LTDE BGE Experiment

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Overview

Is growth efficiency an evolvable trait? Here, we explore patterns in bacterial growth efficiency using a set of isolates from the long-term starvation experiment. Each isolate has an ancestor and at least three derived strains that were isolated after three years of starvation in PBS. We are using 14C succinate as the sole carbon source for these experiments.

Experiment 1: BGE Evolution Experiment

Data Import

```
# Import Data
# KBS0707.data <- read.csv("../data/BGE/20160216_BRBP.csv", header = T)
# Not using this run because it doesn't appear that the cultures grew enough
KBS0707.data <- read.csv("../data/BGE/20160228_BRBP.csv", header = T)
KBS0802.data <- read.csv("../data/BGE/20160214_BRBP.csv", header = T)
```

Data Processing

```
# Raw Data For Intra Vial Test
voucher707  <- KBS0707.data[grep("Voucher", KBS0707.data$Tube), ]
voucher802  <- KBS0802.data[grep("Voucher", KBS0802.data$Tube), ]
control707  <- KBS0707.data[grep("Control", KBS0707.data$Tube), ]
control802  <- KBS0802.data[grep("Control", KBS0802.data$Tube), ]

# Merge Datasets
BGE.data.full <- rbind(KBS0707.data, KBS0802.data)

# Remove Control and Voucher
BGE.data <- BGE.data.full[-which(BGE.data.full$Tube == "Voucher" |
                                   BGE.data.full$Tube == "Control"), ]
BGE.data$CPM.c <- NA

for (i in 1:dim(BGE.data)[1]){
  if (BGE.data$Sample[i] == "Cells"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (6150/1500)
  } else {
  if (BGE.data$Sample[i] == "Trap"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (500/100)
  } else {
  if (BGE.data$Sample[i] == "Sup"){
```

```
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (6150/100)
  }
  else {
    BGE.data$CPM.c[i] <- NA
  }}}
}

BGE.data.m <- melt(BGE.data)
```

Using Tube, Sample, Organism as id variables

```
BGE.data.p <- cast(BGE.data.m, Tube + Organism + Sample ~ variable,
                   subset=variable=="CPM.c",  fun.aggregate = c(mean, CV))

BGE.data.p$CPM.p <- NA
for (i in 1:dim(BGE.data.p)[1]){
  if (grepl("707", BGE.data.p$Organism[i])){
    BGE.data.p$CPM.p <- BGE.data.p$CPM.c_mean / voucher707$CPM
  } else {
  if (grepl("802", BGE.data.p$Organism[i])){
    BGE.data.p$CPM.p <- BGE.data.p$CPM.c_mean / voucher802$CPM
  } else{
    BGE.data.p$CPM.p <- NA
  }}}

mass.l <- melt(as.data.frame(BGE.data.p))
```

Using Tube, Organism, Sample as id variables

```
# Mass Balance
mass <- cast(mass.l, Tube + Organism ~ variable, subset=variable == "CPM.p",
             fun.aggregate = sum)
mean(mass$CPM.p)

# Data For Plots
BGE.707 <- droplevels(BGE.data.p[grep("707", BGE.data.p$Organism), ])
BGE.802 <- droplevels(BGE.data.p[grep("802", BGE.data.p$Organism), ])
levels(BGE.802$Tube)
```

802 Plot

```
BGE.802.w <- reshape(BGE.802, idvar = "Tube", direction="wide",
                     v.names = "CPM.p", timevar = "Sample",
                     drop = c("CPM.c_mean", "CPM.c_CV"))

BGE.802.w$BGE <- BGE.802.w$CPM.p.Cells / (BGE.802.w$CPM.p.Cells +
                                          BGE.802.w$CPM.p.Trap)
BGE.802.m <- melt(data.frame(BGE.802.w[,c(1,2,6)]))
```

