

SASP endpoint survival

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Set working environment; load packages

```
# Clear and set working directory
rm(list = ls())
# setwd("~/GitHub/ssp_phage/data")
library("dplyr")
library("tidyr")
library("readr")
library("broom") #to extract stat results as table
# d <- read_csv("~/GitHub/ssp_phage/data/HeatDecayperc survivals.csv", header = TRUE)
library(here)
```

Load data

```
d <- read_csv(here("data", "ssp_data.csv"))%>%
  # to avoid confusion with R commands change group to g
  mutate(group=gsub("group.", "g", group))%>%

  #remove data from prelims
  filter(group!="preliminary")%>%

  #remove data on filtered samples
  filter(!filter)%>%

  #Calculate titer from counts
  mutate(counts=as.numeric(counts ))%>%
  mutate(no.ml = counts/(dilution*ml.plated))%>%

  #calculate percent survival relative to T=0 (no heat treatment)
  group_by(host, phage, assay, group)%>%
  mutate(perc.survival = 100*no.ml/no.ml[heat.min==0])%>%

  # keep endpoint values
  filter(heat.min==45)%>%

  #add spore type
  mutate(spore=case_when(assay=="PFU" ~ "viral spore",
```

```

        phage=="noPhage" ~ "non-infected host spore",
        TRUE ~ "infected host spore"))%>%

# make a colony variable
mutate(colony=interaction(host,group))%>%
mutate(colony=paste0(host,"-col",group))%>%
mutate(colony=gsub("colg","col",colony))%>%
ungroup()

##
## -- Column specification -----
## cols(
##   host = col_character(),
##   phage = col_character(),
##   assay = col_character(),
##   filter = col_logical(),
##   dilution = col_double(),
##   ml.plated = col_double(),
##   counts = col_character(),
##   heat.min = col_double(),
##   group = col_character(),
##   date = col_double()
## )

#export data
write_csv(d,here("data/percent_T-end.csv"))

```

Does deletion of sspAB diminish perc.survival?

Bacillus culture was split into three phage treatments: (SPO1, Goe2, noPhage). The “group” column refers to spores that were assayed on same day. The “colony” column reflects pairing to be accounted for in analysis.

```

# subset perc.survivals by colony origin
cfu.1 <- filter(d, assay == "CFU" & phage == "noPhage") %>%
  dplyr::select(host, assay, colony, perc.survival)

# extract perc.survivals from wildtype
x.wt <- cfu.1 %>%
  filter(host == "wt") %>%
  pull(perc.survival)

# extract perc.survivals from mutant
y.mut <- cfu.1 %>%
  filter(host == "dsspAB") %>%
  pull(perc.survival)

# conduct one-sided, paired t-test
cfu.1.test1 <- t.test(x.wt, y.mut, alternative = "greater")

# conduct two-sided, paired t-test
cfu.1.test2 <- t.test(x.wt, y.mut, alternative = "two.sided")

```

```
# Answer: deletion of sspAB reduces heat tolerance
# two-sided: P = 0.003983 (alternative = "two.sided")
# one-sided: P = 0.001992 (alternative = "greater")
```

Does infection with phage ameliorate effects of sspAB deletion?

For each colony we calculate the ratio of surviving spores (%) in phage infected vs non-infected cultures. The null hypothesis is that phage has no effect, so that the ratio equals 1. The alternative is that a greater percentage of spores survive in phage infected cultures, so we do a one sided test.

```
# subset perc survivals by host, phage, and group
cfu.2 <- filter(d, assay == "CFU", host == "dsspAB") %>%
  dplyr::select(host, phage, assay, colony, perc.survival)

# convert to wide
cfu.2 <- cfu.2 %>%
  # spread(phage, perc.survival)
  tidyr::pivot_wider(names_from = phage, values_from = perc.survival)

# calculate ratio of perc survivals between no-phage and plus-phage treatments
cfu.2 <- mutate(cfu.2, ratio.sp01 = cfu.2$SP01/cfu.2$noPhage,
  ratio.goe2 = cfu.2$Goe2/cfu.2$noPhage)

# Tests

# 1) Goe2: one-sided, paired t-test
cfu.Goe2.test <- t.test(cfu.2$ratio.goe2, mu = 1, alternative = "greater")
# one-sided: P = 0.1268 (alternative = "greater" -> does phage ameliorate?)
# answer = no

# 2) SP01: one-sided, paired t-test
cfu.SP01.test <- t.test(cfu.2$ratio.sp01, mu = 1, alternative = "greater")
# one-sided: P = 0.0792
# answer = maybe

# 3) Phage (Goe2 and SP01), paired t-test: does "phage" ameliorate
cfu.both.test <- t.test(c(cfu.2$ratio.goe2, cfu.2$ratio.sp01), mu = 1, alternative = "greater")
# one-sided: P = 0.01634
# answer = yes

# 4) Ratio in Goe2 vs SP01 perc survivals, paired
cfu.GvsS.test <- t.test(x=cfu.2$Goe2, y=cfu.2$SP01, mu = 0, alternative = "two.sided")
# two-sided: P = 0.0006878
# answer = yes (SP01 ameliorates more than Goe2)

cfu.table <- dplyr::bind_rows(
  broom::glance(cfu.Goe2.test),
  broom::glance(cfu.SP01.test),
  broom::glance(cfu.both.test),
  broom::glance(cfu.GvsS.test)
)
```

Does infection with phage ameliorate effects of sspAB deletion in virospores?

