### SASP slopes

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#### Set working environment; load packages and data

```
# Clear and set working directory
rm(list = ls())

library("dplyr")
library("tidyr")
library("broom") #to extract stat results as table
# setwd("~/GitHub/ssp_phage/data")
# d <- read.csv("~/GitHub/ssp_phage/data/HeatDecaySlopes.csv", header = TRUE)
library(here)
d <- read.csv(here("data/HeatDecaySlopes.csv"), header = TRUE)</pre>
```

#### Does deletion of sspAB diminish slope?

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 slopes by colony origin
cfu.1 <- filter(d, assay == "CFU" & phage == "noPhage") %>%
  dplyr::select(host, assay, colony, slope)
# extract slopes from wildtype
x.wt <- cfu.1 %>%
 filter(host == "wt") %>%
 pull(slope)
# extract slopes from mutant
y.mut <- cfu.1 %>%
 filter(host == "dsspAB") %>%
 pull(slope)
# conduct one-sided, paired t-test
cfu.1.test <- t.test(x.wt, y.mut, alternative = "greater")</pre>
# Answer: deletion of sspAB reduces heat tolerance
# two-sided: P = 0.005858 (alternative = "two.sided")
# one-sided: P = 0.002929 (alternative = "greater")
```

#### Does infection with phage ameliorate effects of sspAB deletion?

```
# subset slopes by host, phage, and group
cfu.2 <- filter(d, assay == "CFU", host == "dsspAB") %>%
  dplyr::select(host, phage, assay, colony, slope)
# convert to wide
cfu.2 <- cfu.2 %>%
  # spread(phage, slope)
 tidyr::pivot_wider(names_from = phage, values_from = slope)
# calculate difference in slopes between no-phage and plus-phage treatments
cfu.2 <- mutate(cfu.2, dif.goe2 = cfu.2$noPhage-cfu.2$Goe2,
                dif.spo1 = cfu.2$noPhage - cfu.2$SPO1)
# Tests
# 1) Goe2: one-sided, paired t-test
cfu.Goe2.test <- t.test(cfu.2$dif.goe2, mu = 0, alternative = "less")</pre>
# one-sided: P = 0.1314 (alternative = "less" -> does phage ameliorate?)
# answer = no
# 2) SP01: one-sided, paired t-test
cfu.SP01.test <- t.test(cfu.2$dif.spo1, mu = 0, alternative = "less")</pre>
# one-sided: P = 0.06147
# answer = maybe
# 3) Phage (Goe2 and SPO1), paired t-test: does "phage" ameliorate
cfu.both.test <- t.test(c(cfu.2$dif.goe2, cfu.2$dif.spo1), mu = 0, alternative = "less")
# one-sided: P = 0.01357
# answer = yes
# 4) Difference in Goe2 vs SP01 slopes, paired
cfu.GvsS.test <- t.test(x=cfu.2$Goe2, y=cfu.2$SP01, mu = 0, alternative = "two.sided")
# two-sided: P = 0.6836
# answer = no
cfu.table <- dplyr::bind_rows(</pre>
  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 slopes of viral spores to slopes of colony-forming spores in the WT. There are no viral spores in non-infected cultures.

```
# subset slopes 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, slope)
# convert to wide
pfu <- pfu %>%
  # spread(phage, slope)
  tidyr::pivot_wider(names_from = phage, values_from = slope)
# calculate differences in slopes between no-phage and plus-phage treatments
pfu <- mutate(pfu, dif.goe2 = pfu$noPhage-pfu$Goe2,</pre>
                dif.spo1 = pfu$noPhage - pfu$SP01)
# Tests
# 1) Goe2: one-sided, paired t-test
pfu.Goe2.test <- t.test(pfu$dif.goe2, mu = 0, alternative = "less")</pre>
# one-sided: P = 0.3231
# answer: no
# 2) SPO1: one-sided, paired t-test
pfu.SP01.test <- t.test(pfu$dif.spo1, mu = 0, alternative = "less")</pre>
# one-sided: P = 0.4109
# answer: no
# 3) Phage (Goe2 and SP01)
pfu.both.test <- t.test(c(pfu$dif.goe2, pfu$dif.spo1), mu = 0,
                            alternative = "less")
# one-sided: P = 0.2765
# answer: no
# 4) Difference in Goe2 vs SP01
pfu.GvsS.test <- t.test(x=pfu$Goe2, y=pfu$SP01, mu = 0, alternative = "two.sided")
# two-sided: 0.8138
# answer: no
pfu.table <-dplyr::bind_rows(</pre>
  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 slope value: slope.PFU-slopePFU > 0

```
# subset slopes by deletion host, assay type (CFU vs. PFU), and colony
cfu.pfu <- filter(d, host == "dsspAB", phage != "noPhage") %>%
   dplyr::select(host, phage, assay , colony, slope)
# convert to wide
cfu.pfu <- cfu.pfu %>%
  # spread(phage, slope)
 tidyr::pivot_wider(names_from = assay, values_from = slope)
# calculate differences in slopes between CFU and PFU
cfu.pfu <- mutate(cfu.pfu , dif = PFU-CFU)</pre>
cfu.pfu.Goe2.test <-
 cfu.pfu %>%
 filter(phage == "Goe2") %>%
 pull(dif) %>%
 t.test(., mu = 0, alternative = "greater")
# one-sided: P = 0.3425
cfu.pfu.SP01.test <-
  cfu.pfu %>%
 filter(phage == "SP01") %>%
 pull(dif) %>%
 t.test(., mu = 0, alternative = "greater")
# one-sided: P = 0.9197
# answer: no
cfu.pfu.phage.test <-
 cfu.pfu %>%
 t.test(dif~phage, mu = 0, alternative = "two.sided", data =.)
# one-sided: P = 0.4173
# answer: no
cfu.pfu.table <- dplyr::bind_rows(</pre>
 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?

If spores from infected cultures are more resistant, than slope(noPhage)-slope(phage)>0.

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

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

# calculate difference and test
```

```
cfu.3 <- mutate(cfu.3, dif.goe2 = cfu.3$noPhage-cfu.3$Goe2,</pre>
                dif.spo1 = cfu.3$noPhage - cfu.3$SPO1)
cfu.3.Goe2.test <- t.test(cfu.3$dif.goe2, mu = 0, alternative = "greater")</pre>
# one-sided: P = 0.09148
cfu.3.SP01.test <- t.test(cfu.3$dif.spo1, mu = 0, alternative = "greater")</pre>
# one-sided: P = 0.2998
cfu.3.both.test \leftarrow t.test(c(cfu.3\$dif.goe2, cfu.3\$dif.spo1), mu = 0,
                            alternative = "greater")
# one-sided: P = 0.06574
cfu.3.GvsS.test <- t.test(x=cfu.3$dif.goe2, y= cfu.3$dif.spo1, mu = 0,
                            alternative = "two.sided")
# one-sided: P = 0.2114
cfu.wt.table <- dplyr::bind_rows(</pre>
 broom::glance(cfu.3.Goe2.test),
 broom::glance(cfu.3.SP01.test),
 broom::glance(cfu.3.both.test),
 broom::glance(cfu.3.GvsS.test)
```