Using Tube, Organism as id variables

```r
BGE.802.c <- cast(BGE.802.m, Organism ~ variable, fun.aggregate=c(mean, se))

# Plot
png(filename="../figures/BGE802.png",
    width = 1000, height = 800, res = 96*2)

par(mar=c(3,5.5,2.5,1))
bge_plot <- plot(BGE.802.c$BGE_mean, main = "LTDE: KBS0802 BGE",
                 pch = 15, cex = 1.5,
        ylab = "", yaxt= "n", xaxt = "n", xlab = "",
        xlim = c(0.5, 4.5), ylim = c(0.26, 0.33))

arrows(x0 = c(1:4), y0 = BGE.802.c$BGE_mean, y1 = BGE.802.c$BGE_mean -
         BGE.802.c$BGE_se, angle = 90,
       length=0.1, lwd = 2)
arrows(x0 = c(1:4), y0 = BGE.802.c$BGE_mean, y1 = BGE.802.c$BGE_mean +
         BGE.802.c$BGE_se, angle = 90,
       length=0.1, lwd = 2)

axis(side = 1, labels = c("Ancestor", "A-1", "B-1", "C-1"),
     at = c(1:4), lwd.ticks = 2, cex = 2)
axis(side = 2, labels = T, at = c(0.26, 0.28, 0.30, 0.32), las = 1,
     lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
     at = c(0.26, 0.28, 0.30, 0.32))
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
     at = c(0.26, 0.28, 0.30, 0.32))
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
     at = c(0.26, 0.28, 0.30, 0.32))
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
     at = c(0.26, 0.28, 0.30, 0.32))

box(lwd = 2)

mtext("BGE", side = 2 , line =3.5, cex = 1.5)

dev.off() # this writes plot to folder
```

```
## pdf
##   2
```

```r
graphics.off() # shuts down open devices
```

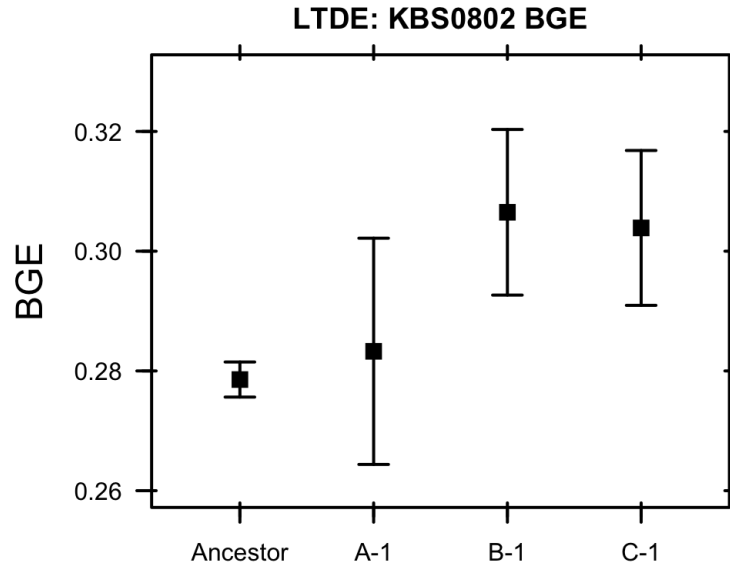## 707 Plot

Figure 1: 14C-Succinate Recovery and BGE

```
BGE.707.w <- reshape(BGE.707, idvar = "Tube", direction="wide",
                     v.names = "CPM.p", timevar = "Sample",
                     drop = c("CPM.c_mean", "CPM.c_CV"))

BGE.707.w$BGE <- BGE.707.w$CPM.p.Cells / (BGE.707.w$CPM.p.Cells +
                                            BGE.707.w$CPM.p.Trap)
BGE.707.m <- melt(data.frame(BGE.707.w[,c(1,2,6)]))
```

```
## Using Tube, Organism as id variables
```

```
BGE.707.c <- cast(BGE.707.m, Organism ~ variable,
                  fun.aggregate=c(mean, se))

# Plot
png(filename="../figures/BGE707.png",
    width = 1000, height = 800, res = 96*2)

par(mar=c(3,5.5,2.5,1))
bge_plot <- plot(BGE.707.c$BGE_mean, main = "LTDE: KBS0707 BGE",
                 pch = 15, cex = 1.5, ylab = "", yaxt= "n",
                 xaxt = "n", xlab = "",
                 xlim = c(0.5, 4.5), ylim = c(0.22, 0.28))

arrows(x0 = c(1:4), y0 = BGE.707.c$BGE_mean, y1 = BGE.707.c$BGE_mean -
         BGE.707.c$BGE_se, angle = 90,
       length=0.1, lwd = 2)
arrows(x0 = c(1:4), y0 = BGE.707.c$BGE_mean, y1 = BGE.707.c$BGE_mean +
         BGE.707.c$BGE_se, angle = 90,
       length=0.1, lwd = 2)
```

```r
axis(side = 1, labels = c("Ancestor", "A-1", "B-1", "C-1"),
     at = c(1:4), lwd.ticks = 2, cex = 2)
axis(side = 2, labels = T, at = c(0.22, 0.24, 0.26, 0.28),
     las = 1, lwd.ticks = 2)
axis(side=1, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1, at = c(1:4))
axis(side=1, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side=3, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1, at = c(1:4))
axis(side = 2, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
axis(side = 4, lwd.ticks = 2, tck = -0.02, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
axis(side = 2, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
axis(side = 4, lwd.ticks = 2, tck = 0.01, labels=F, cex.axis=1,
     at = c(0.22, 0.24, 0.26, 0.28))
box(lwd = 2)

mtext("BGE", side = 2 , line =3.5, cex = 1.5)

dev.off() # this writes plot to folder
```

```
## pdf
##   2
```

```r
graphics.off() # shuts down open devices
```
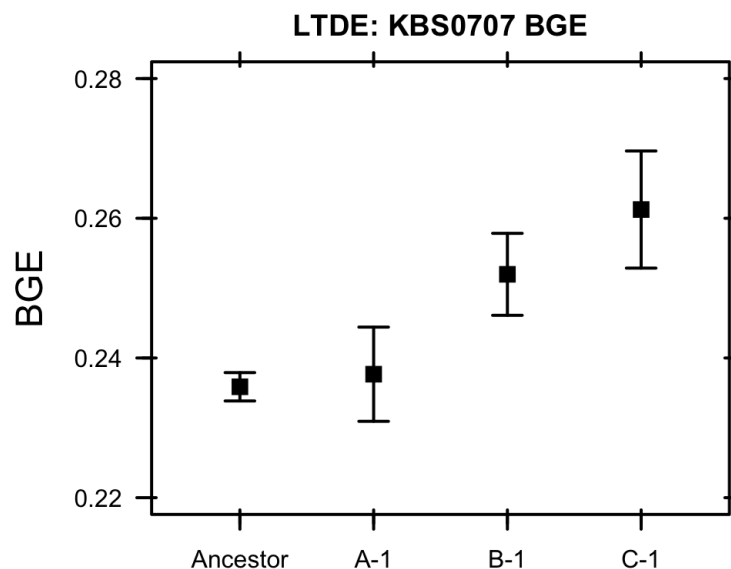

