN40	Blon_0248	Alpha-L-fucosidase (EC 3.2.1.51)
H2	UniRef90_B7GTT2	Blon_0244	Signal transduction histidine kinase-like protein
H3	UniRef90_B7GN40	Blon_0426	Alpha-L-fucosidase (EC 3.2.1.51)
H4	UniRef90_A0A087BR20	Blon_0650	ABC transporter related
H4	UniRef90_B7GPL9	Blon_0642	GntR domain protein
H4	UniRef90_E7CY69	Blon_0625	Beta-glucosidase (EC 3.2.1.21)
H5	UniRef90_B3DQG9	Blon_2177	Extracellular solute-binding protein, family 1
H5	UniRef90_E8MF10	Blon_2171	UDP-glucose 4-epimerase (EC 5.1.3.2)
H5	UniRef90_E8MF11	galT	Galactose-1-phosphate
		Blon_2172	uridylyltransferase (Gal-1-P uridylyltransferase) (EC 2.7.7.12) (UDP-glucose--hexose-1-phosphate uridylyltransferase)
H5	UniRef90_E8MF12	Blon_2173	Aminoglycoside phosphotransferase
Urease	UniRef90_B7GT17	ureC	Urease subunit alpha (EC 3.5.1.5) (Urea
		Blon_0111	amidohydrolase subunit alpha)
Urease	UniRef90_B7GT18	ureE	Urease accessory protein UreE
		Blon_0112	
		BLIJ_0113	
Urease	UniRef90_B7GT19	ureF	Urease accessory protein UreF
		Blon_0113	

[0150] The presence/absence of a *B. longum infantis* marker gene, Blon_0915, and 15 *B. longum* genes involved in human milk oligosaccharide (HMO) metabolism (LoCascio et al. 2010) were determined. 25/27 TA samples detected the marker gene and all 15 HMO genes, with correspondingly high copies per million (CPM) for most genes. By contrast, only 8 non-TA (5 farm and 3 nonfarm) detected Blon_0915. Six HMO genes were detected widely across the non-TA samples, while 9 were conspicuously absent from most. The latter nine were previously identified as uniquely and specifically conserved among *infantis* subspecies compared to other *longum* (LoCascio et al. 2010).

Developing a Microbial Signature for Farm Groups

[0151] In addition to *B. longum,* other microbial taxa and functional pathways were identified that could distinguish the TA from non-TA microbiota. Multiple approaches were used: statistical comparison of microbial abundances and functional pathways, and training machine learning models followed by variable importance ranking.

Machine Learning Models can Discriminate Between TA and Non-TA Samples

[0152] A suite of machine learning approaches was used to attempt to build classifiers to separate the TA from non-TA samples, and to identify important features. All algorithms achieved some success, with PR-AUC well above random guessing in all ten folds of cross-validation. The top performing algorithm was elastic net (implemented in glmnet), with mean PR-AUC=0.91. Two random forest implementations and linear support vector machines essentially tied for second place. The features employed by the glmnet and random forest classifiers to discriminate between the farm groups were examined (elastic net in FIG. **19**). The elastic net approach also provides a sign on each feature that indicates which class (TA or non-TA) the feature is positively correlated to. The top features used by both algorithms were highly concordant, with 15 features appearing in the top 25 of both lists: (species) s_*Actinomyces_sp_oral_taxon* 181, s_*Bacteroides_faecis,* s_*Bacteroides_stercoris,* s_*Bifidobacterium_bifidum,* s_*Bifidobacterium_longum,* s_*Bifidobacterium_pseudocatenulatum,* s_*Bilophila_wadsworthia,* s_*Collinsella_aerofaciens,* s_*Enterococcus_avium,* s_*Enterococcus_durans,* s_*Haemophilus_parainfluenzae,* s_*Parabacteroides_merdae,* s_*Streptococcus_peroris,* s_*Streptococcus_salivarius.* Different members of the same genera, for examples *Bacteroides* and *Bifidobacterium,* were associated by glmnet with either TA or non-TA, suggesting that species-level (and perhaps more granular) genetic diversity varies between the groups.

Differetially Abundant Microbes

[0153] As a companion to the machine learning variable importance analysis, statistical tests were used to identify differentially abundant microbes between the groups. Nonparametric analysis by LEfSE (Segata et al. 2011) identified several taxa that were higher in TA compared to non-TA (FIGS. **32**, **33**), including a substantial overlap with the top features from the machine learning analysis.

Differentiallv Abundant Functional Pathways

[0154] Differentially abundant MetaCyc pathways between TA and non-TA are shown in FIG. **34**. Values shown are row-sealed log 1p(copies per million). Pathway p<0.01 after Benjamini-Hochberg correction. Row annotations: coefficient (positive: higher in TA), negative log 10(adjusted p-value).

EXAMPLE 2

[0155] In one embodiment, for genus-level *Bifidobacterium,* if the total *Bifidobacterium* >80%, then there is a reduced disease risk and if the total *Bifidobacterium* <58%, then there is an increased disease risk.

TABLE 3

Genus	TA	non-TA (exclusive breastfeeding only)	non-TA (all diets)
Bifidobacterium	0.829	0.649	0.579

For species and subspecies level *Bifidobacterium longum,* in one embodiment, the *Bifidobacterium longum* subsp. *infantis* >71% and/or non-*Bifidobacterium* genera <17%, then there is a reduced disease risk while if the *Bifidobacterium longum* (any subspecies) <22% and/or total non-*Bifidobacterium* genera relative abundance >42%, then there is an increased disease risk.

TABLE 4

Bifidobacterium Species	TA	non-TA (exclusive breastfeeding only)	non-TA, all STDs
Bifidobacterium_longum	0.713	0.265	0.219
Other (not *Bifidobacterium*)	0.171	0.351	0.421

Diversity metrics are summaries of the distributions of the relative abundances, where higher "diversity" means more species are represented with more abundance, while higher "dominance" means fewer species have most of the abundance.

[0156] The following table shows exemplary means per group (all diets):

TABLE 5

metric	TA	'non-TA'	Direction
diversity_inverse_simpson (1/(sum of squared relative abundances)	1.87	3.19	TA < non-TA
diversity_coverage (number of species needed to sum up to at least 50% of the relative abundance)	1.04	1.57	TA < non-TA
diversity_gini_simpson ((1 − (sum of squared relative abundances))	0.394	0.606	TA < non-TA
dominance_relative (relative abundance of the single most abundant taxon in each sample)	0.74	0.534	TA > non-TA
dominance_core_abundance (combined relative abundance of taxa that appear in at least half of the samples)	0.736	0.257	TA > non-TA

Metrics higher in non-TA (meaning more diversity, which implies *B. longum infantis* is not dominant):

[0157] If inverse simpson alpha diversity >3, then increased risk

[0158] If inverse simpson alpha diversity <1.9, then decreased risk

[0159] If coverage diversity >1.5, then increased risk

[0160] If coverage diversity <1.04 then decreased risk

[0161] If gini simpson diversity >0.61, then increased risk

[0162] If gini simpson diversity <0.39, then decreased risk

Metrics higher in TA than non-TA:

[0163] If dominance relative abundance (relative abundance of single most abundant taxon) >74%, then decreased risk

[0164] If dominance relative abundance <53%, then increased risk

[0165] If dominance core abundance <74%, then decreased risk

[0166] If dominance core abundance <26%, then increased risk

EXAMPLE 3

[0167] A table of relative abundances for the top features from machine learning analysis (FIG. **13**). The purple ones are more associated with TA (decreased risk) and the yellow ones are more associated with non-TA (increased risk). The other tables in the figure contain the full list of top genera and *Bifido* species. A threshold of 1% relative abundance was set in the associated group. The top 5 in the "TA" set are shown in purple and the top 4 in the "non-TA" set are shown in yellow.

TABLE 6

Top learning machine features

Associated class (based on centered log ratio transformed data (CLR))	Species	TA relative abundance	non-TA relative abundance (all infants)	non-TA relative abundance (breast feeding only)	glmnet score	ranger score	mann whitney - log10(p)	full clade_name	glmnet coefficient sign	rank correlation on CLR
TA	s__*Bifidobacterium_longum*	0.7134	0.2186	0.2650	0.896	4.335	4.098	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Bifidobacteriales|f__Bifidobacteriaceae|g__*Bifidobacterium*|s__*Bifidobacterium_longum*	TA	0.5641155
TA	s__*Bifidobacterium_bifidum*	0.0808	0.0943	0.0942	0.472	0.969	NA	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Bifidobacteriales|f__Bifidobacteriaceae|g__*Bifidobacterium*|s__*Bifidobacterium_bifidum*	TA	0.257873256
TA	s__*Bifidobacterium_breve*	0.0336	0.1756	0.2050	NA	0.578	NA	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Bifidobacteriales|f__Bifidobacteriaceae|g__*Bifidobacterium*|s__*Bifidobacterium_breve*	none	0.068485119
TA	s__*Parabacteroides_distasonis*	0.0168	0.0213	0.0250	NA	0.324	NA	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Tannerellaceae|g__*Parabacteroides*|s__*Parabacteroides_distasonis*	none	0.152581114
TA	s__*Collinsella_aerofaciens*	0.0103	0.0147	0.0114	0.391	0.488	1.312	k__Bacteria|p__Actinobacteria|c__Coriobacteriia|o__Coriobacteriales|f__Coriobacteriaceae|g__*Collinsella*|s__*Collinsella_aerofaciens*	TA	0.333176874
TA	s__*Bacteroides_faecis*	0.0069	0.0004	0.0005	0.246	0.595	1.312	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__*Bacteroides*|s__*Bacteroides_faecis*	TA	0.338732633
TA	s__*Parabacteroides_merdae*	0.0037	0.0025	0.0009	0.221	0.491	NA	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Tannerellaceae|g__*Parabacteroides*|s__*Parabacteroides_merdae*	TA	0.30111717
TA	s__*Bacteroides_stercoris*	0.0027	0.0013	0.0009	0.559	0.658	NA	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__*Bacteroides*|s__*Bacteroides_stercoris*	TA	0.310929705
TA	s__*Enterococcus_avium*	0.0016	0.0016	0.0002	1.127	1.194	1.312	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__*Enterococcus*|s__*Enterococcus_avium*	TA	0.325467951
TA	s__*Bifidobacterium_dentium*	0.0011	0.0156	0.0248	NA	0.266	NA	k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Bifidobacteriales|f__Bifidobacteriaceae|g__*Bifidobacterium*|s__*Bifidobacterium_dentium*	none	0.265321611
TA	s__*Lactobacillus_gasseri*	0.0008	0.0003	0.0004	0.282	NA	NA	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__*Lactobacillus*|s__*Lactobacillus_gasseri*	TA	−0.035738222
TA	s__*Enterococcus_durans*	0.0007	0.0008	0.0000	0.312	0.865	1.617	k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Enterococcaceae|g__*Enterococcus*|s__*Enterococcus_durans*	TA	0.377012091
TA	s__*Bacteroides_ovatus*	0.0007	0.0010	0.0002	NA	0.284	NA	k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__*Bacteroides*|s__*Bacteroides_ovatus*	none	0.054401947

