

# Metagenome Analysis of Preterm Birth

Jaewoong Lee    Semin Lee

Department of Biomedical Engineering  
Ulsan National Institute of Science and Technology

*jwlee230@unist.ac.kr*

2023-01-20

# Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

6 References

# 1. Introduction

# Microbiome

- Microbiota: the microorganisms which live inside & on humans (Turnbaugh et al., 2007)
- Microbiome:  $10^{13}$  to  $10^{14}$  microorganisms whose collective genome (Gill et al., 2006)



Figure: Concept of a core human microbiome (Turnbaugh et al., 2007)

# rRNA

- Ribosomal RNA
- Well-known as a key to phylogeny (Olsen & Woese, 1993)

# Preterm Birth (PTB)

PTB:

- ① PTB  $< 37$  GW (Gestational week)
- ② Normal  $\geq 37$  GW

Detailed PTB:

- ① Early PTB  $< 34$  GW
- ②  $34$  GW  $\leq$  Late PTB  $< 37$  GW
- ③ Normal  $\geq 37$  GW

(J. Tucker & McGuire, 2004; Voronkov, Solonovych, Liashenko, & Revenko, 2018)

## 2. Materials

# 16S rRNA Sequencing

**16S rRNA sequencing** is the *reference method* for bacterial taxonomy & identification (Mignard & Flandrois, 2006)

Three main reasons (Janda & Abbott, 2007):

- 16S rRNA exists in almost all bacteria
- Functions of the 16S rRNA has not changed over evolution.
- 16S rRNA is large enough for bioinformatics

# Data Composition I

## Data composition

50 pregnant women & 59 newborns

### PTB

- Mother ⇒ Early PTB: 17 & Late PTB: 22 & Normal: 29
- Newborn ⇒ Early PTB: 13 & Late PTB: 24 & Normal: 22

# Data Composition II

Table: Clinical characteristics of mothers

Clinical	<34 GW (n=11)	≥34 GW (n=39)	p-value	Remarks
Cholesterol	273.4±37.5	285.3±63.4	0.667	
DBP	84.3±18.1	80.1±11.1	0.863	
Glucose	105.4±38.4	87.4±18.3	0.228	
HDL	82.2±20.6	84.1±26.5	0.957	
Hb	11.8±1.0	11.8±1.5	0.916	
Hct	35.1±2.5	35.0±3.8	0.833	
LDL	162.8±35.0	143.7±33.7	0.345	
Mother Age	31.5±4.8	33.4±4.5	0.264	
SBP	144.2±33.0	132.8±16.9	0.541	
Weight gain	6.7±3.7	12.3±5.8	0.002	*
Advanced maternal age	3 (27.3%)	12 (30.8%)	1.000	
C-section	6 (54.5%)	33 (84.6%)	0.048	*
Gestational Diabetes	0 (0.0%)	3 (7.7%)	1.000	
Hypertension	3 (27.3%)	10 (25.6%)	1.000	
Mother Antibiotics	5 (45.5%)	8 (20.5%)	0.126	
Mother Steroid	11 (100.0%)	1 (2.6%)	0.000	*
Obesity	3 (27.3%)	9 (23.1%)	1.000	
PROM	5 (45.5%)	7 (17.9%)	0.105	
Preterm Labor	4 (36.4%)	8 (20.5%)	0.424	
Too much weight gain	0 (0.0%)	7 (17.9%)	0.324	

# Data Composition III

Table: Clinical characteristics of newborns

Clinical	<34 GW (n=13)	≥34 GW (n=46)	p-value	Remarks
Apgar Score	7.7±0.7	9.5±0.8	0.000	*
Gestational Week	30.1±2.7	36.5±1.7	0.000	*
Hospitalized Day	25.5±15.2	8.4±6.3	0.003	*
Weight	1505.1±407.3	2795.4±476.9	0.000	*
CPAP	7 (53.8%)	5 (10.9%)	0.002	*
Dyspnea	11 (84.6%)	6 (13.0%)	0.000	*
Neonate Antibiotics	8 (61.5%)	8 (17.4%)	0.003	*
PROM	7 (53.8%)	7 (15.2%)	0.008	*
Respirator	8 (61.5%)	1 (2.2%)	0.000	*
Sepsis	4 (30.8%)	6 (13.0%)	0.204	

## Statistical tests

- Continuous: M.W.W.
- Categorical: Fisher exact

### 3. Methods

# QIIME 2 Workflow

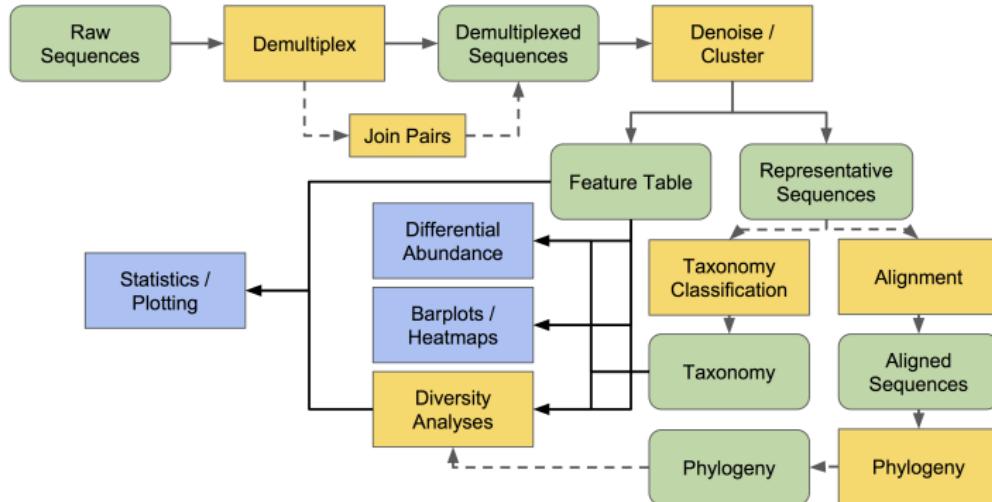


Figure: QIIME 2 workflow (Bolyen et al., 2019; Mandal et al., 2015; McDonald et al., 2012)

## 4. Results

## 4. Results

### 4.1. Taxonomy Overview

# Proportion Distribution

## Proportion

- Minimum: 0.0
- Mean: 0.00008
- Median: 0.0
- Maximum: 0.793

## Proportion without Zero

- Minimum: 0.00002
- Mean: 0.00008
- Median: 0.00153
- Maximum: 0.793

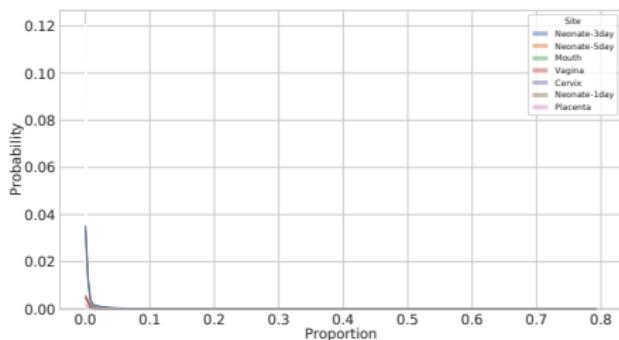


Figure: Proportion distribution

# Microbial community with Proportion

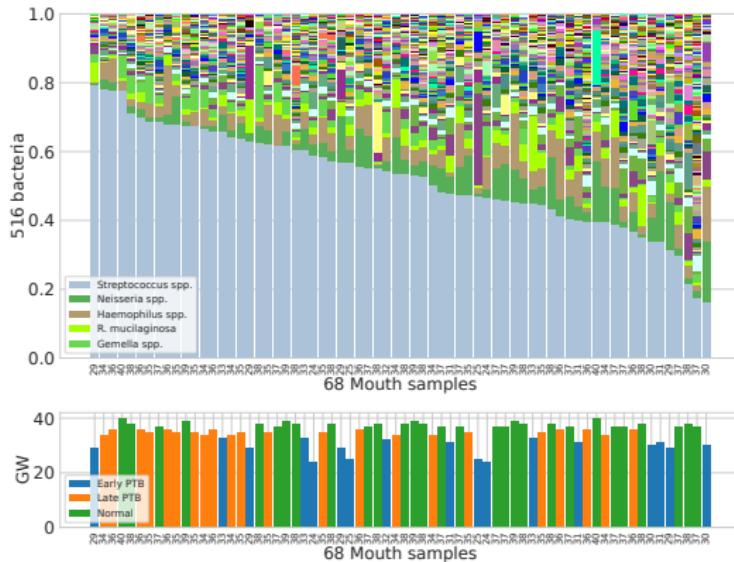
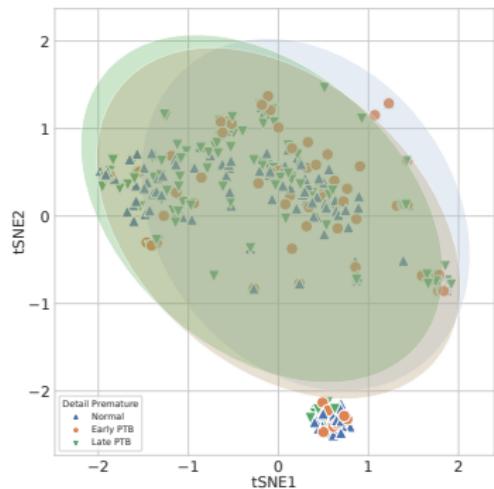
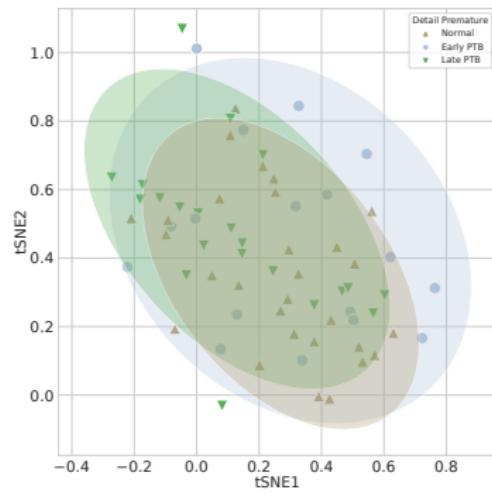