Figure 2: 14C-Succinate Recovery and BGE

Experiment 2: 14C Succinate BGE Using Michaelis-Menten Experiment

Data Import

```
KBS0802.data <- read.csv("../data/BGE/20160306_BRBP.csv", header = T)
```

Data Processing

```
# Raw Data For Intra Vial Test
voucher802  <- KBS0802.data[grep("Voucher", KBS0802.data$Tube), ]
control802  <- KBS0802.data[grep("Control", KBS0802.data$Tube), ]

# Merge Datasets
BGE.data.full <- rbind(KBS0802.data)

# Remove Control and Voucher
BGE.data <- BGE.data.full[-which(BGE.data.full$Tube == "Voucher" |
                                 BGE.data.full$Tube == "Control"), ]
BGE.data$CPM.c <- NA

# Turn Conc into Factor
BGE.data$Conc <- as.factor(BGE.data$Conc)

# Correct CPM data for entire contents of fraction
for (i in 1:dim(BGE.data)[1]){
  if (BGE.data$Sample[i] == "Cells"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (6150/1500)
  } else {
  if (BGE.data$Sample[i] == "Trap"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (500/100)
  } else {
  if (BGE.data$Sample[i] == "Sup"){
    BGE.data$CPM.c[i] <- BGE.data$CPM[i] * (6150/100)
  }
  else {
    BGE.data$CPM.c[i] <- NA
  }}}
}

# Re-organize data
BGE.data.m <- melt(BGE.data)
```

```
## Using Tube, Sample, Organism, Conc as id variables
```

```
BGE.data.p <- cast(BGE.data.m, Tube + Organism + Conc +
                     Sample ~ variable, subset=variable=="CPM.c",
                   fun.aggregate = c(mean, CV))
```

```r
# Calculate Percentages given Voucher
BGE.data.p$CPM.p <- NA
for (i in 1:dim(BGE.data.p)[1]){
    BGE.data.p$CPM.p <- BGE.data.p$CPM.c_mean / voucher802$CPM
  }

# Mass Balance
mass.l <- melt(as.data.frame(BGE.data.p))
```

