

# SASP slopes

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29 December, 2020

## Set working environment; load packages and data

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

## Does deletion of sspAB diminish slope?

```
cfu.1 <- filter(d, assay == "CFU" & phage == "noPhage") %>%
  dplyr::select(host, assay, group, slope)

# convert to wide
cfu.1 <- cfu.1 %>%
  # spread(host, slope)
  tidyr::pivot_wider(names_from=host, values_from=slope)

# calculate difference and test
cfu.1 <- mutate(cfu.1, dif = cfu.1$wt-cfu.1$dsspAB)
cfu.1.test <- t.test(cfu.1$dif, mu = 0, alternative = "greater")

# Answer: yes, as expected, deletion of sspAB reduces heat tolerance
# two-sided: P = 0.01363
# one-sided: P = 0.006816
```

In the above analysis a pairing of samples from the same test group was made. However The groups are not truly paired across host types. Groups refer to spores that were assayed on the same day, colonies reflect true pairing, a single culture that was split into 3 phage treatments (SPO1, Goe2, noPhage). So next I compare slopes of uninfected cultures without pairing groups.

```
cfu.1b <- filter(d, assay == "CFU" & phage == "noPhage") %>%
  dplyr::select(host, assay, group, slope)
```

```
x.wt <- cfu.1b%>%
  filter(host=="wt")%>%
  pull(slope)

y.mut <- cfu.1b%>%
  filter(host=="dsspAB")%>%
  pull(slope)

cfu.1b.test <- t.test(x.wt,y.mut,alternative = "greater")

# Same answer: deletion of sspAB reduces heat tolerance
# two-sided: P = 0.005858
# one-sided: P = 0.002929
```

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

```
cfu.2 <- filter(d, assay == "CFU", host == "dsspAB") %>%
  dplyr::select(host, phage, assay, group, slope)

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

# calculate difference and test
cfu.2 <- mutate(cfu.2, dif.1 = cfu.2$noPhage-cfu.2$Goe2,
  dif.2 = cfu.2$noPhage - cfu.2$SP01)

cfu.2.Goe2.test <- t.test(cfu.2$dif.1, mu = 0, alternative = "less")
# one-sided: P = 0.1314

cfu.2.SP01.test <- t.test(cfu.2$dif.2, mu = 0, alternative = "less")
# one-sided: P = 0.06147

# we dont have an initail hypthesis on the direction of change for comparing the 2 phages.
# so using a two sided test,
cfu.2.phage.test <- t.test(c(cfu.2$dif.1, cfu.2$dif.2), mu = 0,
  alternative = "two.sided")
# one-sided: P = 0.02714

dplyr::bind_rows(
  broom::glance(cfu.2.Goe2.test),
  broom::glance(cfu.2.SP01.test),
  broom::glance(cfu.2.phage.test)
)

## # A tibble: 3 x 8
##   estimate statistic p.value parameter  conf.low conf.high method  alternative
##   <dbl>      <dbl>   <dbl>      <dbl>    <dbl>    <dbl> <chr>      <chr>
## 1 -0.00692    -1.54  0.131         2 -Inf      0.00617 One Samp~ less
## 2 -0.00983    -2.58  0.0615        2 -Inf      0.00129 One Samp~ less
```

```
## 3 -0.00837      -3.09  0.0271          5   -0.0153  -0.00141 One Samp~ two.sided
```

**conclusion:** border-line significance for the hypothesis that infection with SPO1 reduces heat-resistance of colony-forming spores. There is a significant difference (though adjustment for multiple hypothesis still needed) in resistance between spores from cultures infected with the 2 phage types.