Figure: Microbial community with Proportion

# t-SNE with Proportion



(a) All



(b) Mother Mouth

Figure: t-SNE plot of Proportion with PTB

# Notable Taxa I

## *Streptococcus* spp.

- *S. mutans*: pathogen of dental caries
- Membrane vesicles of Group B *Streptococcus* disrupt feto-maternal barrier (Surve et al., 2016).  
∴ Leading to PTB

## *Neisseria* spp.

- *N.* colonize the mucosal surfaces of many animals.
- Only two pathogens in 11 known species
  - *N. meningitidis*: Meningitis & Sepsis
  - *N. gonorrhoeae*: Gonorrhea (sexual transmitted disease)
- *N. gonorrhoeae* results adverse pregnancy outcomes (Heumann, Quilter, Eastment, Heffron, & Hawes, 2017).

## Notable Taxa II

### *Haemophilus* spp.

- *H.* inhabit the mucous membranes of upper respiratory tract, mouth, vagina, & intestinal tract.
- *H. influenzae*: A major cause of systemic infection
- PTB caused by *H. influenzae* (Hills et al., 2022) and *H. parainfluenzae* (Mendz, Petersen, Quinlivan, & Kaakoush, 2014).

### *R. mucilaginosa*

- *Rhodotorula mucilaginosa*
- *R.* is a genus of pigmented yeasts.
- Catheter-related bloodstream infection due to *R. mucilaginosa* (Kitazawa, Ishigaki, Seo, Yoshino, & Ota, 2018).
- ∴ *Rhodotorula* bloodstream infections

## *Gemella* spp.

- *G. bacteria* are primarily found in mucous membranes of human e.g. oral cavity & upper digestive tract.
- *G. haemolysans* causes endocarditis (Mosquera, Zabalza, Laniero, & Blanco, 2000).
- Women who have PTB also have significant lower level of *G.* (Li et al., 2021).

## 4. Results

### 4.2. Diversity Index

# Diversity Index

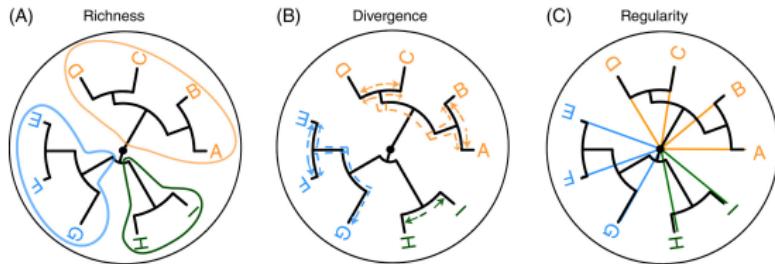


Figure: Three dimensions of phylogenetic information (C. M. Tucker et al., 2017)

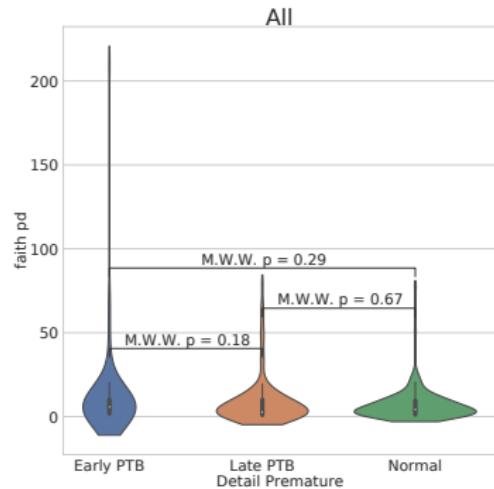
- A quantitative measure that shows richness, divergence, and regularity (C. M. Tucker et al., 2017)
- Alpha diversity: the richness of taxa **at a single community**
- Beta diversity: taxonomy differentiation **between communities**