Using Tube, Organism, Conc, Sample as id variables

```r
mass <- cast(mass.l, Tube + Organism + Conc ~ variable,
             subset=variable == "CPM.p", fun.aggregate = sum)
mean(mass$CPM.p)

# Drop Levels (needed?)
BGE.802 <- droplevels(BGE.data.p[grep("802", BGE.data.p$Organism), ])
levels(BGE.802$Tube)

# Turn percentages into concentrations given experimental concentration
BGE.802.w <- reshape(BGE.802, idvar = "Tube", direction="wide",
                     v.names = "CPM.p", timevar = "Sample",
                     drop = c("CPM.c_mean", "CPM.c_CV"))
BGE.802.w$CPM.p.Resp <- 1 - (BGE.802.w$CPM.p.Cells + BGE.802.w$CPM.p.Sup)
BGE.802.w$Conc <- as.numeric(as.character(BGE.802.w$Conc))
BGE.802.w$Conc.Cells <- BGE.802.w$CPM.p.Cells * BGE.802.w$Conc
BGE.802.w$Conc.Sup <- BGE.802.w$CPM.p.Sup * BGE.802.w$Conc
BGE.802.w$Conc.Trap <- BGE.802.w$CPM.p.Trap * BGE.802.w$Conc
BGE.802.w$Conc.Resp <- BGE.802.w$CPM.p.Resp * BGE.802.w$Conc

# Calculate BGE
BGE.802.w$BGE <- BGE.802.w$CPM.p.Cells / (BGE.802.w$CPM.p.Cells +
                                          BGE.802.w$CPM.p.Resp)

# Recovery
BGE.802.w$Recovery <- rowSums(BGE.802.w[,4:6])
```

Michaelis Menten Plot

```r
# Plot
png(filename="../figures/MM802-14C.png",
    width = 1000, height = 1600, res = 96*2)

par(mfrow = c(1,1), mar = c(0, 6, 0, 0) + 0.5, oma = c(4, 0, 1, 1) + 0.5)
layout(rbind(1, 2, 3), height = c(3, 3, 3))

prod_plot <- plot(Conc.Cells ~ Conc, data = BGE.802.w,
                  pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                  xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                  ylim = c(0.0, 0.15))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
```

```r
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = -0.04,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BP", side = 2 , line = 4, cex = 1.5)
# mtext("(mM Carbon)", side = 2, line = 3, cex = 0.8)
box(lwd = 3)
resp_plot <- plot(Conc.Resp ~ Conc, data = BGE.802.w,
                  pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                  xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                  ylim = c(0.1, 0.9))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = -0.04,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BR", side = 2 , line = 4, cex = 1.5)
# mtext("(mM Carbon)", side = 2, line = 3, cex = 0.8)
box(lwd = 3)
bge_plot <- plot(BGE ~ Conc, data = BGE.802.w,
                 pch = 22, cex = 3, col = "black", bg = "gray", lwd = 2.5,
                 xaxt = 'n', yaxt = 'n', xlab = '', ylab = '',
                 ylim = c(0.05, 0.22))
axis(side=2, lwd.ticks = 2, tck=-0.04, labels=T, las = 1, cex.axis = 1.5)
axis(side=2, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck=-0.02, labels=F)
axis(side=4, lwd.ticks = 2, tck= 0.02, labels=F)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1.5, las = 1, tck = -0.04,
     at = c(0, 2.5, 5, 7.5, 10), labels = T)
axis(side = 1, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = -0.02,
     at = c(0, 2.5, 5, 10), labels = F)
axis(side = 3, lwd.ticks = 2 , cex.axis = 1, las = 1, tck = 0.02,
     at = c(0, 2.5, 5, 7.5, 10), labels = F)
mtext("BGE", side = 2 , line = 4, cex = 1.5)
box(lwd = 3)
mtext("Succinate (mM)", side = 1, line = 3.5, outer=F, cex = 1.5)
```

```
dev.off() # this writes plot to folder
graphics.off() # shuts down open devices
```
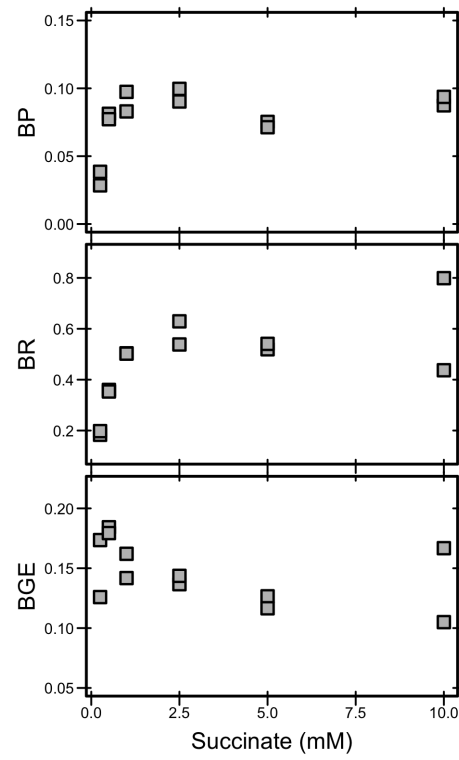

Figure 3: BGE Under Michaelis Menten Dynamics