TABLE 6-continued

Top learning machine features										
Associated class (based on centered log ratio transformed data (CLR))	Species	TA relative abundance	non-TA relative abundance (all infants)	non-TA relative abundance (breast feeding only)	glmnet score	ranger score	mann whitney - log10(p)	full clade_name	glmnet coefficient sign	rank correlation on CLR
TA	s___*Streptococcus_mitis*	0.0006	0.0016	0.0026	NA	0.260	NA	k___Bacteria|p___Firmicutes|c___Bacilli|o___Lactobacillales|f___Streptococcaceae|g___*Streptococcus*|s___*Streptococcus_mitis*	none	0.007843237
TA	s___*Actinomyces*_sp_oral_taxon_181	0.0005	0.0000	0.0000	0.636	3.139	3.359	k___Bacteria|p___Actinobacteria|c___Actinobacteria|o___Actinomycetales|f___Actinomycetaceae|g___*Actinomyces*|s___*Actinomyces*_sp_oral_taxon_181	TA	0.510650315
TA	s___*Bilophila_wadsworthia*	0.0004	0.0001	0.0001	0.361	0.364	NA	k___Bacteria|p___Proteobacteria|c___Deltaproteobacteria|o___Desulfovibrionales|f___Desulfovibrionaceae|g___*Bilophila*|s___*Bilophila_wadsworthia*	TA	0.201570209
TA	s___*Streptococcus_peroris*	0.0003	0.0001	0.0001	0.980	1.653	2.325	k___Bacteria|p___Firmicutes|c___Bacilli|o___Lactobacillales|f___Streptococcaceae|g___*Streptococcus*|s___*Streptococcus_peroris*	TA	0.440807168
TA	s___*Anaerococcus_vaginalis*	0.0002	0.0000	0.0000	NA	0.587	1.312	k___Bacteria|p___Firmicutes|c___Tissierellia|o___Tissierellales|f___Peptoniphilaceae|g___*Anaerococcus*|s___*Anaerococcus_vaginalis*	none	0.336679708
TA	s___*Actinomyces_odontolyticus*	0.0001	0.0002	0.0003	0.355	NA	NA	k___Bacteria|p___Actinobacteria|c___Actinobacteria|o___Actinomycetales|f___Actinomycetaceae|g___*Actinomyces*|s___*Actinomyces_odontolyticus*	TA	0.122690145
TA	s___[*Collinsella*]_*massiliensis*	0.0000	0.0000	0.0000	0.361	NA	NA	k___Bacteria|p___Actinobacteria|c___Coriobacteriia|o___Coriobacteriales|f___Coriobacteriaceae|g___*Enorma*|s___[*Collinsella*]_*massiliensis*	TA	0.252048516
TA	s___*Streptococcus_infantis*	0.0000	0.0001	0.0002	0.209	NA	NA	k___Bacteria|p___Firmicutes|c___Bacilli|o___Lactobacillales|f___Streptococcaceae|g___*Streptococcus*|s___*Streptococcus_infantis*	TA	0.044638423
TA	s___*Finegoldia_magna*	0.0000	0.0000	0.0000	NA	0.271	NA	k___Bacteria|p___Firmicutes|c___Tissierellia|o___Tissierellales|f___Peptoniphilaceae|g___*Finegoldia*|s___*Finegoldia_magna*	none	0.196569753
TA	s___*Streptococcus*_sp_HMSC071D03	0.0000	0.0000	0.0000	0.405	NA	NA	k___Bacteria|p___Firmicutes|c___Bacilli|o___Lactobacillales|f___Streptococcaceae|g___*Streptococcus*|s___*Streptococcus*_sp_HMSC071D03	TA	0.219531894
non-TA	s___*Bifidobacterium_pseudocatenulatum*	0.0000	0.0408	0.0336	0.948	0.469	1.617	k___Bacteria|p___Actinobacteria|c___Actinobacteria|o___Bifidobacteriales|f___Bifidobacteriaceae|g___*Bifidobacterium*|s___*Bifidobacterium_pseudocatenulatum*	non-TA	−0.374039199
non-TA	s___*Escherichia_coli*	0.0222	0.0386	0.0494	0.543	NA	NA	k___Bacteria|p___Proteobacteria|c___Gammaproteobacteria|o___Enterobacterales|f___Enterobacteriaceae|g___*Escherichia*|s___*Escherichia_coli*	non-TA	0.041550877
non-TA	s___*Bacteroides_thetaiotaomicron*	0.0008	0.0242	0.0286	0.235	NA	NA	k___Bacteria|p___Bacteroidetes|c___Bacteroidia|o___Bacteroidales|f___Bacteroidaceae|g___*Bacteroides*|s___*Bacteroides_thetaiotaomicron*	non-TA	−0.190106231
non-TA	s___*Bifidobacterium_adolescentis*	0.0000	0.0201	0.0104	0.499	NA	NA	k___Bacteria|p___Actinobacteria|c___Actinobacteria|o___Bifidobacteriales|f___Bifidobacteriaceae|g___*Bifidobacterium*|s___*Bifidobacterium_adolescentis*	non-TA	−0.264614336

TABLE 6-continued

Top learning machine features										
Associated class (based on centered log ratio transformed data (CLR))	Species	TA relative abundance	non-TA relative abundance (all infants)	non-TA relative abundance (breast feeding only)	glmnet score	ranger score	mann whitney - log10(p)	full clade_name	glmnet coefficient sign	rank correlation on CLR
non-TA	s___*Klebsiella_michiganensis*	0.0000	0.0079	0.0128	0.418	NA	NA	k___Bacteria|p___Proteobacteria|c___Gammaproteobacteria|o___Enterobacterales|f___Enterobacteriaceae|g___*Klebsiella*|s___*Klebsiella_michiganensis*	non-TA	−0.279322423
non-TA	s___*Clostridium_neonatale*	0.0075	0.0060	0.0094	NA	0.303	NA	k___Bacteria|p___Firmicutes|c___Clostridia|o___Clostridiales|f___Clostridiaceae|g___*Clostridium*|s___*Clostridium_neonatale*	none	−0.130726656
non-TA	s___*Streptococcus_salivarius*	0.0011	0.0043	0.0042	0.673	1.397	1.312	k___Bacteria|p___Firmicutes|c___Bacilli|o___Lactobacillales|f___Streptococcaceae|g___*Streptococcus*|s___*Streptococcus_salivarius*	non-TA	−0.326060035
non-TA	s___*Streptococcus_parasanguinis*	0.0003	0.0016	0.0018	NA	0.517	NA	k___Bacteria|p___Firmicutes|c___Bacilli|o___Lactobacillales|f___Streptococcaceae|g___*Streptococcus*|s___*Streptococcus_parasanguinis*	none	−0.12389155
non-TA	s___*Staphylococcus_epidermidis*	0.0003	0.0012	0.0011	NA	0.400	NA	k___Bacteria|p___Firmicutes|c___Bacilli|o___Bacillales|f___Staphylococcaceae|g___Staphylococcus|s___*Staphylococcus_epidermidis*	none	−0.247687847
non-TA	s___*Veillonella_dispar*	0.0000	0.0010	0.0013	NA	0.390	1.312	k___Bacteria|p___Firmicutes|c___Negativicutes|o___Veillonellales|f___Veillonellaceae|g___*Veillonella*|s___*Veillonella_dispar*	none	−0.326597126
non-TA	s___*Haemophilus_parainfluenzae*	0.0000	0.0004	0.0006	0.828	0.742	1.957	k___Bacteria|p___Proteobacteria|c___Gammaproteobacteria|o___Pasteurellales|f___Pasteurellaceae|g___*Haemophilus*|s___*Haemophilus_parainfluenzae*	non-TA	−0.409352834
non-TA	s___*Cutibacterium_avidum*	0.0000	0.0003	0.0005	0.239	NA	NA	k___Bacteria|p___Actinobacteria|c___Actinobacteria|o___Propionibacteriales|f___Propionibacteriaceae|g___*Cutibacterium*|s___*Cutibacterium_avidum*	non-TA	−0.087249315
non-TA	s___*Veillonella*_sp_T11011_6	0.0000	0.0000	0.0001	0.275	NA	1.312	k___Bacteria|p___Firmicutes|c___Negativicutes|o___Veillonellales|f___Veillonellaceae|g___*Veillonella*|s___*Veillonella*_sp_T11011_6	non-TA	−0.336322381

TABLE 7

Top species

Species	TA	non-TA (all diets)	non-TA (exclusive breastfeeding only)
Bifidobacterium_longum	0.713	0.219	0.265
Bifidobacterium_bifidum	0.081	0.094	0.094
Bifidobacterium_breve	0.034	0.176	0.205
Escherichia_coli	0.022	0.039	0.049
Bacteroides_fragilis	0.020	0.022	0.028
Parabacteroides_distasonis	0.017	0.021	0.025
Other	0.016	0.071	0.052
Bacteroides_vulgatus	0.015	0.022	0.018
Bacteroides_dorei	0.011	0.011	0.011
Collinsella_aerofaciens	0.010	0.015	0.011
Clostridium_neonatale	0.008	0.006	0.009
Klebsiella_oxytoca	0.007	0.007	0.006
Lactobacillus_rhamnosus	0.006	0.004	0.005
Enterococcus_faecalis	0.004	0.005	0.005
Parabacteroides_merdae	0.004	0.003	0.001
Veillonella_parvula	0.004	0.005	0.001
Eggerthella_lenta	0.003	0.002	0.001
Bacteroides_stercoris	0.003	0.001	0.001
Erysipelatoclostridium_ramosum	0.003	0.023	0.015
Bacteroides_caccae	0.003	0.007	0.009
Ruminococcus_gnavus	0.002	0.054	0.023
Enterococcus_avium	0.002	0.002	0.000
Bacteroides_uniformis	0.001	0.004	0.003
Bifidobacterium_dentium	0.001	0.016	0.025
Streptococcus_salivarius	0.001	0.004	0.004
Veillonella_atypica	0.001	0.002	0.001
Collinsella_stercoris	0.001	0.001	0.001
Lactobacillus_gasseri	0.001	0.000	0.000
Bacteroides_thetaiotaomicron	0.001	0.024	0.029
Flavonifractor_plautii	0.001	0.004	0.002
Enterococcus_durans	0.001	0.001	0.000
Bacteroides_ovatus	0.001	0.001	0.000
Klebsiella_pneumoniae	0.001	0.009	0.010
Streptococcus_mitis	0.001	0.002	0.003
Actinomyces_sp_oral_taxon_181	0.000	0.000	0.000
Streptococcus_vestibularis	0.000	0.001	0.000
Gordonibacter_pamelaeae	0.000	0.001	0.000
Bilophila_wadsworthia	0.000	0.000	0.000
Klebsiella_variicola	0.000	0.005	0.007
Streptococcus_parasanguinis	0.000	0.002	0.002
Staphylococcus_aureus	0.000	0.000	0.000
Bacteroides_xylanisolvens	0.000	0.001	0.000
Streptococcus_peroris	0.000	0.000	0.000
Staphylococcus_epidermidis	0.000	0.001	0.001
Clostridium_clostridioforme	0.000	0.001	0.000
Clostridium_innocuum	0.000	0.005	0.003
Actinomyces_odontolyticus	0.000	0.000	0.000
Lactobacillus_paragasseri	0.000	0.002	0.001
Enterococcus_faecium	0.000	0.001	0.000
Intestinibacter_bartlettii	0.000	0.001	0.000
Actinomyces_sp_HPA0247	0.000	0.000	0.000
Klebsiella_quasipneumoniae	0.000	0.001	0.001
Enterococcus_gallinarum	0.000	0.003	0.000
Rothia_mucilaginosa	0.000	0.000	0.000
Clostridium_paraputrificum	0.000	0.001	0.000
Clostridium_butyricum	0.000	0.002	0.002
Veillonella_dispar	0.000	0.001	0.001
Enterobacter_cloacae_complex	0.000	0.000	0.000
Clostridium_perfringens	0.000	0.003	0.004
Veillonella_infantium	0.000	0.000	0.000
Streptococcus_thermophilus	0.000	0.000	0.000
Haemophilus_parainfluenzae	0.000	0.000	0.001
Bifidobacterium_adolescentis	0.000	0.020	0.010
Bifidobacterium_pseudocatenulatum	0.000	0.041	0.034
Klebsiella_michiganensis	0.000	0.008	0.013
Streptococcus_lutetiensis	0.000	0.023	0.002