## 4. Results

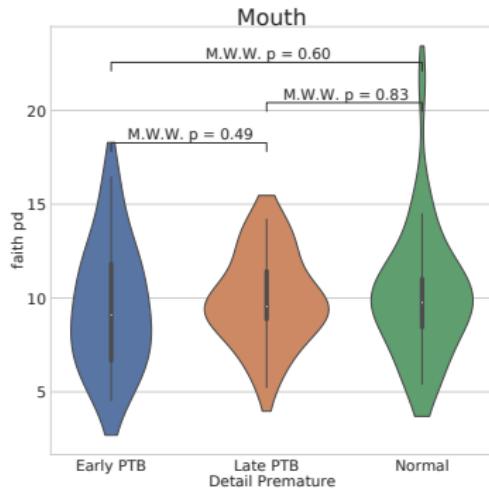
### 4.2. Diversity Index

#### 4.2.1. Alpha-diversity

# Violin Plot with Alpha-diversity I



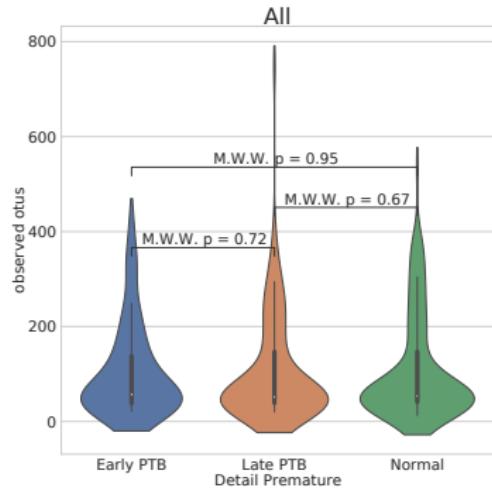
(a) All



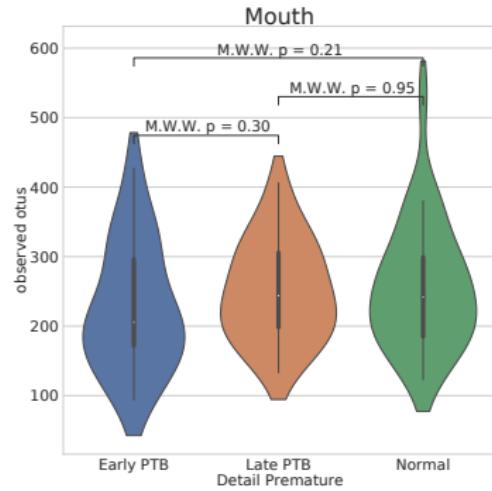
(b) Mother Mouth

Figure: Detail premature & Faith's PD

# Violin Plot with Alpha-diversity II



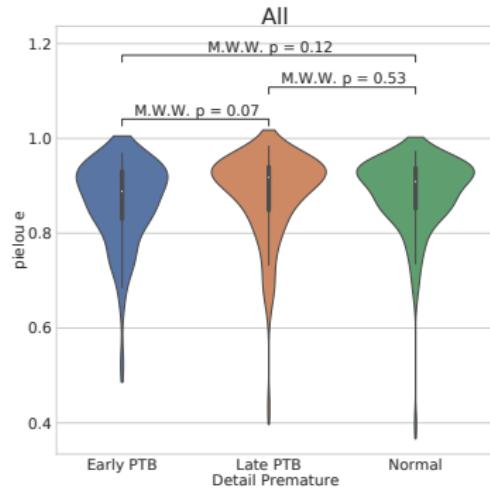
(a) All



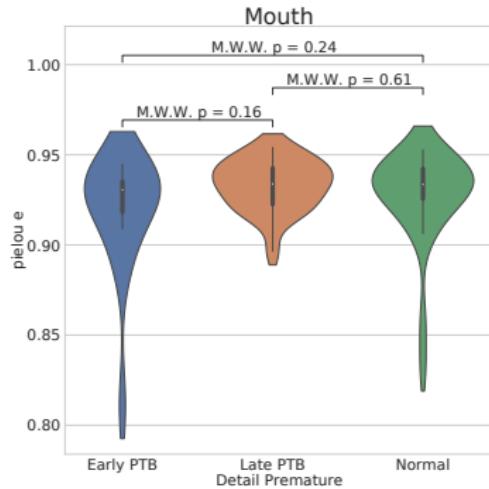
(b) Mother Mouth

Figure: Detail premature & Observed OTUs

# Violin Plot with Alpha-diversity III



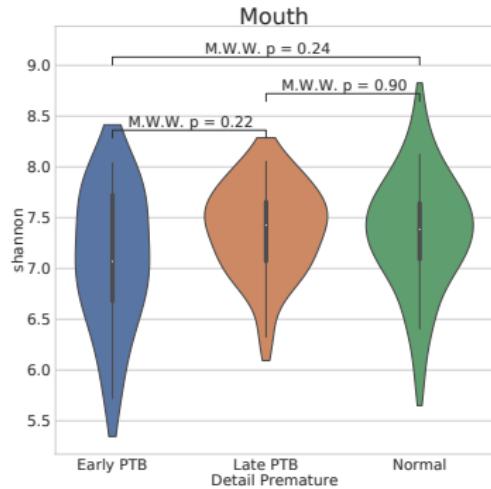
(a) All



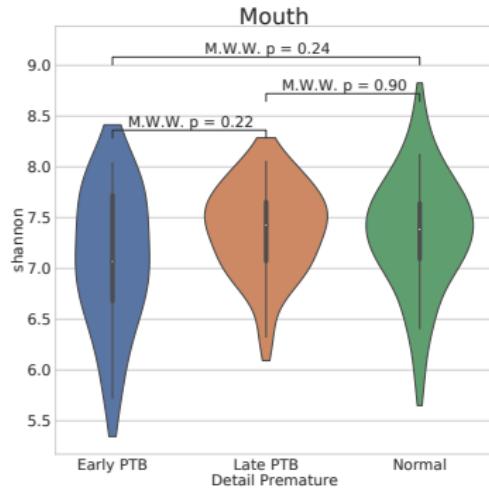
(b) Mother Mouth

Figure: Detail premature & Pielou Evenness

# Violin Plot with Alpha-diversity IV



(a) All



(b) Mother Mouth

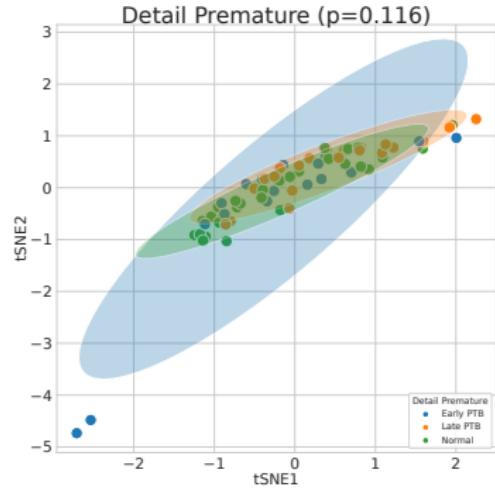
Figure: Detail premature & Shannon Entropy

## 4. Results

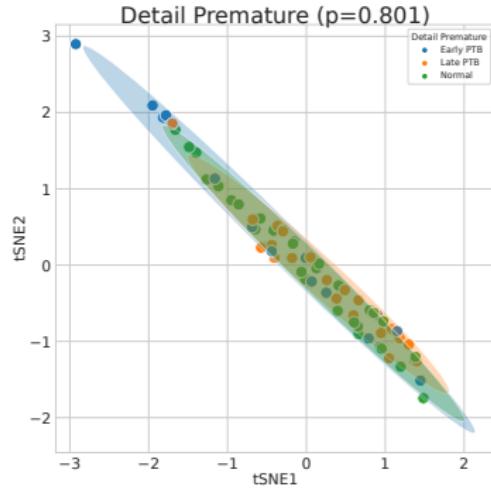
### 4.2. Diversity Index

#### 4.2.2. Beta-diversity

# Beta-diversity t-SNE plots I



(a) Bray-Curtis



(b) Hamming

Figure: Beta-diversity t-SNE plots

## 4. Results

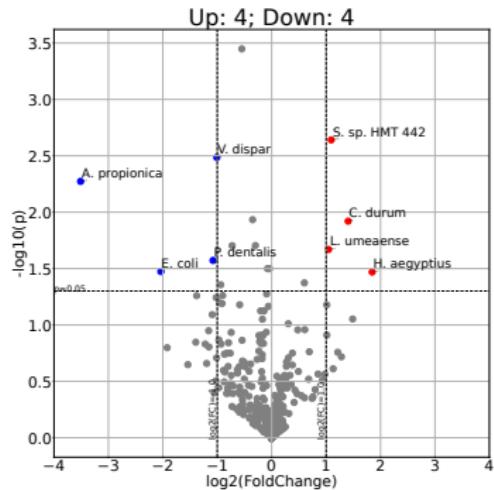
### 4.3. Taxonomy Analyses

## 4. Results

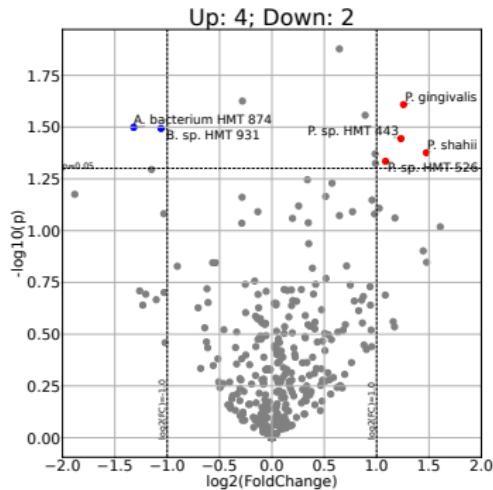
### 4.3. Taxonomy Analyses

#### 4.3.1. Differentially Abundant Taxa

# Volcano plots I



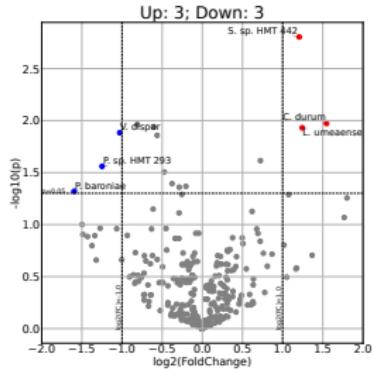
(a) Early PTB vs. other



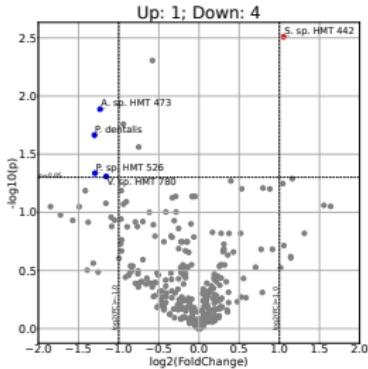
(b) PTB vs. Normal

Figure: DAT in Mouth

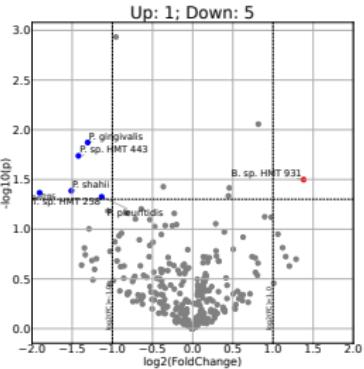
# Volcano plots II



(a) Early vs. Late



(b) Early vs. Normal



(c) Late vs. Normal

Figure: DAT in Mouth

# Venn Diagrams

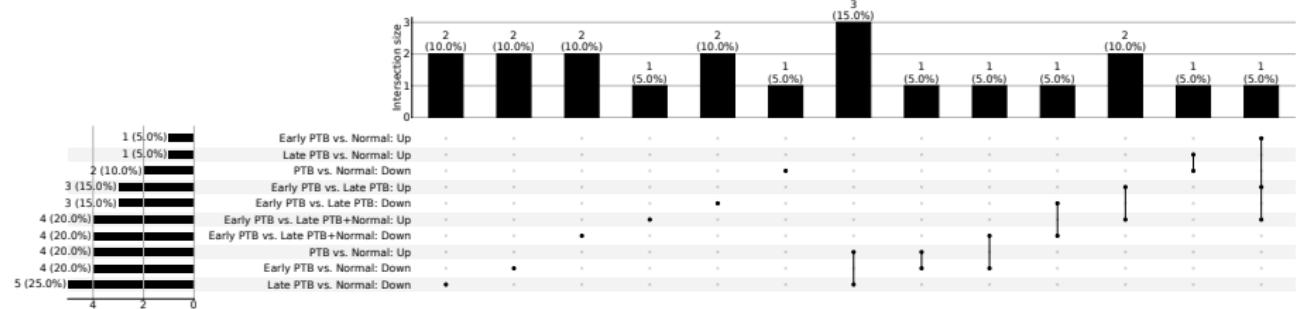
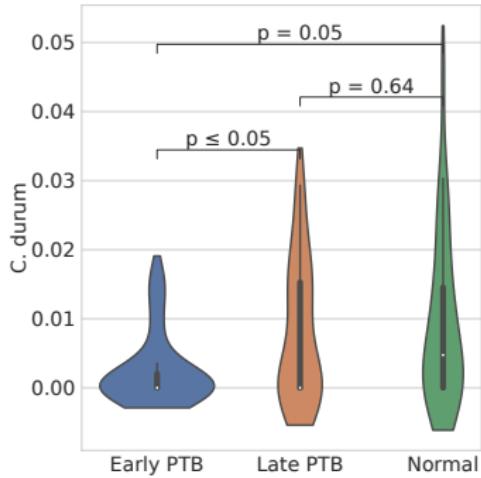


Figure: DAT Upset plot

## Union of DAT

A total of 20 taxa selected as DAT.

# Violin Plots I

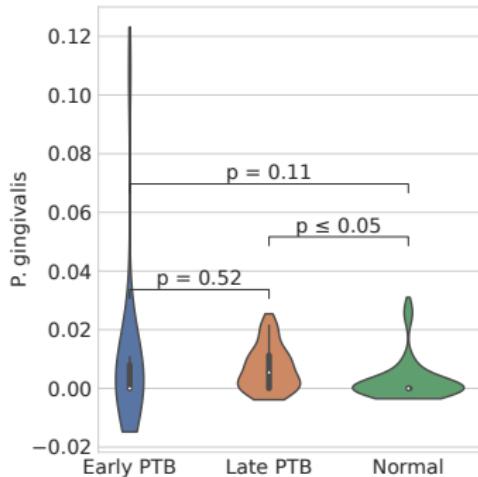


## *C. durum*

- *Corynebacterium durum*
- Poly-microbial interactions  
⇒ Oral mucosal & Gingival cells  
(Redanz et al., 2021).
- Synergism between *C.* & *Streptococcus* in oral commensals (Treerat et al., 2020).