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

In this comparison we are comparing slopes of viral spores to slopes of colony-forming spores in the WT. This is required since there are no viral spores in non-infected cultures.

```
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, group, slope)

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

# calculate difference and test
pfu <- mutate(pfu, dif.1 = pfu$noPhage-pfu$Goe2,
              dif.2 = pfu$noPhage - pfu$SP01)

pfu.Goe2.test <- t.test(pfu$dif.1, mu = 0, alternative = "less")
# one-sided: P = 0.3231

pfu.SP01.test <- t.test(pfu$dif.2, mu = 0, alternative = "less")
# one-sided: P = 0.4109

pfu.phage.test <- t.test(c(pfu$dif.1, pfu$dif.2), mu = 0,
                        alternative = "two.sided")
# one-sided: P = 0.5530

dplyr::bind_rows(
  broom::glance(pfu.Goe2.test),
  broom::glance(pfu.SP01.test),
  broom::glance(pfu.phage.test)
)
```

```
## # A tibble: 3 x 8
##   estimate statistic p.value parameter  conf.low conf.high method  alternative
##   <dbl>      <dbl>   <dbl>    <dbl>    <dbl>    <dbl> <chr>      <chr>
## 1 -0.00459    -0.535   0.323        2 -Inf        0.0205 One Samp~ less
## 2 -0.00189    -0.256   0.411        2 -Inf        0.0197 One Samp~ less
## 3 -0.00324    -0.636   0.553        5  -0.0164    0.00987 One Samp~ two.sided
```

**conclusion:** no support for the hypothesis of difference in slopes between viral spores and spores from non-infected cultures.

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

Our onesided hypothesis is that viral spores are more resistan, i.e have a greater slope value: slope.PFU-slopePFU > 0

```
cfu.pfu <- filter(d, host == "dsspAB", phage != "noPhage") %>%
  dplyr::select(host, phage, assay, group, slope)

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

# calculate difference and test
cfu.pfu <- mutate(cfu.pfu, dif = PFU-CFU)

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

cfu.pfu.phage.test <-
  cfu.pfu%>%
  t.test(dif~phage, mu = 0, alternative = "two.sided", data=.)
# one-sided: P = 0.4173

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

```
## # A tibble: 3 x 10
##   estimate statistic p.value parameter conf.low conf.high method alternative
##   <dbl>      <dbl>    <dbl>    <dbl>    <dbl>      <dbl> <chr>   <chr>
## 1 -0.00233    -0.469    0.657      2    -0.0168    Inf     One S~ greater
## 2 -0.00794    -2.18    0.920      2    -0.0186    Inf     One S~ greater
## 3  0.00561     0.913    0.417     3.67  -0.0121    0.0233 Welch~ two.sided
## # ... with 2 more variables: estimate1 <dbl>, estimate2 <dbl>
```

## Does infection with phage compromise resistance of WT spores?

```
cfu.3 <- filter(d, assay == "CFU", host == "wt") %>%
  dplyr::select(host, phage, assay, group, 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.1 = cfu.3$noPhage-cfu.3$Goe2,
  dif.2 = cfu.3$noPhage - cfu.3$SP01)

cfu.3.Goe2.test <- t.test(cfu.3$dif.1, mu = 0, alternative = "greater")
# one-sided: P = 0.09148

cfu.3.SP01.test <- t.test(cfu.3$dif.2, mu = 0, alternative = "greater")
# one-sided: P = 0.2998

cfu.3.phage.test <- t.test(c(cfu.3$dif.1, cfu.3$dif.2), mu = 0,
  alternative = "two.sided")
# one-sided: P = 0.1315

dplyr::bind_rows(
  broom::glance(cfu.3.Goe2.test),
  broom::glance(cfu.3.SP01.test),
  broom::glance(cfu.3.phage.test)
)
```

```
## # A tibble: 3 x 8
##   estimate statistic p.value parameter conf.low conf.high method alternative
##   <dbl>      <dbl>   <dbl>    <dbl>    <dbl>    <dbl> <chr>      <chr>
## 1  0.00934      2.00  0.0915      2 -0.00427  Inf    One Sampl~ greater
## 2  0.00108      0.618  0.300      2 -0.00403  Inf    One Sampl~ greater
## 3  0.00521      1.80  0.131      5 -0.00222  0.0126 One Sampl~ two.sided
```

**conclusion:** no support for lower resistance between WT colony-forming spores when infected by phage, and no difference in spores between phages.