TABLE 8

Relative abundance *Bifidobacterium* species

Bifidobacterium Species	TA	non-TA (exclusive breastfeeding only)	non-TA, all SIDs
Bifidobacterium_longum (includes infantis and other subspecies)	0.713	0.265	0.219
Other (not *Bifidobacterium*)	0.171	0.351	0.421
Bifidobacterium_bifidum	0.081	0.094	0.094
Bifidobacterium_breve	0.034	0.205	0.176
Bifidobacterium_dentium	0.001	0.025	0.016
Bifidobacterium_adolescentis	0.000	0.010	0.020
Bifidobacterium_animalis	0.000	0.000	0.000
Bifidobacterium_anseris	0.000	0.000	0.000
Bifidobacterium_boum	0.000	0.000	0.000
Bifidobacterium_catenulatum	0.000	0.000	0.000
Bifidobacterium_choerinum	0.000	0.000	0.000
Bifidobacterium_criceti	0.000	0.000	0.000
Bifidobacterium_gallinarum	0.000	0.000	0.000
Bifidobacterium_kashiwanohense	0.000	0.016	0.010
Bifidobacterium_merycicum	0.000	0.000	0.000
Bifidobacterium_minimum	0.000	0.000	0.000
Bifidobacterium_mongoliense	0.000	0.000	0.000
Bifidobacterium_moukalabense	0.000	0.000	0.000
Bifidobacterium_pseudocatenulatum	0.000	0.034	0.041
Bifidobacterium_pseudolongum	0.000	0.000	0.000
Bifidobacterium_pullorum	0.000	0.000	0.003
Bifidobacterium_ruminantium	0.000	0.000	0.000
Bifidobacterium_saeculare	0.000	0.000	0.000
Bifidobacterium_scardovii	0.000	0.000	0.000
Bifidobacterium_subtile	0.000	0.000	0.000
Bifidobacterium_thermacidophilum	0.000	0.000	0.000
Bifidobacterium_thermophilum	0.000	0.000	0.000

TABLE 9

Mean alpha diversities

metric	TA	'non-TA'	Direction
diversity_inverse_simpson	1.87	3.19	−1.32
diversity_coverage	1.04	1.57	−0.53
diversity_gini_simpson	0.394	0.606	−0.212
dominance_relative	0.74	0.534	0.206
dominance_core_abundance	0.736	0.257	0.479

TABLE 10

Relative abundance, top genera (1% in 10% samples)
Relative abundance, top genera (1% in 10% samples)

Genus	TA	non-TA (exclusive breastfeeding only)	non-TA (ALL)
Bifidobacterium	0.829	0.649	0.579
Bacteroides	0.063	0.101	0.096
Escherichia	0.022	0.049	0.039
Parabacteroides	0.021	0.026	0.024
Collinsella	0.011	0.013	0.016
Clostridium	0.008	0.018	0.013
Klebsiella	0.008	0.037	0.030
Lactobacillus	0.007	0.007	0.008
Enterococcus	0.006	0.006	0.012
Other	0.005	0.014	0.024
Veillonella	0.005	0.010	0.013
Eggerthella	0.003	0.001	0.002
Streptococcus	0.003	0.019	0.036
Erysipelatoclostridium	0.003	0.019	0.029

TABLE 10-continued

Relative abundance, top genera (1% in 10% samples)
Relative abundance, top genera (1% in 10% samples)

Genus	TA	non-TA (exclusive breastfeeding only)	non-TA (ALL)
Blautia	0.002	0.023	0.066
Actinomyces	0.002	0.001	0.001
Flavonifractor	0.001	0.002	0.004
Staphylococcus	0.001	0.002	0.002
Eubacterium	0.000		0.000
Gordonibacter	0.000	0.000	0.001
Bilophila	0.000	0.000	0.000
Lachnoclostridium	0.000	0.001	0.003
Phascolarctobacterium	0.000		0.000
Corynebacterium	0.000	0.000	0.000
Intestinibacter	0.000		0.001
Rothia	0.000	0.000	0.000
Gemella	0.000	0.001	0.001
Enterobacter	0.000		0.000
Citrobacter	0.000		0.001
Haemophilus	0.000	0.001	0.001

EXAMPLE 4

[0168] Thus, breastfeeding and traditional agrarian lifestyle influence 2-month-old infants' gut microbiome composition. TA infant gut is dominated by *Bifidobacterium longum* subspecies *infantis. B. infantis* and early gut commensals are selected by breastmilk oligosaccharides to colonize, preventing colonization by more pathogenic bacteria and those bacteria have been shown to produce nutritive and anti-inflammatory metabolites.

[0169] *B. infantis* has a broad capacity to break down human milk oligosaccharides. *B. infantis* is declining in industrialized communities, but still found in agrarian communities. *B. infantis* and potentially other early life gut commensals may influence healthy development that includes protecting against pathogen colonization, e.g., by producing nutritive and immunomodulatory molecules, e.g., B vitamins, short chain fatty acids (SCFAs, e.g., fatty acids with fewer than 6 carbons), folic acid and/or tryptophan metabolites. Bacterially produced aromatic amino acid metabolites and exopolypeptides have a tolerogenic effect on gut epithelial and T cells.

EXAMPLE 5

[0170] Asthma is an immune-mediated chronic illness, and its prevalence is increasing worldwide. It is a lifelong disease and treatment is primarily focused on symptom management. Development of asthma begins in very early life, but it is not diagnosed until later in childhood. It is often preceded by conditions including allergic rhinitis, eczema, and wheezing. People who grow up on farms have reduced rates of asthma and immune-mediated diseases. The histograms in FIG. **1** show data from a study conducted in Wisconsin. In red, children who grew up on farms have lower prevalence of these conditions compared to non-farm in black. Several maternal and infant lifestyle practices have been associated with protection against disease development. They include close contact with farm animals and their stables, especially by milking cows, and frequent ingestion of unpasteurized cow's milk.

[0171] Intriguingly, allergy prevalence is even lower in WI TA children compared to WI farm children (FIG. **1**). In fact, eczema prevalence is 10 times lower in WI TA children compared to non-TA WI farm children. Also, children who moved to farms after the age of 5 did not enjoy the protective effects experienced by those who lived on farms from birth. Very early life exposure starting during pregnancy of animal and farm milk have the highest protection against allergic diseases.

[0172] FIG. **2**A illustrates the Wisconsin Infant Study Cohort (WISC) and Wisconsin Farm Study (WFS). The Wisconsin Infant Study Cohort (WISC) and Wisconsin Farm Study are prospective birth cohort studies that aim to identify molecular contributors of farm exposures on development of asthma and childhood respiratory illness. Together they consist of 3 arms: The Wisconsin Infant Study Cohort (WISC) includes infants from non-farming and dairy farming families in upper and central Wisconsin. Wisconsin Farm Study arm of the project is comprised of infants from Wisconsin TA families who follow a traditional agrarian lifestyle ("TA"). These studies recruited families who were expecting a child and followed the children through the first two years of life, collecting health information and a broad range of environmental and personal biospecimens.

[0173] The gut microbiomes at two months of age were compared between the farm exposure groups (FIG. **2**C). Whole genome shotgun metagenomics sequencing was performed on 116 stool samples with participant characteristics shown in this table. All TA infants were delivered vaginally and are exclusively breastfed, so we enriched for those categories when we selected non-TA samples. It was hypothesized that the microbial communities of the groups would vary with the level of farming exposure, and that the TA infants would harbor unique microbes compared to the non-TA infants.

[0174] Beta diversity from species level features was computed using the Bray distance, and the samples clustered with Dirichlet Multinomial Mixtures to identify latent structure (FIG. **20**). The Beta diversity plot on the left is annotated by the DMM cluster assignment, with cluster 1 in red, cluster 2 in blue and cluster 3 in green. The plot in the middle uses the same coordinates but is labeled by farm group. TA in blue squares, Farm in green triangles, Nonfarm in orange circles. The plot on the right is annotated by the infant's diet at the time the sample was collected. Exclusively breastfed infants are blue stars, exclusively formula-fed infants in red circles, and those with mixed diet of formula and breastfeeding in yellow diamonds. These bar plots show the distribution of farm groups or diet within each DMM cluster. All but two TA fall into cluster 1, and all exclusively formula-fed fall into cluster 2. Clusters 1 and 3 are each driven primarily by one *Bifidobacterium* species (*Bifidobacterium longum* and *breve,* respectively) while Cluster 2 has more diverse drivers with lower weights. In summary, the high-level structure of the data is driven by diet and farm group.

[0175] The bars on the left in FIG. **21** show the exclusively breastfeeding infants, in the middle those with mixed diet, and on the right the exclusively formula-fed. The dominant genus across most of these categories is *Bifidobacterium,* shown in yellow. While all of the breastfed infants had very high *Bifidobacterium,* the TA infants have relatively higher *Bifidobacterium* and lower diversity of other microbes. Other patterns are associated with diet. For example, infants

with any formula have more *Blautia* (light blue). The few exclusively formula fed infants had lower abundance of *Bifidobacterium* and higher abundance of *Streptococcus* (red).

[0176] FIG. **22** shows non-TA infants have more diverse microbiomes at species level. The samples were compared based on species-level alpha diversity. The figure provides two example metrics that are significantly associated with diet and with farm group independent of diet. A pattern was observed that the TA infants had lower diversity but higher dominance metrics, which summarize the relative contribution of the most abundant species. The *Bifidobacterium* species that made up the genus level totals were characterized. The bar plot includes only exclusively breastfeeding infants with any detected *Bifidobacterium* and shows the average distribution of *Bifidobacterium* species. The TA infants are predominantly colonized by longum, shown in light blue, while the farm and nonfarm have a greater diversity of species. Thus, the diversity metrics are capturing this predominance of *Bifidobacterium longum* in the TA samples.

[0177] A pangenome analysis was performed (FIG. **10**) to survey which *Bifidobacterium* genes were present in each sample and to compare them to reference genomes. Each row in the heatmap represents a UniRef90 gene family that was found in at least one publicly available reference genome for *Bifidobacterium longum.* Each column is either a study sample or a reference genome. Red indicates presence and orange indicates absence of the gene. The first bottom annotation indicates reference genomes in light blue, TA by dark blue, farm by green, and nonfarm by orange. A diet annotation with exclusive breastfeeding is also included in blue. For reference genomes, subspecies annotation, if available, is shown in the bottom row annotation. Subspecies *infantis* are shown in pink, suis in green, and *longum* in blue. Using hierarchical clustering to compare the gene family representation in our study samples with reference genomes, this heatmap shows that TA samples clustered next to known *infantis* strains (boxes). However, some differences are observed between the TA study samples compared to *infantis* references, suggesting the TA samples may have different functional capacity.

[0178] Finding more similarity between *infantis* and the TA study samples compared to the non-TA samples is consistent with a body of work that has observed a decline in *infantis* prevalence in cities and Western lifestyles compared to traditional agrarian communities.

[0179] *Bifidobacterium infantis* has a full complement of genes for metabolizing human milk oligosaccharides and other components of breast milk, whereas other related species have fewer genes, although they can perform cross-feeding. TA samples were confirmed to have greater prevalence of HMO metabolism genes compared to the non-TA. The heatmap shows a subset of HMO genes that are found in the reference files packaged with HUMAnN3. The top half of genes are found broadly in *Bifidobacterium longum,* while the bottom half are specific to *infantis.*

[0180] Although profiles for all metagenomics samples that were sequenced were computed, to remove the confounding effect of infant diet, the analysis was restricted to TA and exclusively breastfeeding non-TA only. Although HUMANn3 provides community level as well as species-level abundances, significant pathways at the community level were identified. Benjamini-Hochberg was again used to adjust the p-values for community level pathways and called significant those with adjusted $p<0.25$ (threshold from MaAslin2). After calling significant pathways, the species-level abundances per pathway identified which organisms were involved.

[0181] For the data in FIG. **23**, "pangenome" files were obtained for *B. longum, B. breve,* and *B. bifidum.* These pangenomes do not include all genes found in those organisms, curiously. The three pangenomes were concatenated and for each sample and each reference genome, PanPhlan was used to determine presence/absence of gene in sample. Clustered genes (rows) used k-means, k=8, and hypergeometric tests were run to ask about enrichment of GO terms in clusters (shown in plot on the right). Clusters 2 and 3 are particularly interesting because they have high prevalence in WFS samples and *B. infantis* references.