Figure: *C. durum*

# Violin Plots II



## *P. gingivalis*

- *Porphyromonas gingivalis*
- *P. gingivalis* is well-known periodontitis pathogen.
- *P. gingivalis* is associated with bacterial vaginosis (Africa, Nel, & Stemmet, 2014).
- *P. gingivalis* ⇒ Adverse pregnancy outcomes (Miyauchi et al., 2018).

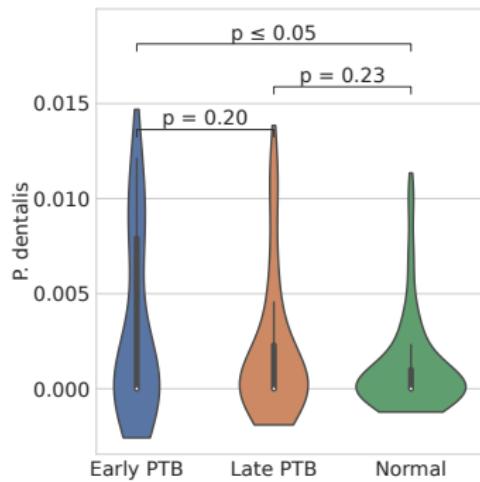
Figure: *P. gingivalis*

# Violin Plots III

## *Prevotella* spp.

- *P.* interactions with human health (Tett, Pasolli, Masetti, Ercolini, & Segata, 2021).
- *P.* spp. might have different role in PTB (Freitas, Bocking, Hill, & Money, 2018).
- *P.* spp. are associated with human infections (Sharma, Garg, Hasan, & Shirodkar, 2022).

# Violin Plots IV



## *P. dentalis*

- *Prevotella dentalis*
- *P. dentalis* is observed as colorectal cancer-promoting bacterium (Shi et al., 2022).

Figure: *P. dentalis*

# Violin Plots V

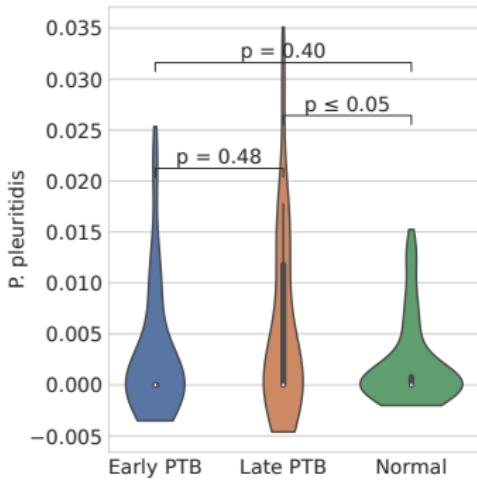


Figure: *P. pleuritidis*

## *P. pleuritidis*

- *Prevotella pleuritidis*
- *P. pleuritidis* causes lung abscess (Asif, Roy, & Ahmad, 2020).
- *P. pleuritidis* is abundant in oral microbiome of smokers (Al Bataineh et al., 2020).
- *P. pleuritidis* associated with gastric cancer (Yang et al., 2022).

## 4. Results

### 4.4. Machine Learning

# ML algorithm comparison

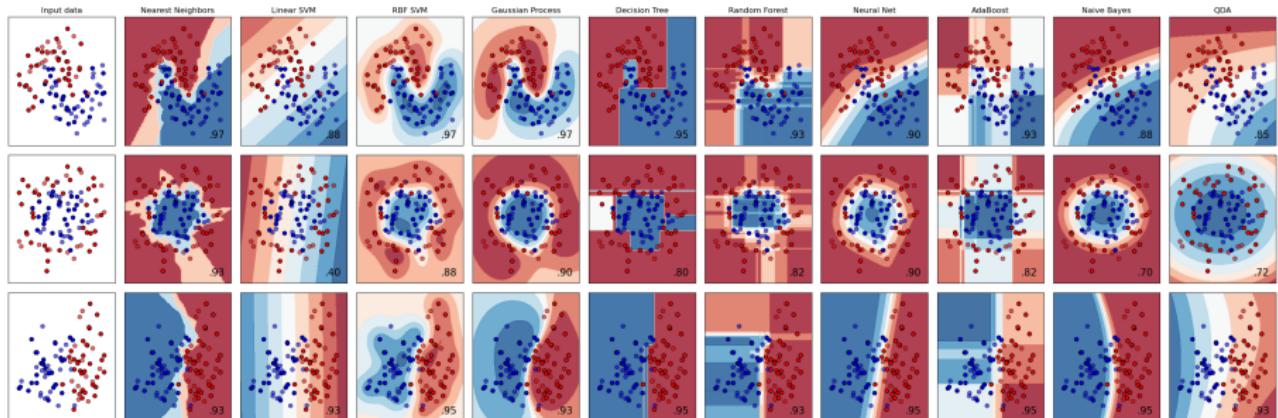


Figure: Classification Comparison (Pedregosa et al., 2011)

# Oversampling

## SMOTE (Chawla, Bowyer, Hall, & Kegelmeyer, 2002)

- Synthetic Minority Oversampling Technique
- An algorithm that makes pseudo-sample
- Using  $K$ -Nearest Neighbor algorithm

