

Probiotic pilot

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Overview: pilot experiment with *Bifidobacterium thermophilus* on inulin with degrees of polymerization (DP)

Setup Work Environment

```
rm(list=ls())
getwd()
setwd("~/GitHub/Gut/")

# Require and/or install packages
package.list <- c('plyr', 'grid', 'png', 'ggplot2', 'tidyverse')
for (package in package.list) {
  if (!require(package, character.only=T, quietly=T)) {
    install.packages(package)
    library(package, character.only=T)
  } }

sem <- function(x, ...){sd(x, na.rm = TRUE)/sqrt(length(na.omit(x)))}

# Load t-test custom functions
ttest <- function(reg, coefnum, val){
  co <- coef(summary(reg))
  tstat <- (co[coefnum,1]-val)/co[coefnum,2]
  pstat <- 2 * pt(abs(tstat), reg$df.residual, lower.tail = FALSE)
  return(list = c(t = tstat, df = reg$df.residual, p = pstat))
}
```

Load data

```
data <- read.table("~/GitHub/Gut/data/inulin/Bt_pilot.txt", sep="\t", header=TRUE)
```

Calculate means, sem, and sample size by resource type

```
carbon.means <- aggregate(OD600 ~ carbon, data = data, FUN = mean)
carbon.n <- aggregate(OD600 ~ carbon, data = data, FUN = length)
carbon.sem <- aggregate(OD600 ~ carbon, data = data, FUN = sem)
carbon.95.LL <- aggregate(OD600 ~ carbon, data = data,
  FUN = function(x) t.test(x)$conf.int[1])
carbon.95.UL <- aggregate(OD600 ~ carbon, data = data,
  FUN = function(x) t.test(x)$conf.int[2])
```

```

# Make table of proportion eDNA by environment
carbon.table.a <- data.frame(carbon.means, carbon.sem[,2], carbon.n[,2],
                             carbon.95.LL[,2], carbon.95.UL[,2])
colnames(carbon.table.a) <- c("carbon", "mean", "sem", "n", "95LL", "95UL")

# Sort table by carbon type from smallest to largest DP
carbon.table <- carbon.table.a[order(factor(carbon.table.a$carbon, levels=c("gluc", "kest", "CLR", "TEX23", "sensus")))]

```

ANOVA

```

carbon.aov <- aov(OD600 ~ carbon, data = data)
summary(carbon.aov)

```

```

##           Df Sum Sq Mean Sq F value    Pr(>F)
## carbon      4  30.15    7.538   17.82 3.16e-06 ***
## Residuals   19   8.04    0.423
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

```
TukeyHSD(carbon.aov)
```

```

##    Tukey multiple comparisons of means
##      95% family-wise confidence level
##
## Fit: aov(formula = OD600 ~ carbon, data = data)
##
## $carbon
##           diff          lwr          upr      p adj
## gluc-CLR     -1.081 -2.393231  0.2312311 0.1379043
## kest-CLR     -0.924 -2.161183  0.3131833 0.2057765
## sensus-CLR   -2.592 -3.829183 -1.3548167 0.0000424
## TEX23-CLR    -2.956 -4.193183 -1.7188167 0.0000072
## kest-gluc     0.157 -1.155231  1.4692311 0.9960970
## sensus-gluc  -1.511 -2.823231 -0.1987689 0.0194202
## TEX23-gluc   -1.875 -3.187231 -0.5627689 0.0031640
## sensus-kest  -1.668 -2.905183 -0.4308167 0.0053916
## TEX23-kest   -2.032 -3.269183 -0.7948167 0.0007710
## TEX23-sensus -0.364 -1.601183  0.8731833 0.8990141

```

Make scatter plot with error bars by carbon type with individual data points

```

# carbon data for plotting
carbon.glucose <- data[ which(data$carbon == "gluc"),]
carbon.kestose <- data[ which(data$carbon == "kest"),]
carbon.CLR <- data[ which(data$carbon == "CLR"),]
carbon.TEX <- data[ which(data$carbon == "TEX23"),]
carbon.sensus <- data[ which(data$carbon == "sensus"),]

png(filename="~/GitHub/Gut/output/Bifido.all.carbon.png",

```

```

width = 800, height = 800, res = 96*2)

par(mar = c(4, 5, 1, 1))

# start with glucose
all.carbon <- plot(jitter(rep(1, length(carbon.glucose$OD600))), amount = 0.1),
  carbon.glucose$OD600, ylim = c(-0.1, 7), xlim = c(0.5, 5.5), pch = 21,
  col = "lightgrey", bg = "lightgrey", lwd = 2, cex = 1.7, yaxt = "n",
  xaxt = "n", cex.lab = 2, cex.axis = 1.5, las = 1, ylab = "", xlab = "")
box(lwd = 2)

# add kestose
points(jitter(rep(2, length(carbon.kestose$OD600))), amount = 0.1), carbon.kestose$OD600,
  pch = 21, bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# add CLR
points(jitter(rep(3, length(carbon.CLR$OD600))), amount = 0.1), carbon.CLR$OD600, pch = 21,
  bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# add TEX23
points(jitter(rep(4, length(carbon.TEX$OD600))), amount = 0.1), carbon.TEX$OD600,
  pch = 21, bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