[0182] FIG. **24** shows WISC/WFS HMO profiles. Values are log 10(CPM+1). Top annotation is gene cluster, which is based on the organization of the genes on the *B. infantis* genome. Most of these genes, e.g., most of the genes in clusters H1 (Blon_2331-2361), H2 (Blon_0243-Blon_0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), and Urease (Blon_0104-Blon_0115), are highly prevalent among TA samples and not among WISC samples. The H5 cluster of genes is, e.g., Blon_2171-Blon_2177. LoCascio reports that H5 is found commonly in other *B. longum* strains, so it is not surprising to see that it is prevalent in Farm and Nonfarm as well.

[0183] FIG. **25** illustrates differential functional capacities. The analysis was altered by comparing the genetic capacities for metabolic pathways between the groups. The heatmap summarizes the significant pathways, where we took the intersection of hits from two different statistical tests, Maaslin2 and Limma. One of the most significant pathways was folate transformations II, which pertains to B vitamin metabolism. The TA infants have a higher abundance of reads for this pathway than the non-TA infants. Both bacteria and humans need these B vitamins for development. Differences in pathways for short chain fatty acids and amino acid metabolism were also seen.

[0184] Machine learning models trained on stool metagenomics profiles can distinguish TA from non-TA (FIG. **26**). Machine learning models were trained on other organisms or interactions among organisms distinguished the groups and it was observed that it was possible to distinguish the TA samples with high accuracy. ML method performance from 10 repeats of 10-fold CV are shown in the area under the precision-recall curve (PR-AUC) on top and ROC-AUC on the bottom. This is a three-way classification task, and the performance with respect to each target class is shown. For AUPR, the dashed line in each panel is set at the fraction of examples with that class label. For ROC-AUC it is always at 0.5. Elastic networks (glmnet) and random forests (ranger) perform very well on classifying TA samples, and have some more modest signal to distinguish the farm and non-farm groups.

[0185] Next, the features used in the elastic network models distinguishing TA from non-TA were inspected. The heatmap shows the top features as well as top differentially abundant microbes. The bottom half is higher in TA and includes *Bifidobacterium longum* as well as some less abundant distinguishing microbes. The top set of microbes are higher in non-TA samples than TA.

[0186] Additional machine learning analysis of metabolites and lipids provided in the Tables below.

[0187] Cross-validated machine learning analysis was also used to identify metabolites and lipids associated with TA versus non-TA, considering exclusively breastfeeding infants only. Although the untargeted mass spectrometry experiments identified many features, only features with a confident identification were used for this analysis to improve interpretability of the results. For metabolites, methods performed comparably to metagenomics features. Elastic net (glmnet) achieved average PR-AUC 0.95 and random forest achieved average PR-AUC 0.90 (ROC-AUC 0.95 and 0.90, respectively). Performance using lipid features was slightly lower: elastic net achieved average PR-AUC 0.80 and random forest PR-AUC 0.76 (ROC-AUC 0.82 and 0.78). The union of the top 25 metabolite features prioritized by elastic net and random forest is given in the tables below.

TABLE 12

Stool metabolites for distinguishing TA from non-TA infants.

Associated group	Identification	HMDB ID	Retention Time	Molecular Weight
TA higher	5-Aminovaleric acid	HMDB0003355	12.526	117.07934
TA higher	Adenine	HMDB0000034	6.364	135.05405
TA higher	Cytosine	HMDB0000630	8.497	111.04364
TA higher	DL-Carnitine	HMDB0000062	10.615	161.10532
TA higher	L-Citrulline	HMDB0000904	13.141	175.09567
TA higher	L-Methionine	HMDB0000696	9.964	149.05106
TA higher	L-Phenylalanine	HMDB0000159	9.07	165.07874
TA higher	L-Serine	HMDB0000187	13.113	105.04303
TA higher	L-Tryptophan	HMDB0000929	9.941	204.08986
TA higher	N-Acetylhexosamine_RT13.797		13.797	301.04642
TA higher	N-Acetylhistamine	HMDB0013253	3.37	153.09022
TA higher	N-Acetylornithine	HMDB0003357	12.375	174.10024
TA higher	Uracil	HMDB0000300	4.389	112.02633
non-TA higher	2-Deoxyuridine	HMDB0000012	4.742	228.07423
non-TA higher	9-HpODE	HMDB0242602	1.92	312.22994
non-TA higher	Acamprosate	HMDB0014797	8.228	181.0402
non-TA higher	Adenosine	HMDB0000050	6.767	267.09683
non-TA higher	Alanylalanine	HMDB0028680	11.035	160.08452
non-TA higher	Dihydrosphingosine	HMDB0251517	2.239	301.29814
non-TA higher	Glyceric acid	HMDB0000139; HMDB0006372	12.007	106.02564
non-TA higher	Hexose		13.41	180.06272
non-TA higher	Imidazolelactic acid	HMDB0002320	11.604	156.05283
non-TA higher	L-Proline	HMDB0000162	10.222	115.06372
non-TA higher	Lenticin	HMDB0061115	7.032	246.13695
non-TA higher	Methylnicotinamide	HMDB0059711; HMDB0000699; HMDB0003152; HMDB0246826	9.392	136.06381
non-TA higher	N-Acetylaspartic acid	HMDB0000812	14.431	175.04735
non-TA higher	N-Acetylhexosamine_RT12.28		12.28	301.04644
non-TA higher	N-alpha-L-Acetyl-arginine	HMDB0004620	11.992	216.12238
non-TA higher	Succinic acid	HMDB0000254	14.695	118.02564
non-TA higher	Sugar acid 6C_RT13.449		13.449	194.04204
non-TA higher	Sugar alcohol 5C		9.897	152.06765
non-TA higher	Sugar alcohol 6C		11.161	182.07834
non-TA higher	Triethanolamine	HMDB0032538	4.288	149.10535
non-TA higher	Uridine	HMDB0000296	7.628	244.06919

TABLE 13

Stool lipids for distinguishing TA from non-TA infants.

Associated group	Identification	Lipid Class	Retention Time	Quant Ion	Polarity
TA higher	(2E,4E,14E)-13-Hydroperoxy-N-(2-methylpropyl)icosa-2,4,14-trienamide	FAA	4.962	394.33173	+
TA higher	Cer[AP] t40:0	Cer[AP]	11.815	654.60638	–
TA higher	Cer[AS] d18:2_23:0	Cer[AS]	11.9	648.59442	–
TA higher	Cer[NS] d18:1_17:0	Cer[NS]	10.035	534.52539	+
TA higher	Cer[NS] d34:1 (s2lip_121)	Cer[NS]	8.574	520.50848	+
TA higher	Cer[NS] d42:2 (s2lip_276)	Cer[NS]	11.34	630.61792	+
TA higher	Docosahexaenoic acid (DHA)	FA	8.546	329.24762	+
TA higher	PG 22:6_22:6	PG	6.9	865.50311	–
TA higher	Plasmanyl-PC O-38:1 (s2lip_303)	Plasmanyl-PC	12.024	802.6698	+
TA higher	Plasmanyl-PC O-40:4	Plasmanyl-PC	10.811	824.65356	+
TA higher	SP d17:1	SP	2.473	286.27399	+
TA higher	TG 22:4 22:4 22:4 (s2lip_408)	TG	16.527	1057.81787	+
non-TA higher	Alkanyl-DG O-34:3 (s2lip_229)	Alkanyl-DG	10.232	577.51941	+
non-TA higher	Alkenyl-TG P-52:1	Alkenyl-TG	18.795	862.82202	+
non-TA higher	CE 20:3	CE	17.265	692.63446	+
non-TA higher	Cer[AP] t42:1	Cer[AP]	12.229	680.62048	–
non-TA higher	Cer[NS] d18:1_24:0	Cer[NS]	14.171	708.65216	–
non-TA higher	Cer[NS] d18:2_24:0	Cer[NS]	13.597	706.63623	–
non-TA higher	Cer[NS] d36:3 (s2lip_174)	Cer[NS]	9.316	544.50946	+
non-TA higher	Cer[NS] d38:0 (s2lip_306)	Cer[NS]	12.12	654.60553	–
non-TA higher	Cer[NS] d40:1 (s2lip_310)	Cer[NS]	12.204	620.59949	–
non-TA higher	LysoPE 16:0	LysoPE	1.668	452.27853	–
non-TA higher	LysoPE 16:1	LysoPE	1.244	450.26312	–
non-TA higher	LysoPE 17:1	LysoPE	1.482	464.27853	–
non-TA higher	LysoPG 16:0 (s2lip_17)	LysoPG	1.276	483.27298	–
non-TA higher	PC 33:1 (s2lip_179)	PC	9.364	746.57843	+
non-TA higher	PC 35:2 (s2lip_188)	PC	9.518	772.59277	+
non-TA higher	PE 16:0_17:1	PE	9.51	702.50928	–
non-TA higher	PE 16:0_18:1 (s2lip_204)	PE	9.794	718.53839	+
non-TA higher	PE 16:0_18:2	PE	9.089	714.50909	–
non-TA higher	PE 28:0	PE	8.005	636.45966	+
non-TA higher	PE 29:0	PE	8.277	648.46161	–
non-TA higher	PE 30:1	PE	8.116	660.46185	–
non-TA higher	PE 31:0	PE	9.119	678.50629	+
non-TA higher	PE 32:1	PE	8.917	688.49292	–
non-TA higher	PE 34:2 (s2lip_148)	PE	8.949	716.52179	+
non-TA higher	PE 34:2 (s2lip_171)	PE	9.256	716.52295	+
non-TA higher	PE 36:5	PE	9.068	738.50354	+

TABLE 13-continued

Stool lipids for distinguishing TA from non-TA infants.

Associated group	Identification	Lipid Class	Retention Time	Quant Ion	Polarity
non-TA higher	PG 18:1_18:1	PG	8.481	773.5354	–
non-TA higher	PI 16:0_18:1	PI	8.256	835.53711	–

TABLE 14

Stool lipids from untargeted mass spectrometry analysis that are correlated to TA-associated microbial pathways and may be indicative of decreased risk of allergic disease.