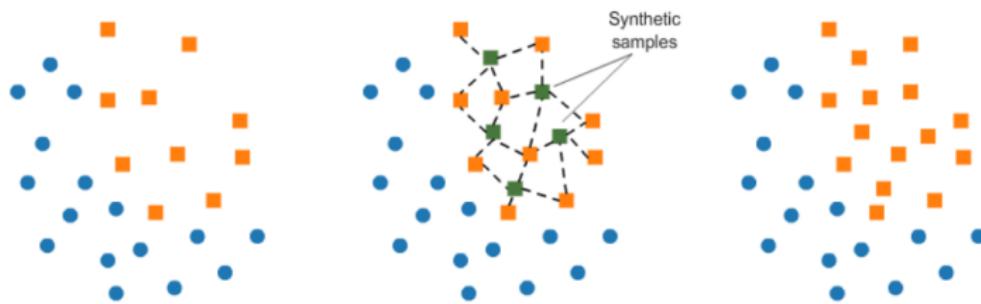


Figure: Workflow of SMOTE

## 4. Results

### 4.4. Machine Learning

#### 4.4.1. Random Forest Classifier on Proportion

# Random Forest with (Early vs. Late vs. Normal) I

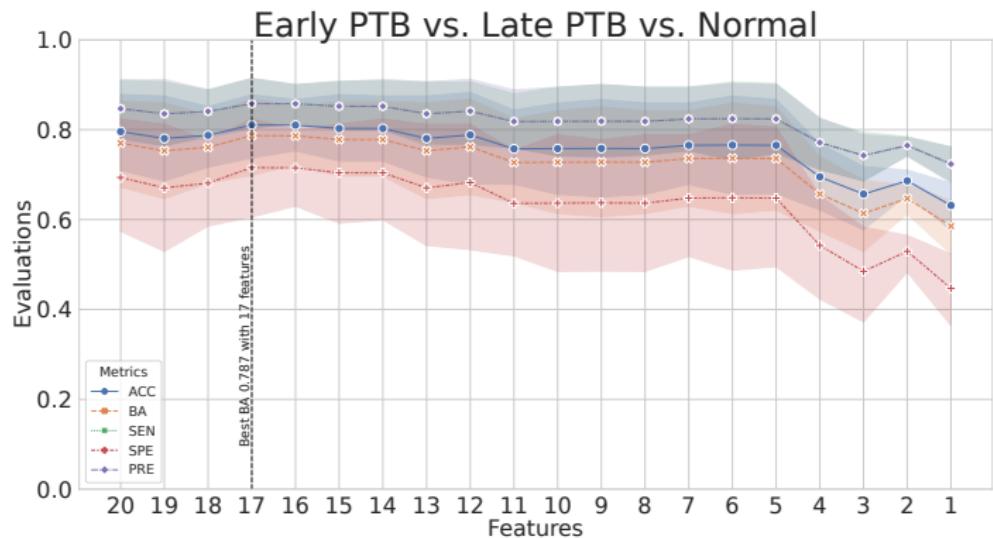


Figure: RF evaluations with feature counts

# Random Forest with (Early vs. Late vs. Normal) II

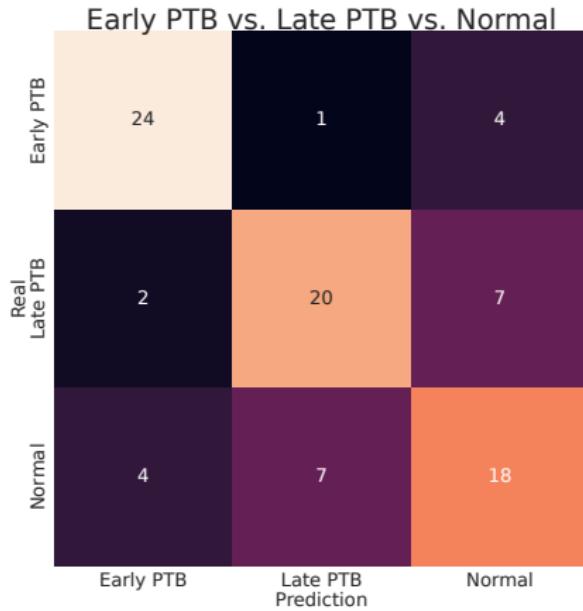


Figure: RF confusion matrix

# Random Forest with (Early vs. Late vs. Normal) III

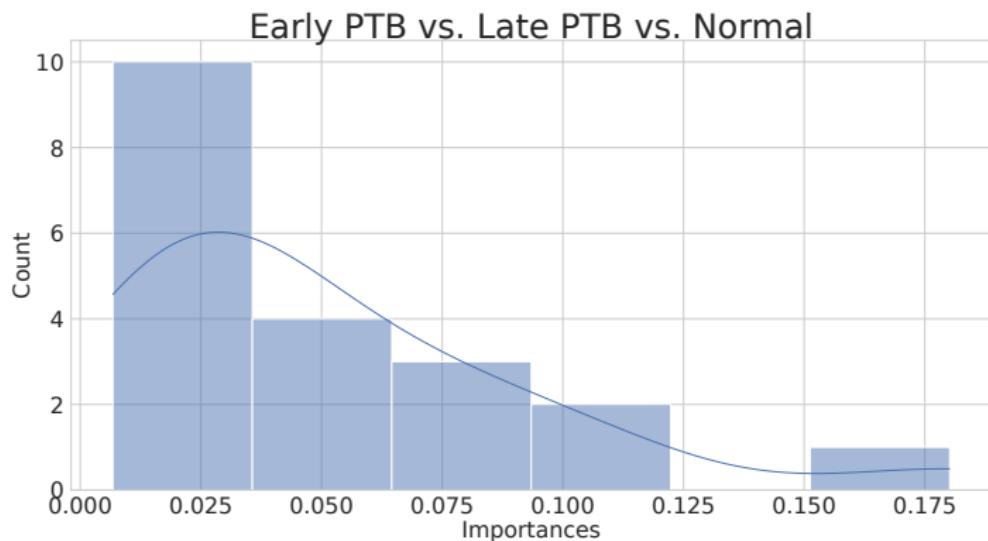


Figure: RF importances

## Highest Importances

- ① *L. umeaense*
- ② *S. sp. HMT 442*
- ③ *V. dispar*

# Random Forest with (Early vs. Late + Normal) I

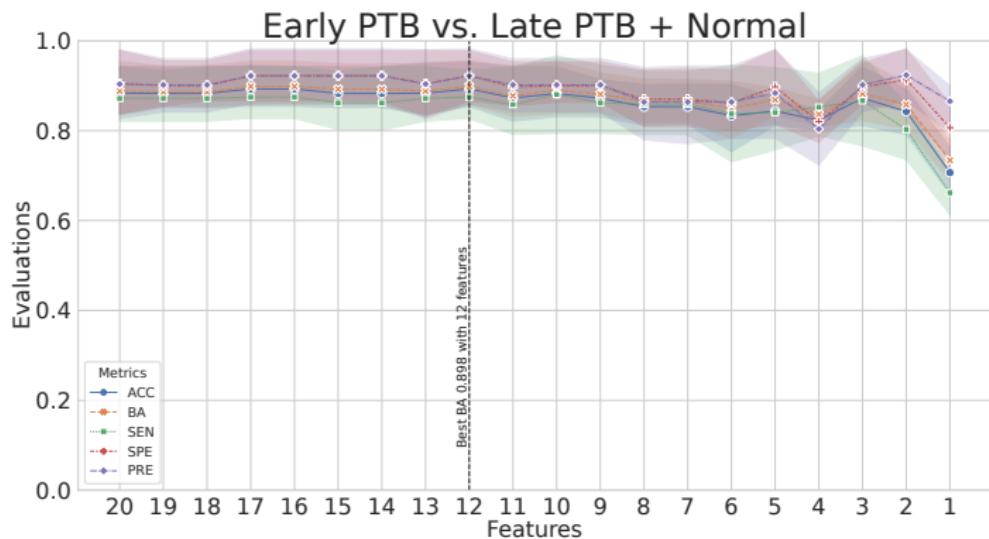


Figure: RF evaluations with feature counts

# Random Forest with (Early vs. Late + Normal) II

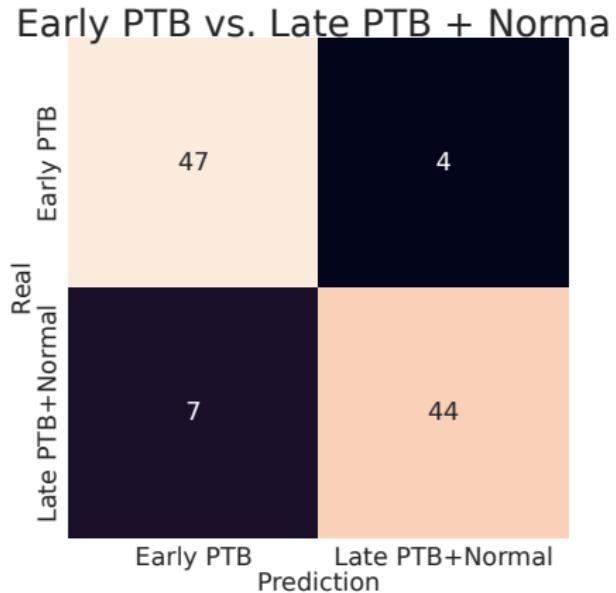


Figure: RF confusion matrix

# Random Forest with (Early vs. Late + Normal) III

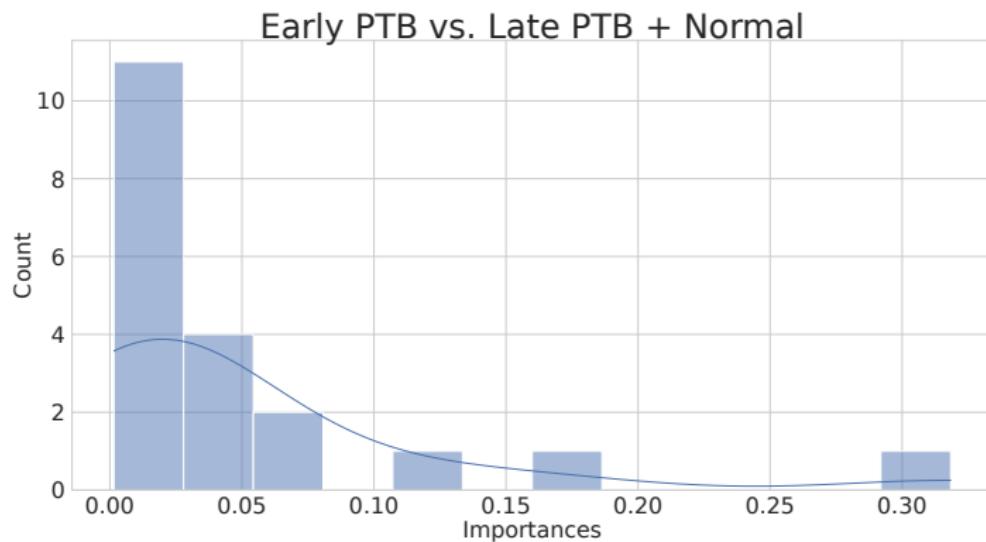


Figure: RF importances

## Highest Importances

- ① *L. umeaense*
- ② *S. sp. HMT 442*
- ③ *C. durum*

# Random Forest with (PTB vs. Normal) I

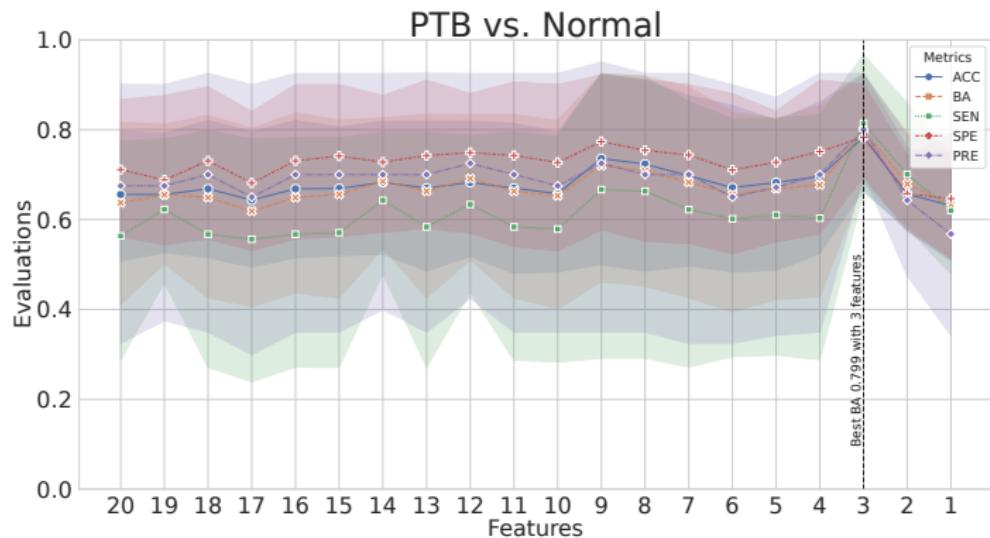


Figure: RF evaluations with feature counts

# Random Forest with (PTB vs. Normal) II

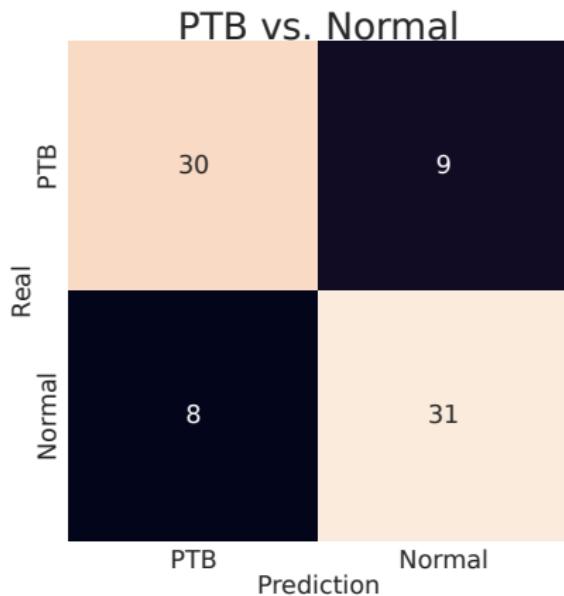


Figure: RF confusion matrix

# Random Forest with (PTB vs. Normal) III

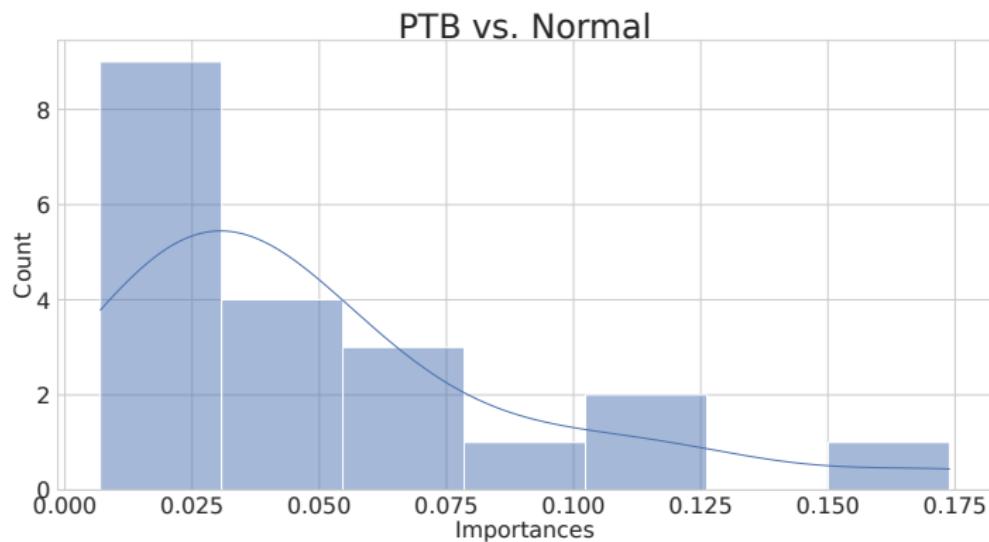


Figure: RF importances

## Highest Importances

- ① *L. umeaense*
- ② *P. gingivalis*
- ③ *B. sp. HMT 931*

## 4. Results

### 4.5. Pathway Enrichment Prediction

# PICRUSt2

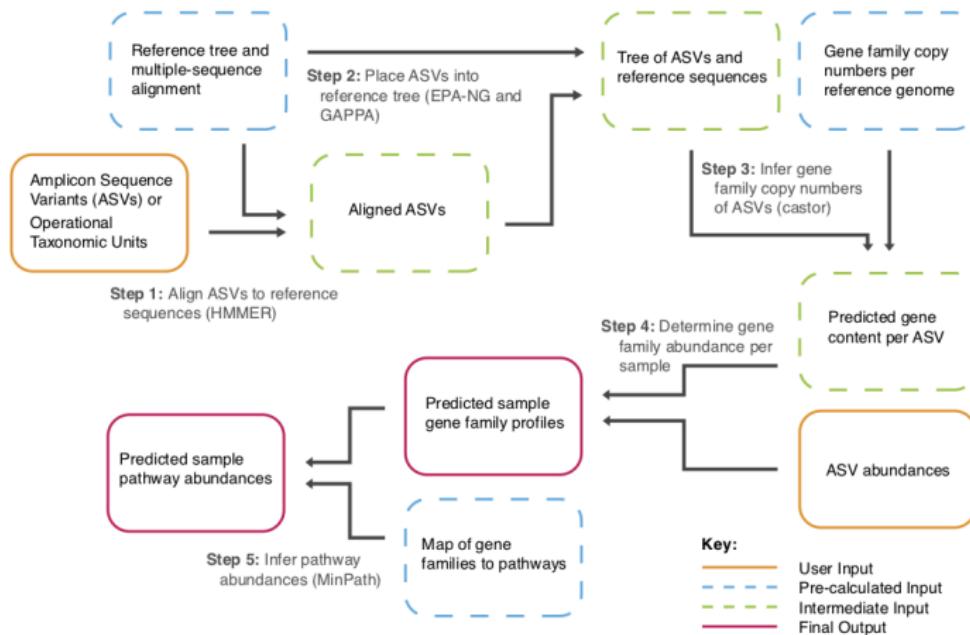


Figure: PICRUSt2 flowchart (Douglas et al., 2020)

# Pathway Predictions: Early PTB vs. Late PTB I

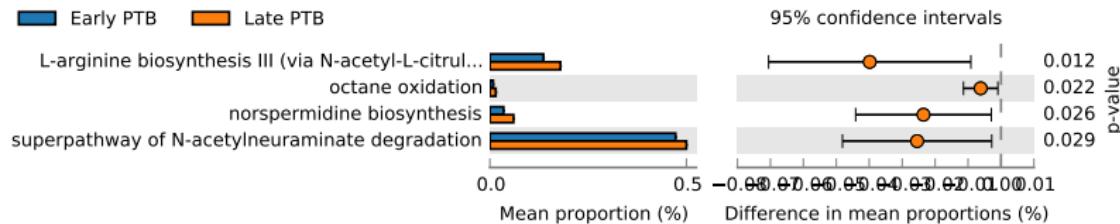