Compare perc survivals of viral spores to perc survivals of colony-forming spores in the WT. There are no viral spores in non-infected cultures.

```
# subset perc survivals by deletion host, phage treatment, and group
pfu <- filter(d, host == "dsspAB") %>%
  # select CFU for noPhage and PFU for infected cultures
  filter((phage == "noPhage" & assay == "CFU") | (assay == "PFU")) %>%
  dplyr::select(host, phage, colony, perc.survival)

# convert to wide
pfu <- pfu %>%
  # spread(phage, perc.survival)
  tidyr::pivot_wider(names_from = phage, values_from = perc.survival)

# calculate differences in perc survivals between no-phage and plus-phage treatments
pfu <- mutate(pfu, ratio.sp01 = pfu$SP01/pfu$noPhage,
              ratio.goe2 = pfu$Goe2/pfu$noPhage)

# Tests

# 1) Goe2: one-sided, paired t-test
pfu.Goe2.test <- t.test(pfu$ratio.goe2, mu = 1, alternative = "greater")
# one-sided: P = 0.1521
# answer: no

# 2) SP01: one-sided, paired t-test
pfu.SP01.test <- t.test(pfu$ratio.sp01, mu = 1, alternative = "greater")
# one-sided: P = 0.1802
# answer: no

# 3) Phage (Goe2 and SP01)
pfu.both.test <- t.test(c(pfu$ratio.goe2, pfu$ratio.sp01), mu = 1,
                       alternative = "greater")
# one-sided: P = 0.05531
# answer: maybe

# 4) Difference in Goe2 vs SP01
pfu.GvsS.test <- t.test(x=pfu$Goe2, y=pfu$SP01, mu = 0, alternative = "two.sided")
# two-sided: 0.8137
# answer: no

pfu.table <- dplyr::bind_rows(
  broom::glance(pfu.Goe2.test),
  broom::glance(pfu.SP01.test),
  broom::glance(pfu.both.test),
  broom::glance(pfu.GvsS.test)
)
```

Do colony-forming spores and viral spores differ from each other when infecting the mutant host?

One-sided hypothesis: viral spores are more resistant. That is, they have a greater perc.survival value: $\text{perc.survival.PFU} / \text{perc.survival.CFU} > 1$

```
# subset perc survivals by deletion host, assay type (CFU vs. PFU), and colony
cfu.pfu <- filter(d, host == "dsspAB", phage != "noPhage") %>%
  dplyr::select(host, phage, assay, colony, perc.survival)

# convert to wide
cfu.pfu <- cfu.pfu %>%
  # spread(phage, perc.survival)
  tidyr::pivot_wider(names_from = assay, values_from = perc.survival)

# calculate differences in perc survivals between CFU and PFU
cfu.pfu <- mutate(cfu.pfu, dif = PFU/CFU)

cfu.pfu.Goe2.test <-
  cfu.pfu %>%
  filter(phage == "Goe2") %>%
  pull(dif) %>%
  t.test(., mu = 1, alternative = "greater")
# one-sided: P = 0.507

cfu.pfu.SP01.test <-
  cfu.pfu %>%
  filter(phage == "SP01") %>%
  pull(dif) %>%
  t.test(., mu = 1, alternative = "greater")
# one-sided: P = 0.958
# answer: no

cfu.pfu.phage.test <-
  cfu.pfu %>%
  t.test(dif~phage, mu = 0, alternative = "two.sided", data =.)
# one-sided: P = 0.427
# answer: no

cfu.pfu.table <- dplyr::bind_rows(
  broom::glance(cfu.pfu.Goe2.test),
  broom::glance(cfu.pfu.SP01.test),
  broom::glance(cfu.pfu.phage.test)
)
```

Does infection with phage compromise resistance of WT spores?

```
cfu.3 <- filter(d, assay == "CFU", host == "wt") %>%
  dplyr::select(host, phage, assay, colony, perc.survival)
```

```

# convert to wide
cfu.3 <- cfu.3 %>%
  # spread(phage, perc.survival)
  tidyr::pivot_wider(names_from=phage, values_from=perc.survival)

# calculate difference and test
cfu.3 <- mutate(cfu.3, ratio.spo1 = cfu.3$SP01/cfu.3$noPhage,
               ratio.goe2 = cfu.3$Goe2/cfu.3$noPhage)

cfu.3.Goe2.test <- t.test(cfu.3$ratio.goe2, mu = 1, alternative = "less")
# one-sided: P = 0.0794

cfu.3.SP01.test <- t.test(cfu.3$ratio.spo1, mu = 1, alternative = "less")
# one-sided: P = 0.374

cfu.3.phage.test <- t.test(c(cfu.3$ratio.goe2, cfu.3$ratio.spo1), mu = 0,
                        alternative = "two.sided")
# one-sided: P = 0.00898

cfu.wt.table <- dplyr::bind_rows(
  broom::glance(cfu.3.Goe2.test),
  broom::glance(cfu.3.SP01.test),
  broom::glance(cfu.3.phage.test)
)

```