Unique Lipid ID	Retention time	Quant ion	Polarity	Identification
s2lip_1856	7.031	547.40125	–	Unknown_mz547.40125_–_RT7.031
s2lip_1571	5.95	581.36212	–	Unknown_mz581.36212_–_RT5.95
s2lip_1855	7.031	583.37781	–	Unknown_mz583.37781_–_RT7.031
s2lip_1860	7.033	593.40692	–	Unknown_mz593.40692_–_RT7.033
s2lip_1570	5.95	605.40637	–	Unknown_mz605.40637_–_RT5.95
s2lip_1858	7.033	607.42255	–	Unknown_mz607.42255_–_RT7.033
s2lip_2013	7.277	609.43762	–	Unknown_mz609.43762_–_RT7.277
s2lip_6153	12.462	793.57672	–	Unknown_mz793.57672_–_RT12.462
s2lip_1868	7.035	803.6405	–	Unknown_mz803.6405_–_RT7.035
s2lip_1569	5.949	815.64166	–	Unknown_mz815.64166_–_RT5.949
s2lip_6154	12.463	817.62134	–	Unknown_mz817.62134_–_RT12.463
s2lip_1853	7.03	831.67328	–	Unknown_mz831.67328_–_RT7.03
s2lip_6177	12.503	843.63611	–	Unknown_mz843.63611_–_RT12.503
s2lip_6637	13.432	845.65088	–	Unknown_mz845.65088_–_RT13.432
s2lip_1842	7.022	845.68896	–	Unknown_mz845.68896_–_RT7.022
s2lip_6643	13.441	847.62115	–	Unknown_mz847.62115_–_RT13.441
s2lip_6642	13.441	857.65302	–	Unknown_mz857.65302_–_RT13.441
s2lip_594	0.754	861.60938	–	Unknown_mz861.60938_–_RT0.754
s2lip_6997	14.137	861.63867	–	Unknown_mz861.63867_–_RT14.137
s2lip_6213	12.573	869.65259	–	Unknown_mz869.65259_–_RT12.573
s2lip_6647	13.443	871.6684	–	Unknown_mz871.6684_–_RT13.443
s2lip_7102	14.399	873.68286	–	Unknown_mz873.68286_–_RT14.399
s2lip_6999	14.138	885.68402	–	Unknown_mz885.68402_–_RT14.138
s2lip_7668	15.847	1053.81946	–	Unknown_mz1053.81946_–_RT15.847
s2lip_3638	9.164	1079.67383	–	Unknown_mz1079.67383_–_RT9.164
s2lip_5721	11.725	1119.77075	–	Unknown_mz1119.77075_–_RT11.725
s2lip_1578	5.957	367.33621	+	Unknown_mz367.33621_+_RT5.957
s2lip_1851	7.029	369.35153	+	Unknown_mz369.35153_+_RT7.029
s2lip_6650	13.444	409.29474	+	Unknown_mz409.29474_+_RT13.444
s2lip_6651	13.446	427.30508	+	Unknown_mz427.30508_+_RT13.446
s2lip_1865	7.034	566.4422	+	Unknown_mz566.4422_+_RT7.034
s2lip_1573	5.954	569.38165	+	Unknown_mz569.38165_+_RT5.954
s2lip_1852	7.03	571.39697	+	Unknown_mz571.39697_+_RT7.03
s2lip_1575	5.955	585.35571	+	Unknown_mz585.35571_+_RT5.955
s2lip_1849	7.028	587.37134	+	Unknown_mz587.37134_+_RT7.028
s2lip_1579	5.957	610.40771	+	Unknown_mz610.40771_+_RT5.957
s2lip_1861	7.033	612.42352	+	Unknown_mz612.42352_+_RT7.033
s2lip_6152	12.462	781.59595	+	Unknown_mz781.59595_+_RT12.462
s2lip_6655	13.455	809.62524	+	Unknown_mz809.62524_+_RT13.455
s2lip_6225	12.598	828.67358	+	Unknown_mz828.67358_+_RT12.598
s2lip_6639	13.436	830.68719	+	Unknown_mz830.68719_+_RT13.436
s2lip_6221	12.586	833.62671	+	Unknown_mz833.62671_+_RT12.586
s2lip_6644	13.442	835.64264	+	Unknown_mz835.64264_+_RT 13.442
s2lip_7004	14.143	849.65796	+	Unknown_mz849.65796_+_RT14.143
s2lip_6649	13.444	851.61694	+	Unknown_mz851.61694_+_RT13.444
s2lip_3561	9.112	866.66913	+	Unknown_mz866.66913_+_RT9.112
s2lip_6646	13.442	911.62836	+	Unknown_mz911.62836_+_RT13.442
s2lip_6648	13.443	917.64435	+	Unknown_mz917.64435_+_RT13.443
s2lip_7667	15.847	1012.83838	+	Unknown_mz1012.83838_+_RT15.847
s2lip_7669	15.847	1017.79352	+	Unknown_mz1017.79352_+_RT15.847
s2lip_7662	15.823	1038.85388	+	Unknown_mz1038.85388_+_RT15.823
s2lip_3637	9.164	1098.71521	+	Unknown_mz1098.71521_+_RT9.164
s2lip_3647	9.168	1103.67017	+	Unknown_mz1103.67017_+_RT9.168
s2lip_1577	5.957	1115.7738	+	Unknown_mz1115.7738_+_RT5.957

TABLE 14-continued

Stool lipids from untargeted mass spectrometry analysis that are correlated to TA-associated microbial pathways and may be indicative of decreased risk of allergic disease.

Unique Lipid ID	Retention time	Quant ion	Polarity	Identification
s2lip__1850	7.029	1119.80518	+	Unknown__mz1119.80518__+__RT7.029
s2lip__1863	7.034	1121.81189	+	Unknown__mz1121.81189__+__RT7.034

TABLE 15

Stool metabolites from untargeted mass spectrometry analysis that are correlated to TA-associated microbial pathways and may be indicative of decreased risk of allergic disease. HMDB ID: Entry for metabolite in Human Metabolome Database (hmdb.ca).

Unique metabolite ID	Retention time	Molecular weight	HMDB ID	Identification
s2met__893	14.064	89.04671		unknown__mass89.04671__RT14.064
s2met__872	13.804	101.04815		unknown__mass101.04815__RT13.804
s2met__54	8.497	111.04364	HMDB0000630	Cytosine
s2met__951	15.055	130.02569		unknown__mass130.02569__RT15.055
s2met__593	9.987	134.05697		unknown__mass134.05697__RT9.987
s2met__34	6.364	135.05405	HMDB0000034	Adenine
s2met__440	6.33	136.07265		unknown__mass136.07265__RT6.33
s2met__906	14.206	145.07306		unknown__mass145.07306__RT14.206
s2met__619	10.971	146.05709		unknown__mass146.05709__RT10.971
s2met__920	14.477	148.03633		unknown__mass148.03633__RT14.477
s2met__952	15.055	148.03633		unknown__mass148.03633__RT15.055
s2met__745	12.606	150.05199		unknown__mass150.05199__RT12.606
s2met__706	12.094	163.08413		unknown__mass163.08413__RT12.094
s2met__623	10.987	164.0677		unknown__mass164.0677__RT10.987
s2met__663	11.635	164.0677		unknown__mass164.0677__RT11.635
s2met__899	14.135	171.05244		unknown__mass171.05244__RT14.135
s2met__502	7.977	173.06821		unknown__mass173.06821__RT7.977
s2met__436	6.251	176.06775		unknown__mass176.06775__RT6.251
s2met__67	10.097	182.0572	HMDB0000755	4-Hydroxyphenyllactic acid
s2met__618	10.952	188.11623		unknown__mass188.11623__RT10.952
s2met__95	14.201	189.0631	HMDB0001138	N-Acetyl-DL-glutamic acid
s2met__438	6.323	196.09406		unknown__mass196.09406__RT6.323
s2met__637	11.187	204.06115		unknown__mass204.06115__RT11.187
s2met__909	14.271	205.05806		unknown__mass205.05806__RT14.271
s2met__59	9.004	205.0733	HMDB0000671	Indole-3-lactic acid
s2met__905	14.19	211.04578		unknown__mass211.04578__RT14.19
s2met__626	11.006	217.09509		unknown__mass217.09509__RT11.006
s2met__845	13.525	218.09034		unknown__mass218.09034__RT13.525
s2met__896	14.098	219.07376		unknown__mass219.07376__RT14.098
s2met__907	14.239	227.01973		unknown__mass227.01973__RT14.239
s2met__860	13.762	248.1008		unknown__mass248.1008__RT13.762
s2met__947	14.903	259.03589		unknown__mass259.03589__RT14.903
s2met__886	13.911	268.07928		unknown__mass268.07928__RT13.911
s2met__778	12.897	277.0618		unknown__mass277.0618__RT12.897
s2met__82	12.229	278.09357	HMDB0034367	gamma-Glutamylmethionine
s2met__953	15.082	283.96981		unknown__mass283.96981__RT15.082
s2met__813	13.305	287.11181		unknown__mass287.11181__RT13.305
s2met__839	13.424	294.08869		unknown__mass294.08869__RT13.424
s2met__883	13.869	309.10591		unknown__mass309.10591__RT13.869
s2met__790	13.093	312.04481		unknown__mass312.04481__RT13.093
s2met__624	10.997	328.13667		unknown__mass328.13667__RT10.997
s2met__857	13.696	331.18566		unknown__mass331.18566__RT13.696
s2met__850	13.588	335.1327		unknown__mass335.1327__RT13.588
s2met__612	10.881	350.11846		unknown__mass350.11846__RT10.881
s2met__541	8.782	393.12686		unknown__mass393.12686__RT8.782
s2met__92	13.605	398.13746	HMDB0001185	S-Adenosylmethionine (SAM-e)
s2met__973	15.541	422.07266		unknown__mass422.07266__RT15.541

[0188] In the comparative genomics analysis by LoCascio et al, specific gene clusters for HMO metabolism were found in *infantis* but not in *longum* subspecies. *Infantis* has greater genetic capacity to perform HMO metabolism reactions compared to other *Bifidobacteria*. Other Bifidos can metabolize HMOs but may do so less efficiently or require cooperation between different bacteria to perform different steps of the pathway.

[0189] The gene clusters that are more prevalent in *infantis* are also more prevalent in the TA samples (FIG. **24**). They are clusters H1 (Blon_2331-2361), H2 (Blon_0243-Blon_

0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), and Urease (Blon_0104-Blon_0115). ("Blon_----" are gene names for *_B_ifidobacterium_lon_gum.*)

TABLE 16

HMO Gene List. H5 genes (not specific to infantis) are italicized.

Ensembl Bacteria Gene ID	Blon_list	Cluster
ACJ53389	Blon_2331	H1
ACJ53390	Blon_2332	H1
ACJ53392	Blon_2334	H1
ACJ53394	Blon_2336	H1
ACJ53400, ACJ53403	Blon_2342 Blon_2345	H1
ACJ53401, ACJ53404	Blon_2343 Blon_2346	H1
ACJ53402	Blon_2344	H1
ACJ53405	Blon_2347	H1
ACJ53406	Blon_2348	H1
ACJ53408	Blon_2350	H1
ACJ53409	Blon_2351	H1
ACJ53410	Blon_2352	H1
ACJ53412	Blon_2354	H1
ACJ53413	Blon_2355	H1
ACJ53415	Blon_2357	H1
ACJ53417	Blon_2359	H1
ACJ53418	Blon_2360	H1
ACJ53419	Blon_2361	H1
ACJ51372	Blon_0243	H2
ACJ51373	Blon_0244	H2
ACJ51374	Blon_0245	H2
ACJ51376	Blon_0248	H2
ACJ51375, ACJ51545	Blon_0247 Blon_0425	H3
ACJ51544	Blon_0423	H3
ACJ51546	Blon_0426	H3
ACJ51732	Blon_0625	H4
ACJ51748	Blon_0641	H4
ACJ51749	Blon_0642	H4
ACJ51750	Blon_0643	H4
ACJ51751	Blon_0644	H4
ACJ51752	nanE Blon_0645	H4
ACJ51753	Blon_0646	H4
ACJ51754	Blon_0647	H4
ACJ51755	Blon_0648	H4
ACJ51756	Blon_0649	H4
ACJ51757	Blon_0650	H4
ACJ51758	Blon_0651	H4
ACJ53232	*Blon_2171*	*H5*
ACJ53233	*galT Blon_2172*	*H5*
ACJ53234	*Blon_2173*	*H5*
ACJ53235	*Blon_2174*	*H5*
ACJ53236	*Blon_2175*	*H5*
ACJ53237	*Blon_2176*	*H5*
ACJ53238	*Blon_2177*	*H5*
ACJ51233	Blon_0104	Urease
ACJ51234	Blon_0105	Urease
ACJ51235	Blon_0106	Urease
ACJ51236	Blon_0107	Urease
ACJ51237	Blon_0108	Urease
ACJ51238	Blon_0109	Urease
ACJ51239	Blon_0110	Urease
ACJ51240	ureC Blon_0111	Urease
ACJ51241	ureE Blon_0112 BLIJ_0113	Urease
ACJ51242	ureF Blon_0113	Urease
ACJ51243	ureG Blon_0114	Urease
ACJ51244	ureD Blon_0115	Urease

[0190] A subsequent cross-validated machine learning analysis was also used to identify metabolites and lipids associated with level of *Bifidobacterium longum* (rCLR transformed) or total *Bifidobacterium* genus (rCLR). "High" and "Low" were determined by dividing rCLR values into two quantiles around the median across all 116 profiled metagenomics samples. Only features with a confident identification were used for this analysis to improve interpretability of the results. The union of the top 25 metabolite features prioritized by elastic net and random forest for each of species-level *B. longum* or genus-level *Bifidobacterium* is given in the tables below. A blank cell for "Associated with . . . " means the feature was not prioritized by the machine learning analysis for that outcome.