Figure: Early PTB vs. Late PTB

# Pathway Predictions: Early PTB vs. Late PTB II

## L-arginine biosynthesis III (via N-acetyl-L-citrulline) (PWY-5154)

- PWY-5154 is enriched in late PTB than early PTB.
- PWY-5154 is enriched in normal than early PTB.
- PWY-5154 is enriched in other than early PTB.
- PWY-5154 is increased with age in rats (Yang, Chen, Zhang, & Wang, 2021).
- Arginine deficiency ⇒ PTB (SNYDERMAN, HOLT JR, NORTON, & PHANSALKAR, 1970; Zamora et al., 1997; Wu, Jaeger, Bazer, & Rhoads, 2004).

## Octane oxidation (P221-PWY)

- P221-PWY is enriched in late PTB than early PTB.
- P221-PWY is enriched in normal than early PTB.
- P221-PWY is enriched in other than early PTB.
- P221-PWY is important for classifying the PTB (Deutsch et al., 2022):  
higher at PTB than normal ?

## Norspermidine biosynthesis (PWY-6562)

- PWY-6562 is enriched in late PTB than early PTB.
- PWY-6562 is enriched in other than early PTB.
- Norspermidine has antitumor activity via regulating DNA (Nishio et al., 2019).
- Norspermidine is used as a biofilm disassembly trigger (Böttcher, Kolodkin-Gal, Kolter, Losick, & Clardy, 2013).

## Superpathway of N-acetylneuraminate degradation (P441-PWY)

- P441-PWY is enriched in late PTB than early PTB.
- P441-PWY is enriched in late PTB than normal.
- NPL gene ⇒ N-acetylneuraminate ↑  
∴ Viral pathogenicity ↑ (Bomba et al., 2022).
- N-acetylneuraminate level increased at 24-28 GW in PTB women  
(Ghartey, Bastek, Brown, Anglim, & Elovitz, 2015) ??

# Pathway Predictions: Early PTB vs. Normal I

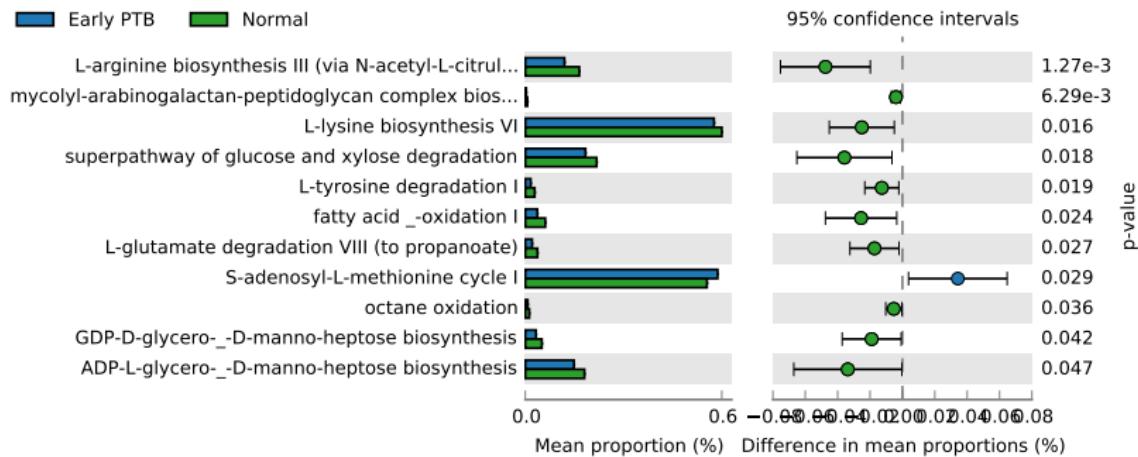


Figure: Early PTB vs. Normal

# Pathway Predictions: Early PTB vs. Normal II

## S-adenosyl-L-methionine cycle (PWY-6151)

- PWY-6151 is enriched in early PTB than normal.
- PWY-6151 is enriched in early PTB than other.