# add sensus
points(jitter(rep(5, length(carbon.sensus$OD600))), amount = 0.1), carbon.sensus$OD600,
  pch = 21, bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

points(1, mean(carbon.glucose$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(carbon.kestose$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)
points(3, mean(carbon.CLR$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)
points(4, mean(carbon.TEX$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)
points(5, mean(carbon.sensus$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)

mtext(expression('Bacterial Biomass (OD600)'), side = 2,
  outer = FALSE, cex = 1.5, line = 3, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,
  labels = c("0", "2", "4", "6"), at = c(0.0, 2, 4, 6))

axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
  at=c(0, 2, 4, 6), labels = F, tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
  labels = c("Glu", "Kest", "CLR", "TEX", "SENS"), at = c(1, 2, 3, 4, 5))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
  at = c(1, 2, 3, 4, 5), labels = F, tck = -0.02)

```

```

arrows(x0 = c(1,2,3,4,5), y0 = carbon.table$mean, y1 = carbon.table$`95LL`, angle = 90,
       length = 0.1, lwd = 2)

arrows(x0 = c(1,2,3,4,5), y0 = carbon.table$mean, y1 = carbon.table$`95UL`, angle = 90,
       length=0.1, lwd = 2)

mtext(text = expression(italic("P")~" < 0.0001") , side =3, line = -2, adj = 0.925, col="black", cex=1.1)

# Close Plot Device
dev.off()

## pdf
## 2

graphics.off()

# Show Plot
img <- readPNG("~/GitHub/Gut/output/Bifido.all.carbon.png")
grid.raster(img)

```

Make scatter plot with error bars for CLR and TEX

```

png(filename="~/GitHub/Gut/output/Bifido.low-high.carbon.png",
     width = 800, height = 800, res = 96*2)

par(mar = c(4, 5, 1, 1))

# start with CLR
all.carbon <- plot(jitter(rep(1, length(carbon.CLR$OD600))), amount = 0.1),
  carbon.CLR$OD600, ylim = c(-0.1, 7), xlim = c(0.5, 2.5), pch = 21,
  col = "lightgrey", bg = "lightgrey", lwd = 2, cex = 1.7, yaxt = "n",
  xaxt = "n", cex.lab = 2, cex.axis = 1.5, las = 1, ylab = "", xlab = "")
box(lwd = 2)

# add TEX23
points(jitter(rep(2, length(carbon.TEX$OD600))), amount = 0.1), carbon.TEX$OD600,
  pch = 21, bg = "lightgrey", col = "lightgrey", lwd = 2, cex = 1.7)

points(1, mean(carbon.CLR$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)
points(2, mean(carbon.TEX$OD600), pch = 21, col = "black",
  bg = "NA", lwd = 2, cex = 2.5)

mtext(expression('Bacterial Biomass (OD600)'), side = 2,
  outer = FALSE, cex = 1.5, line = 2.7, adj = 0.5)

mtext(expression('Inulin Size (DP)'), side = 1,
  outer = FALSE, cex = 1.5, line = 3, adj = 0.5)

# Major Axes
axis(side = 2, lwd.ticks = 2, cex.axis = 1.25, las = 1,

```

```

    labels = c("0", "2", "4", "6"), at = c(0.0, 2, 4, 6))

axis(side = 4, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at=c(0, 2, 4, 6), labels = F, tck = -0.02)

axis(side = 1, lwd.ticks = 2, cex.axis = 0.9, las = 1,
     labels = c("Low", "High"), at = c(1, 2))

axis(side = 3, lwd.ticks = 2, cex.axis = 1.5, las = 1,
     at = c(1, 2), labels = F, tck = -0.02)

arrows(x0 = c(1,2), y0 = carbon.table$mean[c(3,4)], y1 = carbon.table$`95LL`[c(3,4)],
       angle = 90,length = 0.1, lwd = 2)

arrows(x0 = c(1,2), y0 = carbon.table$mean[c(3,4)], y1 = carbon.table$`95UL`[c(3,4)],
       angle = 90, length=0.1, lwd = 2)

mtext(text = expression(italic("P")~" < 0.0001") , side =3, line = -2, adj = 0.925, col="black", cex=1.5)

# Close Plot Device
dev.off()

## pdf
## 2

graphics.off()

# Show Plot
img <- readPNG("~/GitHub/Gut/output/Bifido.low-high.carbon.png")
grid.raster(img)

```

t-test

```

low.high.carbon <- rbind(carbon.CLR, carbon.TEX)
carbon.ttest <- t.test(OD600 ~ carbon, data = low.high.carbon)
carbon.ttest

##
## Welch Two Sample t-test
##
## data: OD600 by carbon
## t = 10.121, df = 7.758, p-value = 9.668e-06
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## 2.278846 3.633154
## sample estimates:
## mean in group CLR mean in group TEX23
## 5.456 2.500

```