TABLE 17

Stool metabolites for distinguishing high from low *B. longum* or total *Bifidobacterium*

Unique metabolite ID	Associated with *B. longum* level (rCLR)	Associated with total genus *Bifidobacterium* (rCLR)	Identification	HMDB ID	Retention Time	Molecular Weight
s2met_67	High	High	4-Hydroxyphenyllactic acid	HMDB0000755	10.097	182.0572
s2met_19	High	High	5'-S-Methyl-5'-thioadenosine	HMDB0001173	3.093	297.08963
s2met_34	High	High	Adenine	HMDB0000034	6.364	135.05405
s2met_73	High	High	Alanylalanine	HMDB0028680	11.035	160.08452
s2met_54	High	High	Cytosine	HMDB0000630	8.497	111.04364
s2met_15	High	High	Dehydrocholic acid	HMDB0304121; HMDB0000502	2.549	402.24026
s2met_82	High	High	gamma-Glutamylmethionine	HMDB0034367	12.229	278.09357
s2met_59	High	High	Indole-3-lactic acid	HMDB0000671	9.004	205.0733
s2met_91	High	High	L-Citrulline	HMDB0000904	13.141	175.09567
s2met_95	High	High	N-Acetyl-DL-glutamic acid	HMDB0001138	14.201	189.0631
s2met_81	High	High	N-Alpha-acetyllysine	HMDB0000446	12.161	188.11613
s2met_92	High	High	S-Adenosylmethionine	HMDB0001185	13.605	398.13746
s2met_112	High	High	Sugar acid 6C_RT14.576		14.576	194.04202

TABLE 17-continued

Stool metabolites for distinguishing high from low *B. longum* or total *Bifidobacterium*

Unique metabolite ID	Associated with *B. longum* level (rCLR)	Associated with total genus *Bifidobacterium* (rCLR)	Identification	HMDB ID	Retention Time	Molecular Weight
s2met_16	High	Low	Bilirubin	HMDB0000054; HMDB0240584; HMDB0000488	2.866	584.26343
s2met_29	High		2'-Deoxyadenosine	HMDB0000101	4.65	251.10196
s2met_35	High		3-Phenyllactic acid	HMDB0000748; HMDB0000779; HMDB0000563	6.435	166.06223
s2met_23	High		4-Pyridoxic acid	HMDB0000017	3.633	183.05256
s2met_85	High		5-Aminovaleric acid	HMDB0003355	12.526	117.07934
s2met_33	High		Alpha-Ketovaline	HMDB0000019	6.036	116.04641
s2met_84	High		gamma-Aminobutyric acid (GABA)	HMDB0000112	12.42	103.06379
s2met_106	High		Hexosamine		11.625	179.07951
s2met_52	High		Kynurenic acid	HMDB0000715	8.371	189.04201
s2met_51	High		Levulinic acid	HMDB0000720	8.269	116.04641
s2met_111	High		N-Acetylhexosamine_RT13.797		13.797	301.04642
s2met_38	High		N-Acetylisoleucine OR N-Acetylleucine	HMDB0061684; HMDB0011756	6.945	173.10451
s2met_83	High		N-Acetylornithine	HMDB0003357	12.375	174.10024
s2met_62	High		N-Acetylputrescine	HMDB0002064	9.423	130.11083
s2met_78	High		N-alpha-L-Acetyl-arginine	HMDB0004620	11.992	216.12238
s2met_72	High		Nicotinamide	HMDB0001406	10.972	122.0483
s2met_70	Low	High	Acetyl-beta-methylcholine	HMDB0015654	10.54	159.12605
s2met_74	Low	High	L-Tyrosine	HMDB0000158	11.225	181.07387
s2met_44	Low	Low	6-Methylquinoline	HMDB0033115	7.614	143.07361
s2met_36	Low	Low	Creatinine	HMDB0000562	6.663	113.05929
s2met_8	Low	Low	FA 12:0	HMDB0000638	1.861	200.17708
s2met_9	Low		9-HpODE	HMDB0242602	1.92	312.22994
s2met_50	Low		Acamprosate	HMDB0014797	8.228	181.0402
s2met_100	Low		DL-Malic acid	HMDB0000156; HMDB0031518	15.601	134.02061
s2met_6	Low		FA 18:2	HMDB0005048; HMDB0003797; HMDB0000673; HMDB0005047	1.612	280.2401
s2met_109	Low		Hexose		13.41	180.06272
s2met_40	Low		Lenticin	HMDB0061115	7.032	246.13695
s2met_63	Low		Xanthine	HMDB0000292	9.45	152.0326
s2met_57		High	3-Hydroxybutyric Acid	HMDB0000011; HMDB0000442	8.792	104.04638
s2met_55		High	Betaine	HMDB0000043	8.649	117.07933
s2met_43		High	Choline	HMDB0000097	7.469	103.10018
s2met_71		High	DL-Carnitine	HMDB0000062	10.615	161.10532
s2met_102		High	L-Arginine	HMDB0000517	17.771	174.11175
s2met_93		High	L-Glutamic acid	HMDB0000148	13.803	147.05264
s2met_87		High	L-Glutamine	HMDB0000641	12.691	146.06905
s2met_80		High	L-Threonine	HMDB0000167	12.125	119.05778
s2met_69		High	L-Valine	HMDB0000883	10.258	117.07933
s2met_12		High	N-(5-acetamidopentyl)acetamide		2.156	186.13699
s2met_97		High	N-Acetylaspartic acid	HMDB0000812	14.431	175.04735
s2met_103		High	N-Acetylhexosamine_RT9.787		9.787	221.08947
s2met_14		High	Palmitoylcarnitine	HMDB0000222; HMDB0240774; HMDB0240783	2.396	399.33508
s2met_110		High	Sugar acid 6C_RT13.449		13.449	194.04204
s2met_7		High	Testosterone sulfate	HMDB0002833	1.835	368.16544
s2met_25		Low	3,4-Dimethylbenzoic acid	HMDB0002237	3.914	150.06727
s2met_2		Low	FA 20:2	HMDB0061864; HMDB0005060	1.558	308.27134
s2met_79		Low	Glyceric acid	HMDB0000139; HMDB0006372	12.007	106.02564
s2met_60		Low	L-Phenylalanine	HMDB0000159	9.07	165.07874

TABLE 17-continued

Stool metabolites for distinguishing high from low *B. longum* or total *Bifidobacterium*

Unique metabolite ID	Associated with *B. longum* level (rCLR)	Associated with total genus *Bifidobacterium* (rCLR)	Identification	HMDB ID	Retention Time	Molecular Weight
s2met_65		Low	L-Tryptophan	HMDB0000929	9.941	204.08986
s2met_61		Low	Methylnicotinamide	HMDB0059711; HMDB0000699; HMDB0003152; HMDB0246826	9.392	136.06381
s2met_31		Low	Phenethylamine	HMDB0012275	5.742	121.08944
s2met_28		Low	Serotonin	HMDB0000259	4.549	176.09507

TABLE 18

Stool lipids for distinguishing high from low *B. longum* or total *Bifidobacterium*

Unique lipid ID	Top feature for *B. longum* level (rCLR)	Top feature for total genus *Bifidobacterium* (rCLR)	Identification	Lipid.Class	Retention. Time..min.	Quant.Ion	Polarity
s2lip_327	High	High	Cer[AS] d18:2_24:0	Cer[AS]	12.776	662.61029	–
s2lip_1405	High		(2E,4E,14E)-13-Hydroperoxy-N-(2-methylpropyl)icosa-2,4,14-trienamide	FAA	4.962	394.33173	+
s2lip_42	High		AC 17:1 (s2lip_42)	AC	1.814	412.34161	+
s2lip_2935	High		Arachidonic acid	FA	8.631	305.24744	+
s2lip_413	High		CE 20:4	CE	16.65	690.61957	+
s2lip_69	High		Cer[NS] d36:3 (s2lip_69)	Cer[NS]	7.48	562.51965	+
s2lip_2869	High		Docosahexaenoic acid (DHA)	FA	8.546	329.24762	+
s2lip_330	High		HexCer[NS] d18:1_24:0	HexCer[NS]	12.84	810.68359	–
s2lip_138	High		HexCer[NS] d36:3	HexCer[NS]	8.85	724.57214	+
s2lip_87	High		PC 32:2	PC	8.089	730.5387	+
s2lip_81	High		PE 36:2	PE	7.977	742.53992	–
s2lip_82	High		PG 18:0_22:6	PG	7.985	821.53497	–
s2lip_63	High		SHexCer d34:2	SHexCer	7.033	776.49957	–
s2lip_401	High		TG 20:3_22:4_22:4	TG	16.432	1026.85168	+
s2lip_36	Low	Low	LysoPE 16:0	LysoPE	1.668	452.27853	–
s2lip_166	Low	Low	PE 15:0_18:1	PE	9.198	702.50873	–
s2lip_187	Low	Low	PE 16:0_17:1	PE	9.51	702.50928	–
s2lip_204	Low	Low	PE 16:0_18:1 (s2lip_204)	PE	9.794	718.53839	+
s2lip_156	Low	Low	PE 16:0_18:2	PE	9.089	714.50909	–
s2lip_145	Low	Low	PE 32:1	PE	8.917	688.49292	–
s2lip_171	Low	Low	PE 34:2 (s2lip_171)	PE	9.256	716.52295	+
s2lip_155	Low	Low	PE 36:5	PE	9.068	738.50354	+
s2lip_94	Low	Low	PG 16:0_17:1	PG	8.251	733.50378	–
s2lip_210	Low	Low	Plasmenyl-PC P-16:0_16:0	Plasmenyl-PC	9.96	776.58173	–
s2lip_153	Low		Alkanyl-DG O-18:2_18:2 (s2lip_153)	Alkanyl-DG	9.049	603.5351	+
s2lip_194	Low		Alkanyl-DG O-34:3 (s2lip_194)	Alkanyl-DG	9.584	577.5188	+
s2lip_270	Low		Alkanyl-DG O-34:3 (s2lip_270)	Alkanyl-DG	11.264	577.51978	+
s2lip_349	Low		Cer[NS] d18:1_24:0	Cer[NS]	14.171	708.65216	–
s2lip_316	Low		Cer[NS] d40:2 (s2lip_316)	Cer[NS]	12.32	620.59821	+
s2lip_318	Low		Cer[NS] d41:2 (s2lip_318)	Cer[NS]	12.362	692.6214	–
s2lip_5944	Low		cis-12-Octadecenoic acid methyl ester	FA	12.134	297.27847	+
s2lip_1103	Low		Linoleoyl ethanolamide	FAA	2.601	324.2897	+
s2lip_8	Low		LysoPG 18:2	LysoPG	1.106	507.27301	–
s2lip_35	Low		LysoPI 18:0	LysoPI	1.62	599.32062	–
s2lip_14	Low		LysoPI 18:1	LysoPI	1.197	597.30585	–
s2lip_85	Low		PE 28:0	PE	8.005	636.45966	+
s2lip_90	Low		PE 30:1	PE	8.116	660.46185	–
s2lip_71	Low		PG 16:1_16:0	PG	7.539	719.48798	–
s2lip_61	Low		SP d18:1 (s2lip_61)	SP	3.545	300.28958	+
s2lip_27		High	AC 20:3	AC	1.479	450.3577	+

TABLE 18-continued

Stool lipids for distinguishing high from low *B. longum* or total *Bifidobacterium*