## L-lysine biosynthesis VI (PWY-5097)

- PWY-5097 is enriched in normal than early PTB.
- PWY-5097 is enriched in normal than late PTB.
- PWY-5097 negatively associated with severity of multiple sclerosis (Zhou et al., 2022).
- Lysine level in serum was higher in term deliveries with PTB symptoms than without PTB symptoms (Lizewska et al., 2018).
- Lysine level in the first trimester are associated with adverse birth outcomes in Rhea cohort (Maitre et al., 2014).

# Pathway Predictions: Early PTB vs. Normal III

## Superpathway of glucose and xylose degradation (PWY-6901)

- PWY-6901 is enriched in normal than early PTB.
- PWY-6901 is enriched in other than early PTB.
- PWY-6901 associated with *zonulin* level (Kaczmarczyk et al., 2021)  
   $\Leftarrow$  a marker of small intestinal paracellular permeability  
∴ Development of immunity during early life.

## L-tyrosine degradation I (TYRFUMCAT-PWY)

- TYRFUMCAT-PWY is enriched in normal than early PTB.
- Oral antibiotics ⇒ microbiome alteration  
∴ Promote oral squamous cell carcinoma (Wei et al., 2022).
- TYRFUMCAT-PWY was depleted in moderate & high activity rheumatoid arthritis patients (Ruiz-Limón et al., 2022).
- A tyrosine-related gene mutation impresses to PTB (Gómez et al., 2010).  
∴ Alteration in inflammation response

## Fatty acid $\beta$ -oxidation I (FAO-PWY)

- FAO-PWY is enriched in normal than early PTB.
- FAO-PWY is enriched in other than early PTB.
- FAO-PWY is associated with fecal calprotectin level and IL12 (Davrandi, Harris, Smith, Murray, & Lowe, 2022).
- $\Omega$ -3 fatty acid  $\Rightarrow$  Prevent recurrent PTB (Harper et al., 2010).

## L-glutamate degradation VIII (to propanoate) (PWY-5088)

- PWY-5088 is enriched in normal than early PTB.
- PWY-5088 is enriched in other than early PTB.
- PWY-5088 is enriched in gut microbiome of Parkinson's disease (Wallen et al., 2022).
- GABA & glutamate play a major role in fetal neonatal brain development (Basu, Pradhan, du Plessis, Ben-Ari, & Limperopoulos, 2021).

# Pathway Predictions: Early PTB vs. Other I

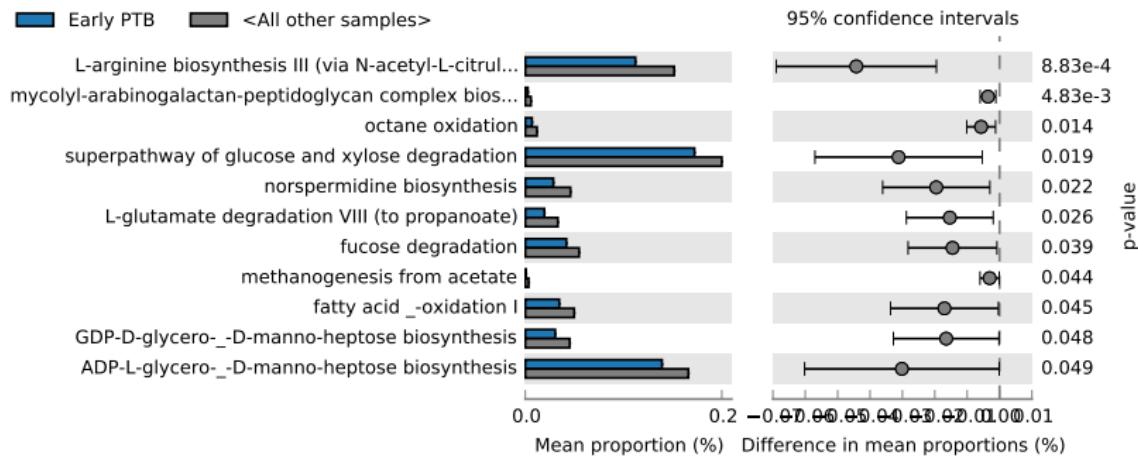


Figure: Early PTB vs. Late PTB + Normal

## Fucose degradation (FUCCAT-PWY)

- FUCCAT-PWY is enriched in other than early PTB.
- Fucose have a linkage with intestinal health & disease (Garber, Hennet, & Szymanski, 2021).
- FUCCAT-PWY is enriched in upper respiratory microbiome of pregnant women (Solazzo et al., 2022).
- Porous cervical mucus plug ⇒ Vaginal infection ⇒ Fucose pathway  
∴ PTB in mice (Lacroix, Gouyer, Rocher, Gottrand, & Desseyn, 2022).

## Methanogenesis from acetate (METH-ACETATE-PWY)

- METH-ACETATE-PWY is enriched in other than early PTB.
- METH-ACETATE-PWY is enriched in upper respiratory microbiome of pregnant women (Solazzo et al., 2022).
- Methanogenesis have a major role in preventing acid accumulation (Abou Chacra & Fenollar, 2021).

## 5. Discussion

## 6. References

## References |

- Abou Chacra, L., & Fenollar, F. (2021). Exploring the global vaginal microbiome and its impact on human health. *Microbial Pathogenesis*, 160, 105172.
- Africa, C. W., Nel, J., & Stemmet, M. (2014). Anaerobes and bacterial vaginosis in pregnancy: virulence factors contributing to vaginal colonisation. *International journal of environmental research and public health*, 11(7), 6979–7000.
- Al Bataineh, M. T., Dash, N. R., Elkhazendar, M., Alnusairat, D. M. H., Darwish, I. M. I., Al-Hajjaj, M. S., & Hamid, Q. (2020). Revealing oral microbiota composition and functionality associated with heavy cigarette smoking. *Journal of translational medicine*, 18(1), 1–10.
- Asif, A. A., Roy, M., & Ahmad, S. (2020). Rare case of prevotella pleuritidis lung abscess. *BMJ Case Reports CP*, 13(9), e235960.

## References II

- Basu, S. K., Pradhan, S., du Plessis, A. J., Ben-Ari, Y., & Limperopoulos, C. (2021). Gaba and glutamate in the preterm neonatal brain: In-vivo measurement by magnetic resonance spectroscopy. *NeuroImage*, 238, 118215.
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. *Nature Biotechnology*, 37(8), 852-857. Retrieved from <https://doi.org/10.1038/s41587-019-0209-9> doi: 10.1038/s41587-019-0209-9
- Bomba, L., Walter, K., Guo, Q., Surendran, P., Kundu, K., Nongmaithem, S., ... others (2022). Whole-exome sequencing identifies rare genetic variants associated with human plasma metabolites. *The American Journal of Human Genetics*.

## References III

- Böttcher, T., Kolodkin-Gal, I., Kolter, R., Losick, R., & Clardy, J. (2013). Synthesis and activity of biomimetic biofilm disruptors. *Journal of the American Chemical Society*, 135(8), 2927–2930.
- Chawla, N. V., Bowyer, K. W., Hall, L. O., & Kegelmeyer, W. P. (2002). Smote: synthetic minority over-sampling technique. *Journal of artificial intelligence research*, 16, 321–357.
- Davrandi, M., Harris, S., Smith, P. J., Murray, C. D., & Lowe, D. M. (2022). The relationship between mucosal microbiota, colitis, and systemic inflammation in chronic granulomatous disorder. *Journal of Clinical Immunology*, 42(2), 312–324.
- Deutsch, L., Debevec, T., Millet, G. P., Osredkar, D., Opara, S., Šket, R., ... Stres, B. (2022). Urine and fecal 1h-nmr metabolomes differ significantly between pre-term and full-term born physically fit healthy adult males. *Metabolites*, 12(6), 536.

## References IV

- Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., ... Langille, M. G. (2020). Picrust2 for prediction of metagenome functions. *Nature biotechnology*, 38(6), 685–688.
- Freitas, A. C., Bocking, A., Hill, J. E., & Money, D. M. (2018). Increased richness and diversity of the vaginal microbiota and spontaneous preterm birth. *Microbiome*, 6(1), 1–15.
- Garber, J. M., Hennet, T., & Szymanski, C. M. (2021). Significance of fucose in intestinal health and disease. *Molecular Microbiology*, 115(6), 1086–1093.
- Ghartey, J., Bastek, J. A., Brown, A. G., Anglim, L., & Elovitz, M. A. (2015). Women with preterm birth have a distinct cervicovaginal metabolome. *American journal of obstetrics and gynecology*, 212(6), 776–e1.

## References V

- Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., ... Nelson, K. E. (2006). Metagenomic analysis of the human distal gut microbiome. *science*, 312(5778), 1355–1359.
- Gómez, L. M., Sammel, M. D., Appleby, D. H., Elovitz, M. A., Baldwin, D. A., Jeffcoat, M. K., ... Parry, S. (2010). Evidence of a gene-environment interaction that predisposes to spontaneous preterm birth: a role for asymptomatic bacterial vaginosis and dna variants in genes that control the inflammatory response. *American journal of obstetrics and gynecology*, 202(4), 386–e1.
- Harper, M., Thom, E., Klebanoff, M. A., Thorp Jr, J., Sorokin, Y., Varner, M. W., ... others (2010). Omega-3 fatty acid supplementation to prevent recurrent preterm birth: a randomized controlled trial. *Obstetrics and gynecology*, 115(2 0 1), 234.