Unique lipid ID	Top feature for *B. longum* level (rCLR)	Top feature for total genus *Bifidobacterium* (rCLR)	Identification	Lipid.Class	Retention. Time..min.	Quant.Ion	Polarity
s2lip_23		High	AC 22:6 (s2lip_23)	AC	1.36	472.34222	+
s2lip_41		High	AC 22:6 (s2lip_41)	AC	1.787	472.34149	+
s2lip_105		High	Cer[NS] d34:2 (s2lip_105)	Cer[NS]	8.354	536.50433	+
s2lip_251		High	Cer[NS] d36:0 (s2lip_251)	Cer[NS]	10.655	550.55536	+
s2lip_389		High	Cer[NS] d36:1 (s2lip_389)	Cer[NS]	16.035	548.54065	+
s2lip_152		High	HexCer[NS] d18:2_18:0	HexCer[NS]	8.987	724.57458	–
s2lip_207		High	HexCer[NS] d36:1	HexCer[NS]	9.821	728.60339	+
s2lip_245		High	HexCer[NS] d40:3 (s2lip_245)	HexCer[NS]	10.516	780.63568	+
s2lip_12		High	LysoPG 16:0 (s2lip_12)	LysoPG	1.186	483.27313	–
s2lip_25		High	LysoPG 18:1	LysoPG	1.405	509.2887	–
s2lip_231		High	PC 33:0	PC	10.242	748.59351	+
s2lip_263		High	PC 35:1 (s2lip_263)	PC	10.961	774.60895	+
s2lip_79		High	PC 42:10	PC	7.863	854.5827	+
s2lip_62		High	PG 22:6_22:6	PG	6.9	865.50311	–
s2lip_89		High	PG 36:2 (s2lip_89)	PG	8.112	773.53448	–
s2lip_266		High	Plasmanyl-PC O-38:2	Plasmanyl-PC	11.152	800.65381	+
s2lip_375		High	TG 54:6 (s2lip_375)	TG	15.718	896.7702	+
s2lip_205		Low	Cer[NS] d36:2 (s2lip_205)	Cer[NS]	9.812	546.52563	+
s2lip_80		Low	Cer[NS] d36:2 (s2lip_80)	Cer[NS]	7.87	564.53558	+
s2lip_34		Low	LysoPC 20:3	LysoPC	1.608	546.3551	+
s2lip_9		Low	LysoPE 14:0	LysoPE	1.117	424.24725	–
s2lip_75		Low	PE 16:0_16:0	PE	7.793	690.5036	–
s2lip_98		Low	PE 29:0	PE	8.277	648.46161	–
s2lip_161		Low	PE 31:0	PE	9.119	678.50629	+
s2lip_160		Low	PE-NMe 30:0	PE-NMe	9.117	676.49347	–
s2lip_225		Low	PE-NMe2 17:1_17:1	PE-NMe2	10.18	742.54071	–
s2lip_114		Low	PG 18:1_18:1	PG	8.481	773.5354	–
s2lip_233		Low	Plasmenyl-PE P-34:1	Plasmenyl-PE	10.274	700.52997	–
s2lip_243		Low	SM d38:1	SM	10.501	759.63776	+

[0191] FIG. **27** illustrates correlated module of 2mo stool microbial pathway capacity and measured metabolites that are associated with *Bifidobacterium longum*-dominated microbiome and TA status. Partial correlations between microbial pathways and stool metabolites. * adjusted p<0.05. Partial Kendall tau correlations were computed between two month stool microbial metabolic pathway capacity (rows) and metabolites measured with respect to farm status (aiming to mitigate spurious correlations due to a third variable, farm status). The resulting correlation matrix was filtered to rows and columns with at least three significant correlations. Then, modules were identified by hierarchical clustering on the metabolites and manually identifying modules enriched for farm differences. This module is enriched for microbial pathways that are elevated in TA infants and metabolites that are associated with *Bifidobacterium longum* dominated microbiomes (DMM_*longum*), TA status, or exclusive breastfeeding. Identified metabolites are indicated in purple and given names on the bottom.

[0192] FIG. **28** illustrates correlated module of 2mo stool microbial pathway capacity and measured lipids that are associated with *Bifidobacterium longum*-dominated microbiome and TA status. Partial correlations between microbial pathways and stool lipids. *adjusted p<0.05. Partial Kendall tau correlations were computed between two month stool microbial metabolic pathway capacity (rows) and measured lipids with respect to farm status (aiming to mitigate spurious correlations due to a third variable, farm status). The resulting correlation matrix were filtered to rows and columns with at least three significant correlations. Modules were identified by hierarchical clustering on the lipids and manual selection for modules enriched for farm-related differences. This module is enriched for microbial pathways that are elevated in TA infants and lipids that are associated with *Bifidobacterium longum* dominated microbiomes (DMM_longum) or TA status.

[0193] FIG. **29** illustrates farm status vs. selected tryptophan pathway metabolites (MW or KW test). Top row is Metabolon data from PLASMA12 (blue) and STOOLO2 (orange). Bottom Row is STOOL02. These metabolites can be used alone or in combination with the metagenomics data to predict future respiratory disease. Datasets PLASMA00: includes WFS and WISC; PLASMA12: WFS and WISC or WISC only. STOOL02: either WFS and WISC or WISC only.

[0194] Metabolites in the tryptophan pathway, starting with L-tryptophan are higher in TA (differences in kynurenine were identified in TA plasma at birth): Tryptophan (Trp) is an essential amino acid and is also the obligatory substrate for the production of several important bioactive substances. For example, tryptophan is a substrate for the synthesis of serotonin (5-hydroxytryptpamine, 5-HT) in the brain and gut, and melatonin in the pineal gland. In vertebrates, central 5-HT plays an integrative role in the behavioral and neuroendocrine stress response. Accordingly, effects of dietary

Trp on the neuroendocrine stress response have been reported in a variety of species, spanning from teleosts to humans.

[0195] Linoleic acid (LA) is a polyunsaturated fatty acid (PUFA) precursor to the longer n-6 fatty acids commonly known as omega-6 fatty acids. An essential fatty acid, is metabolized to gamma linolenic acid (GLA), which serves as an important constituent of neuronal membrane phospholipids and also as a substrate for prostaglandin formation, seemingly important for preservation of nerve blood flow. This pathway leads to the production of 9-Hpode.

[0196] 9-Hpode Hydroxyoctadecadienoic acids (HODEs) are stable oxidation products of linoleic acid, the generation of which is increased where oxidative stress is increased, such as in diabetes. In early atherosclerosis, 13-HODE is generated in macrophages by 15-lipoxygenase-1. This enhances protective mechanisms through peroxisome proliferator-activated receptor (PPAR)-g activation leading to increased clearance of lipid and lipid-laden cells from the arterial wall. In later atherosclerosis, both 9-HODE and 13-HODE are generated nonenzymatically. At this stage, early protective mechanisms are overwhelmed and pro-inflammatory effects of 9-HODE, acting through the receptor GPR132, and increased apoptosis predominate leading to a fragile, acellular plaque. Increased HODE levels thus contribute to atherosclerosis progression and the risk of clinical events such as myocardial infarction or stroke. Better understanding of the role of HODEs may lead to new pharmacologic approaches to modulate their production or action, and therefore lessen the burden of atherosclerotic disease in high-risk patients.

[0197] FIG. **30** shows farm score vs tryptophan metabolites. Farm score is a function of number and frequency of farm animal exposures. These metabolites can be used alone or in combination with the metagenomics data to predict future respiratory disease.

[0198] The metabolites identified herein can be used alone or in combination with the metagenomics data to predict future respiratory disease, or my be employed in a prebiotic or probiotic supplement to pregnant females, infants, toddlers or children under the age of 5 years old.

[0199] FIG. **31** shows microbiome-immune PLS regression. There is a stronger correlation between these *Bifidobacterium* amplicon sequence variants (ASVs) and lipopolysaccharide (LPS) monocyte responses, but not R848 monocyte responses.

[0200] FIG. **32** shows mixed effects model. Classified WISC infants as "high", "medium", or "low" *Bifidobacterium* based on 16S. Linear mixed effects model was used to make comparisons between groups at 1 yr and 2 yr. mDC response to LPS at 2 years of age was positively associated to Bifido abundance in early life

[0201] FIG. **33**. PCA on STOOLO2 metabolomics, lipidomics. Control samples in gray.

[0202] FIG. **34** illustrates microbe-metabolomics module in network form (edges for significant partial Kendall correlations). The map shows connections between pathway (squares) and metabolites (circles). The ones with a wider outline around the circles and squares indicate they are higher in TA.

[0203] In the comparative genomics analysis by LoCascio et al, specific gene clusters for HMO metabolism were found in *infantis* but not in *longum* subspecies. *Infantis* has greater genetic capacity to perform HMO metabolism reactions compared to other *Bifidobacteria*. Other Bifidos can metabolize HMOs but may do so less efficiently or require cooperation between different bacteria to perform different steps of the pathway. The gene clusters that are more prevalent in *infantis* are also more prevalent in the TA samples (FIG. **24**). They are clusters H1 (Blon_2331-2361), H2 (Blon_0243-Blon_0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), and Urease (Blon_0104-Blon_0115).

[0204] *Infantis*-specific human milk oligosaccharide (HMO) genes include but are not limited to HMO clusters H1 (Blon_2331-2361), H2 (Blon_0243-Blon_0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), and Urease (Blon_0104-Blon_0115). That is, the gene clusters that are more prevalent in *infantis* are also more prevalent in the TA samples (as shown in FIG. **24**). They are clusters H1 (Blon_2331-2361), H2 (Blon_0243-Blon_0248), H3 (Blon_0247, Blon_0244-Blon_0248), H4 (Blon_0625; Blon_0641-Blon_0651), and Urease (Blon_0104-Blon_0115).

REFERENCES

[0205] Alfvén, T., C. Braun-Fahrländer, B. Brunekreef, E. von Mutius, J. Riedler, A. Scheynius, M. van Hage, et al. 2006. "Allergic Diseases and Atopic Sensitization in Children Related to Farming and Anthroposophic Lifestyle—the PARSIFAL Study." Allergy 61 (4): 414-21.

[0206] Arrieta, Marie-Claire, Leah T. Stiemsma, Pedro A. Dimitriu, Lisa Thorson, Shannon Russell, Sophie Yurist-Doutsch, Boris Kuzeljevic, et al. 2015. "Early Infancy Microbial and Metabolic Alterations Affect Risk of Childhood Asthma." *Science Translational Medicine* 7 (307): 307ra152.

[0207] Beghini, Francesco, Lauren J. McIver, Aitor Blanco-Míguez, Leonard Dubois, Francesco Asnicar, Sagun Maharjan, Ana Mailyan, et al. 2021. "Integrating Taxonomic, Functional, and Strain-Level Profiling of Diverse Microbial Communities with bioBakery 3." *eLife* 10 (May). https://doi.org/10.7554/eLife.65088.

[0208] Davis, Jasmine C. C., Zachery T. Lewis, Sridevi Krishnan, Robin M. Bernstein, Sophie E. Moore, Andrew M. Prentice, David A. Mills, Carlito B. Lebrilla, and Angela M. Zivkovic. 2017. "Growth and Morbidity of Gambian Infants Are Influenced by Maternal Milk Oligosaccharides and Infant Gut Microbiota." *Scientific Reports* 7 (January): 40466.

[0209] DeAngelis, Kristen M., Eoin L. Brodie, Todd Z. DeSantis, Gary L. Andersen, Steven E. Lindow, and Mary K. Firestone. 2009. "Selective Progressive Response of Soil Microbial Community to Wild Oat Roots." *The ISME Journal* 3 (2): 168-78.

[0210] Ege, Markus J., Melanie Mayer, Anne-Cécile Normand, Jon Genuneit, William O. C. M. Cookson, Charlotte Braun-Fahrländer, Dick Heederik, Renaud Piarroux, Erika von Mutius, and GABRIELA Transregio 22 Study Group. 2011. "Exposure to Environmental Microorganisms and Childhood Asthma." *The New England Journal of Medicine* 364 (8): 701-9.

[0211] Franzosa, Eric A., Lauren J. McIver, Gholamali Rahnavard, Luke R. Thompson, Melanie Schirmer, George Weingart, Karen Schwarzberg Lipson, et al. 2018. "Species-Level Functional Profiling of Metagenomes and Metatranscriptomes." *Nature Methods* 15 (11): 962-68.