## References VI

- Heumann, C. L., Quilter, L. A., Eastment, M. C., Heffron, R., & Hawes, S. E. (2017). Adverse birth outcomes and maternal neisseria gonorrhoeae infection: a population-based cohort study in washington state. *Sexually transmitted diseases*, 44(5), 266.
- Hills, T., Sharpe, C., Wong, T., Cutfield, T., Lee, A., McBride, S., ... others (2022). Fetal loss and preterm birth caused by intraamniotic haemophilus influenzae infection, new zealand. *Emerging Infectious Diseases*, 28(9), 1747–1754.
- Janda, J. M., & Abbott, S. L. (2007). 16s rrna gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, 45(9), 2761–2764.

## References VII

- Kaczmarczyk, M., Löber, U., Adamek, K., Węgrzyn, D., Skonieczna-Żydecka, K., Malinowski, D., ... others (2021). The gut microbiota is associated with the small intestinal paracellular permeability and the development of the immune system in healthy children during the first two years of life. *Journal of translational medicine*, 19(1), 1–26.
- Kitazawa, T., Ishigaki, S., Seo, K., Yoshino, Y., & Ota, Y. (2018). Catheter-related bloodstream infection due to *Rhodotorula mucilaginosa* with normal serum  $(1 \rightarrow 3)$ - $\beta$ -d-glucan level. *Journal de Mycologie Médicale*, 28(2), 393–395.
- Lacroix, G., Gouyer, V., Rocher, M., Gottrand, F., & Desseyen, J.-L. (2022). A porous cervical mucus plug leads to preterm birth induced by experimental vaginal infection in mice. *Iscience*, 25(7), 104526.

## References VIII

- Li, D., Huang, Y., Sadykova, A., Zheng, W., Lin, L., Jin, C., ... Pan, S. (2021). Composition of the microbial communities at different body sites in women with preterm birth and their newborns. *Medicine in Microecology*, 9, 100046.
- Lizewska, B., Teul, J., Kuc, P., Lemancewicz, A., Charkiewicz, K., Goscik, J., ... Laudanski, P. (2018). Maternal plasma metabolomic profiles in spontaneous preterm birth: preliminary results. *Mediators of inflammation*, 2018.
- Maitre, L., Fthenou, E., Athersuch, T., Coen, M., Toledano, M. B., Holmes, E., ... Keun, H. C. (2014). Urinary metabolic profiles in early pregnancy are associated with preterm birth and fetal growth restriction in the rhea mother-child cohort study. *BMC medicine*, 12(1), 1–14.

## References IX

- Mandal, S., Van Treuren, W., White, R. A., Eggesbø, M., Knight, R., & Peddada, S. D. (2015). Analysis of composition of microbiomes: a novel method for studying microbial composition. *Microbial ecology in health and disease*, 26(1), 27663. doi: 10.3402/mehd.v26.27663
- McDonald, D., Clemente, J. C., Kuczynski, J., Rideout, J. R., Stombaugh, J., Wendel, D., ... Caporaso, J. G. (2012). The biological observation matrix (biom) format or: how i learned to stop worrying and love the ome-ome. *GigaScience*, 1(1), 7. doi: 10.1186/2047-217X-1-7
- Mendz, G. L., Petersen, R., Quinlivan, J. A., & Kaakoush, N. O. (2014). Potential involvement of campylobacter curvus and haemophilus parainfluenzae in preterm birth. *Case Reports*, 2014, bcr2014205282.
- Mignard, S., & Flandrois, J.-P. (2006). 16s rrna sequencing in routine bacterial identification: a 30-month experiment. *Journal of microbiological methods*, 67(3), 574–581.

## References X

- Miyauchi, M., Ao, M., Furusho, H., Chea, C., Nagasaki, A., Sakamoto, S., ... Takata, T. (2018). Galectin-3 plays an important role in preterm birth caused by dental infection of *porphyromonas gingivalis*. *Scientific reports*, 8(1), 1–12.
- Mosquera, J., Zabalza, M., Laniero, M., & Blanco, J. (2000). Endocarditis due to *gemella haemolysans* in a patient with hemochromatosis. *Clinical microbiology and infection*, 6(10), 566–568.
- Nishio, T., Yoshikawa, Y., Shew, C.-Y., Umezawa, N., Higuchi, T., & Yoshikawa, K. (2019). Specific effects of antitumor active norspermidine on the structure and function of dna. *Scientific reports*, 9(1), 1–12.
- Olsen, G. J., & Woese, C. R. (1993). Ribosomal rna: a key to phylogeny. *The FASEB journal*, 7(1), 113–123.

## References XI

- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., ... others (2011). Scikit-learn: Machine learning in python. *the Journal of machine Learning research*, 12, 2825–2830.
- Redanz, U., Redanz, S., Treerat, P., Prakasam, S., Lin, L.-J., Merritt, J., & Kreth, J. (2021). Differential response of oral mucosal and gingival cells to corynebacterium durum, streptococcus sanguinis, and porphyromonas gingivalis multispecies biofilms. *Frontiers in Cellular and Infection Microbiology*, 11, 686479.
- Ruiz-Limón, P., Mena-Vázquez, N., Moreno-Indias, I., Manrique-Arija, S., Lisboa-Montanez, J. M., Cano-García, L., ... Fernández-Nebro, A. (2022). Collinsella is associated with cumulative inflammatory burden in an established rheumatoid arthritis cohort. *Biomedicine & Pharmacotherapy*, 153, 113518.

## References XII

- Sharma, G., Garg, N., Hasan, S., & Shirodkar, S. (2022). Prevotella: An insight into its characteristics and associated virulence factors. *Microbial Pathogenesis*, 105673.
- Shi, F., Liu, G., Lin, Y., Han, J., Chu, E. S., Shi, C., ... others (2022). Altered gut microbiome composition by appendectomy contributes to colorectal cancer. *Oncogene*, 1–11.
- SNYDERMAN, S. E., HOLT JR, L. E., NORTON, P. M., & PHANSALKAR, S. V. (1970). Protein requirement of the premature infant: II. influence of protein intake on free amino acid content of plasma and red blood cells. *The American Journal of Clinical Nutrition*, 23(7), 890–895.
- Solazzo, G., Iodice, S., Mariani, J., Persico, N., Bollati, V., & Ferrari, L. (2022). Upper respiratory microbiome in pregnant women: Characterization and influence of parity. *Microorganisms*, 10(11), 2189.

## References XIII

- Surve, M. V., Anil, A., Kamath, K. G., Bhutda, S., Sthanam, L. K., Pradhan, A., ... others (2016). Membrane vesicles of group b streptococcus disrupt feto-maternal barrier leading to preterm birth. *PLoS pathogens*, 12(9), e1005816.
- Tett, A., Pasolli, E., Masetti, G., Ercolini, D., & Segata, N. (2021). Prevotella diversity, niches and interactions with the human host. *Nature Reviews Microbiology*, 19(9), 585–599.
- Treerat, P., Redanz, U., Redanz, S., Giacaman, R. A., Merritt, J., & Kreth, J. (2020). Synergism between corynebacterium and streptococcus sanguinis reveals new interactions between oral commensals. *The ISME journal*, 14(5), 1154–1169.
- Tucker, C. M., Cadotte, M. W., Carvalho, S. B., Davies, T. J., Ferrier, S., Fritz, S. A., ... others (2017). A guide to phylogenetic metrics for conservation, community ecology and macroecology. *Biological Reviews*, 92(2), 698–715.

## References XIV

- Tucker, J., & McGuire, W. (2004). Epidemiology of preterm birth. *Bmj*, 329(7467), 675–678.
- Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, 449(7164), 804–810.
- Voronkov, L., Solonovych, A., Liashenko, A., & Revenko, I. (2018). Prognostic value of cognitive tests and their combination in patients with chronic heart failure and reduced left ventricular ejection fraction. *Eureka: health sciences*(6), 36–45.
- Wallen, Z. D., Demirkan, A., Twa, G., Cohen, G., Dean, M. N., Standaert, D. G., ... Payami, H. (2022). Metagenomics of parkinson's disease implicates the gut microbiome in multiple disease mechanisms. *Nature Communications*, 13(1), 1–20.

## References XV

- Wei, W., Li, J., Liu, F., Wu, M., Xiong, K., He, Q., ... Li, Y. (2022). Alteration of intestinal microecology by oral antibiotics promotes oral squamous cell carcinoma development. *Molecular Immunology*, 149, 94–106.
- Wu, G., Jaeger, L. A., Bazer, F. W., & Rhoads, J. M. (2004). Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications. *The Journal of nutritional biochemistry*, 15(8), 442–451.
- Yang, Y., Chen, T., Zhang, X., & Wang, X. (2021). Age-related functional changes of intestinal flora in rats. *FEMS Microbiology Letters*, 368(10), fnab051.
- Yang, Y., Long, J., Wang, C., Blot, W. J., Pei, Z., Shu, X., ... others (2022). Prospective study of oral microbiome and gastric cancer risk among asian, african american and european american populations. *International journal of cancer*, 150(6), 916–927.

## References XVI

- Zamora, S. A., Amin, H. J., McMillan, D. D., Kubes, P., Fick, G. H., Butzner, J. D., ... others (1997). Plasma l-arginine concentrations in premature infants with necrotizing enterocolitis. *The Journal of pediatrics*, 131(2), 226–232.
- Zhou, X., Baumann, R., Gao, X., Mendoza, M., Singh, S., Sand, I. K., ... others (2022). Gut microbiome of multiple sclerosis patients and paired household healthy controls reveal associations with disease risk and course. *Cell*, 185(19), 3467–3486.