[0212] Fujimura, Kei E., Alexandra R. Sitarik, Suzanne Haystad, Din L. Lin, Sophia Levan, Douglas Fadrosh, Ariane R. Panzer, et al. 2016. "Neonatal Gut Microbiota Associates with Childhood Multisensitized Atopy and T Cell Differentiation." *Nature Medicine* 22 (10): 1187-91.

[0213] Haahtela, T., T. Laatikainen, H. Alenius, P. Auvinen, N. Fyhrquist, I. Hanski, L. von Hertzen, et al. 2015. "Hunt for the Origin of Allergy—Comparing the Finnish and Russian Karelia." *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 45 (5): 891-901.

[0214] Hiippala, Kaisa, Veera Kainulainen, Maiju Suutarinen, Tuomas Heini, Jolene R. Bowers, Daniel Jasso-Selles, Darrin Lemmer, et al. 2020. "Isolation of Anti-Inflammatory and Epithelium Reinforcing *Bacteroides* and *Parabacteroides* Spp. from A Healthy Fecal Donor." *Nutrients* 12 (4). https://doi.org/10.3390/nu12040935.

[0215] Jatzlauk, G., S. Bartel, H. Heine, M. Schloter, and S. Krauss-Etschmann. 2017. "Influences of Environmental Bacteria and Their Metabolites on Allergies, Asthma, and Host Microbiota." *Allergy* 72 (12): 1859-67.

[0216] Kuhn, Max, and Hadley Wickham. 2020. "Tidymodels: A Collection of Packages for Modeling and Machine Learning Using Tidyverse Principles." https://www.tidymodels.org.

[0217] LoCascio, Riccardo G., Prerak Desai, David A. Sela, Bart Weimer, and David A.

[0218] Mills. 2010. "Broad Conservation of Milk Utilization Genes in *Bifidobacterium longum* Subsp. *infantis* as Revealed by Comparative Genomic Hybridization." *Applied and Environmental Microbiology* 76 (22): 7373-81.

[0219] Mallick, Himel, Ali Rahnavard, Lauren J. McIver, Siyuan Ma, Yancong Zhang, Long H. Nguyen, Timothy L. Tickle, et al. 2021. "Multivariable Association Discovery in Population-Scale Meta-Omics Studies." *bioRxiv.* https://doi.org/10.1101/2021.01.20.427420.

[0220] McMurdie, Paul J., and Susan Holmes. 2013. "Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data." *PloS One* 8 (4): e61217.

[0221] Milani, Christian, Sabrina Duranti, Francesca Bottacini, Eoghan Casey, Francesca Turroni, Jennifer Mahony, Clara Belzer, et al. 2017. "The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota." *Microbiology and Molecular Biology Reviews: MMBR* 81 (4). https://doi.org/10.1128/MMBR.00036-17.

[0222] Mutius, Erika von, and Donata Vercelli. 2010. "Farm Living: Effects on Childhood Asthma and Allergy." *Nature Reviews. Immunology* 10 (12): 861-68.

[0223] Nishiyama et al., *Environ. Appl. Microbiol.,* 86:e01464-020 (2020).

[0224] Riedler, J., C. Braun-Fahrländer, W. Eder, M. Schreuer, M. Waser, S. Maisch, D. Carr, et al. 2001. "Exposure to Farming in Early Life and Development of Asthma and Allergy: A Cross-Sectional Survey." *The Lancet* 358 (9288): 1129-33.

[0225] Rocha Martin, Vanesa Natalin, Clarissa Schwab, Lukasz Krych, Evelyn Voney, Annelies Geirnaert, Christian Braegger, and Christophe Lacroix. 2019. "Colonization of Cutibacterium Avidum during Infant Gut Microbiota Establishment." *FEMS Microbiology Ecology* 95 (1). https://doi.org/10.1093/femsec/fiy215.

[0226] Scholz, Matthias, Doyle V. Ward, Edoardo Pasolli, Thomas Tolio, Moreno Zolfo, Francesco Asnicar, Duy Tin Truong, Adrian Tett, Ardythe L. Morrow, and

[0227] Nicola Segata. 2016. "Strain-Level Microbial Epidemiology and Population Genomics from Shotgun Metagenomics." *Nature Methods* 13 (5): 435-38.

[0228] Segata, Nicola, Jacques Izard, Levi Waldron, Dirk Gevers, Larisa Miropolsky, Wendy S. Garrett, and Curtis Huttenhower. 2011. "Metagenomic Biomarker Discovery and Explanation." *Genome Biology* 12 (6): R60.

[0229] Segata, Nicola, Levi Waldron, Annalisa Ballarini, Vagheesh Narasimhan, Olivier Jousson, and Curtis Huttenhower. 2012. "Metagenomic Microbial Community Profiling Using Unique Clade-Specific Marker Genes." *Nature Methods* 9 (8): 811-14.

[0230] Seppo, Antti E., Kevin Bu, Madina Jumabaeva, Juilee Thakar, Rakin A. Choudhury, Chloe Yonemitsu, Lars Bode, et al. 2021. "Infant Gut Microbiome Is Enriched with *Bifidobacterium longum* Ssp. *infantis* in Old Order Mennonites with Traditional Farming Lifestyle." *Allergy, April. https://doi.org/*10.1111/all.14877.

[0231] Seroogy, Christine M., Jeffrey J. VanWormer, Brent F. Olson, Michael D. Evans, Tara Johnson, Deanna Cole, Kathrine L. Barnes, et al. 2019. "Respiratory Health, Allergies, and the Farm Environment: Design, Methods and Enrollment in the Observational Wisconsin Infant Study Cohort (WISC): A Research Proposal." *BMC Research Notes* 12 (1): 423.

[0232] Stein, Michelle M., Cara L. Hrusch, Justyna Gozdz, Catherine Igartua, Vadim Pivniouk, Sean E. Murray, Julie G. Ledford, et al. 2016. "Innate Immunity and Asthma Risk in TA and Hutterite Farm Children." *The New England Journal of Medicine* 375 (5): 411-21.

[0233] Stokholm, Jakob, Martin J. Blaser, Jonathan Thorsen, Morten A. Rasmussen,

[0234] Johannes Waage, Rebecca K. Vinding, Ann-Marie M. Schoos, et al. 2018. "Maturation of the Gut Microbiome and Risk of Asthma in Childhood." *Nature Communications* 9 (1): 141.

[0235] Tantoco, Jamee C., Jordan Elliott Bontrager, Qianqian Zhao, James DeLine, and Christine M. Seroogy. 2018. "The TA Have Decreased Asthma and Allergic Diseases Compared with Old Order Mennonites." *Annals of Allergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma, & Immunology* 121 (2): 252-53.el.

[0236] Von Ehrenstein, O. S., E. Von Mutius, S. Illi, L. Baumann, O. Bohm, and R. von Kries. 2000. "Reduced Risk of Hay Fever and Asthma among Children of Farmers." *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 30 (2): 187-93.

[0237] All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.

1. A method to detect immune health status in a human infant or child, comprising:

providing a stool sample from a human infant or child; and

determining in the sample i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of two or more of Blon_0915, Blon_2177, Blon_0625. Blon_0244, Blon_0248; Blon_0426, ureF, Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0612, Blon_2336, Blon_2344, or Blon_0650.

2. The method of claim **1** wherein a relative abundance of *Bacteroides* of 10%, of *Bifidobacterium* of <60% or of *Blautia* of >10% is indicative of an infant or child at increased risk of allergies or a relative abundance of *Bacteroides* of >8%, of *Bifidobacterium* of <65% or of *Blautia* of >2% is indicative of an infant or child at increased risk of allergies.

3. (canceled)

4. The method of claim **1** wherein a relative abundance of *Bacteroides* of <10%, of *Bifidobacterium* of >60% or of *Blautia* of <10% is indicative of an infant or child at decreased risk of allergies or *Bacteroides* of <10%, of *Bifidobacterium* of >65% or of *Blautia* of <2% is indicative of an infant or child at decreased risk of allergies.

5-6. (canceled)

7. The method of claim **1** wherein a relative abundance of *Bifidobacterium bifidum* of 10% or less, *Bifidobacterium breve* of 25% or less, *Bifidobacterium longum* of 25% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 2% is indicative of immune health in the infant or child or of *Bifidobacterium breve* of 15% or less, *Bifidobacterium longum* of 65% or greater, or of *Bifidobacterium pseudocatenulatum* of less than 3% is indicative of immune health in the infant or child.

8-9. (canceled)

10. The method of claim **1** wherein a relative abundance of *Bifidobacterium bifidum* of less than 5%, *Bifidobacterium breve* of greater than 20?% *Bifidobacterium longum* of less than 50%, or of *Bifidobacterium pseudocatenulatum* of greater than 2% is indicative of impaired immune health in the infant or child or of *Bifidobacterium breve* of greater than 15%, *Bifidobacterium longum* of less than 30%, or of *Bifidobacterium pseudocatenulatum* of greater than 3% is indicative of impaired immune health in the infant or child.

11. The method of claim **1** wherein an increase in the relative abundance of expression of two or more of Blon_0915, Blon_2171, Blon_2173, Blon_2334, galT Blon_2172, Blon_0244, Blon_0248; Blon_0426, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650 is indicative of immune health in the infant or child.

12. (canceled)

13. The method of claim **1** wherein the sample is from a newborn to a 3 month old infant.

14. The method of claim **1** wherein the sample is from a 3 month old to a 9 month old infant.

15. The method of claim **1** wherein the sample is from an infant or child treated with a drug.

16. (canceled)

17. The method of claim **1** wherein the infant or child has necrotizing enterocolitis.

18. The method of claim **1** further comprising administering to the mother of the infant or child, or a pregnant mother a prebiotic or a probiotic.

19. The method of claim **18** wherein the prebiotic or probiotic comprises one or more bacteria, one or more antibodies, or one or more molecules that enhance the relative abundance of *Bifidobacterium longum.*

20-21. (canceled)

22. The method of claim **1** wherein the sample is analyzed using a nucleic acid amplification reaction.

23. The method of claim **1** wherein the sample is analyzed using genome sequencing.

24. A method to identify a human infant or child at higher risk of developing allergies as an adolescent or adult, comprising:

providing a stool sample from a human infant or child; and

determining in the sample i) the relative abundance of bacteria including two or more of *Bacteroides, Bifidobacterium,* or *Blautia,* ii) the relative abundance of bacteria including two or more of *Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium longum,* or *Bifidobacterium pseudocatenulatum,* or iii) the relative abundance or expression of two or more of Blon_0915, Blon_2177, Blon_0625, Blon_0244, Blon_0248; Blon_0=126, ureF Blon_0113, ureC Blon_0111, ureE Blon_0112 BLIJ_0113, Blon_0642, Blon_2336, Blon_2344, or Blon_0650.

25. The method of claim **24** further comprising administering to the infant or child at higher risk of developing allergies a composition comprising one or more prebiotics or one or more probiotics comprising *Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium breve,* and/or *Bifidobacterium bifidum,* or combinations thereof.

26-30. (canceled)

31. A method to enhance immune health comprising administering to a pregnant female, infant or child having or at risk of compromised immune health, an effective amount of a composition comprising i) a plurality of: one or more B vitamins, one or more short chain fatty acids, linoleic said, linolenic acid, tryptophan, one or more tryptophan metabolites, indole-3-methylacetate, or one or more hydroxyoctadecadienoic acids, or combinations thereof, or ii) one or more isolated *Bifidobacteria* or one or more isolated bacteria genetically modified to overexpress human breast milk oligosaccharide metabolizing enzymes, or modified with galT, ureF, ureC or ureE genes.

32. The method of claim **31** wherein the composition is orally administered.

33. The method of claim **31** wherein the composition for the infant is baby formula.

34. (canceled)

35. The method of claim **31** wherein the pregnant female, infant or child is determined to have or be at risk of compromised immune health using the method of claim **1**.

* * * * *