

Pseudomonas Epiphytic Growth and Virulence Analysis

2024 Summer

Contents

Load Libraries	1
Load Data	2
Load colors	2
Goals	3
Virulence Analysis	3
Caclulcate survival probabilities for each strain and create dataframe	3
Make Kaplan-Meier Plot	8
Epiphytic Growth Analysis	10
Calculate mean/variance epiphytic growth ability	10
Plot Epiphytic Growth	11
Epiphytic growth stats	13
Combine epiphytic and virulence data	17
Plot epi virulence plot	18
Make Phylogenetic Tree of Strains	19
Combine my tree and my barplot	20
Session Information	20

Load Libraries

```
pacman::p_load(ggplot2, readxl, BiocManager, RColorBrewer, paletteer, aplot, ggbeeswarm,
  dplyr, tidyverse, devtools, emmeans, cowplot, phangorn, knitr, survival, here,
  tibble, survminer, lubridate, formatR, gridExtra, ggsurvfit, ggtree, treeio,
  gtsummary, phytools, tidycmprsk, install = FALSE)
```

Load Data

```
# Define the desired order of the treatments
strains_order <- c("194", "200", "204", "205", "215", "216", "220", "221", "227",
  "228", "B728a", "Cit7", "pisi", "Control")

strains_df <- read_excel("data/strains_R.xlsx")

epi_growth_data <- read_excel("data/pseud_epi_growth_2024summer_R.xlsx")

# Convert the strain variable to a factor with the specified order
epi_growth_data$treatment <- factor(epi_growth_data$treatment, levels = strains_order)

# Join the strains dataframe to epi growth
epi_growth_data <- left_join(epi_growth_data, strains_df, by = "treatment")

# Load virulence data
aphid_virulence_data <- read_csv("data/virulence_new_all.csv")

## Rows: 4973 Columns: 6
## -- Column specification -----
## Delimiter: ","
## chr (3): date, treatment, replicate
## dbl (3): individual, censored, time
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.

# Convert the treatment variable to a factor with the specified order
aphid_virulence_data$treatment <- factor(aphid_virulence_data$treatment, levels = strains_order)

# Join the strains dataframe to virulence
aphid_virulence_data <- left_join(aphid_virulence_data, strains_df, by = "treatment")

# Set a seed for this analysis
set.seed(3132001)
```

Load colors

```
# Extract the default ggplot colors
default_colors <- (scales::hue_pal())(length(strains_order) - 1)

# Create a named vector for strain colors, ensuring 'Control' is black
strain_colors <- setNames(c(default_colors, "black"), strains_order)

# Load species colors
species_colors <- c(`P. fluorescens` = "dodgerblue2", `P. syringae` = "springgreen3",
  `P. putida` = "sienna4", Control = "black")
```

```
species_colors_manual <- c(`194` = "sienna4", `200` = "dodgerblue2", `204` = "dodgerblue2",
  `205` = "dodgerblue2", `215` = "springgreen3", `216` = "dodgerblue2", `220` = "sienna4",
  `221` = "dodgerblue2", `227` = "dodgerblue2", `228` = "dodgerblue2", B728a = "springgreen3",
  Cit7 = "springgreen3", pisi = "springgreen3", Control = "black")
```

Goals

- Create Kaplan-Meier curve for Pseud. virulence data
- Use stats (Wilcox?) to determine statistical significance of each strain
- Compare virulence data with epiphytic growth ability

Virulence Analysis

Note: *In order to help me with this analysis, I am using the following sites - Survival Analysis in R and Hazard Ratio: Interpretation & Definition.*

Calculate survival probabilities for each strain and create dataframe

Survival model

```
# Fit the survival model
km_fit <- survfit(Surv(time, censored) ~ treatment, data = aphid_virulence_data)
```

Extract survival probability

```
# Fit the survival model
km_fit <- survfit(Surv(time, censored) ~ treatment, data = aphid_virulence_data)

# Extract survival probabilities at specific time points
time_points <- c(72)
km_summary <- summary(km_fit, times = time_points)

# Calculate standard error (assuming fit includes the necessary information)
std_error <- summary(km_fit, times = time_points)$std.err

# Initialize empty lists to store the results
times_list <- list()
treatment_list <- list()
surv_prob_list <- list()
std_error_list <- list()

# Loop over each treatment group and extract survival probabilities at
# specified time points
for (i in 1:length(km_fit$strata)) {
  treatment_name <- names(km_fit$strata)[i]
  for (t in time_points) {
```

```

idx <- which(km_summary$time == t & km_summary$strata == treatment_name)
if (length(idx) > 0) {
  times_list <- c(times_list, t)
  treatment_list <- c(treatment_list, treatment_name)
  surv_prob_list <- c(surv_prob_list, km_summary$surv[idx])
  std_error_list <- c(std_error_list, km_summary$std.err[idx])
} else {
  times_list <- c(times_list, t)
  treatment_list <- c(treatment_list, treatment_name)
  surv_prob_list <- c(surv_prob_list, NA)
  std_error_list <- c(std_error_list, NA)
}
}
}

# Create the data frame
surv_probs <- data.frame(time = unlist(times_list), treatment = unlist(treatment_list),
  surv_prob = unlist(surv_prob_list), std_error_data = unlist(std_error_list))

# Replace 'treatment=' with an empty string
surv_probs$treatment <- gsub("treatment=", "", surv_probs$treatment)

# Convert the treatment variable to a factor with the specified order
surv_probs$treatment <- factor(surv_probs$treatment, levels = strains_order)

# Join the survival dataframe to strains
surv_probs <- left_join(surv_probs, strains_df, by = "treatment")

# Print the data frame
print(surv_probs)

```

```

##      time treatment  surv_prob std_error_data      strain      -80
## 1    72      194 0.12244898   0.019117897 field sample #8    194
## 2    72      200 0.02230483   0.009003784 field sample #14   200
## 3    72      204 0.05281690   0.013272241 field sample #17   204
## 4    72      205 0.07118644   0.014971038 field sample #18-1  205
## 5    72      215 0.22222222   0.024497697 field sample #26-1  215
## 6    72      216 0.15625000   0.021395412 field sample #26-2  216
## 7    72      220 0.17229730   0.021949800 field sample #29-2  220
## 8    72      221 0.09363296   0.017828335 field sample #29-3  221
## 9    72      227 0.16949153   0.021844143 field sample #35    227
## 10   72      228 0.10447761   0.018684542 field sample #36    228
## 11   72      B728a 0.10996564   0.018339411      B728a ΔFlgK    B728a
## 12   72      Cit7 0.17073171   0.022210773      Cit7          14
## 13   72      Control 0.81678082   0.011319228      Control    Control
## 14   72      pisi 0.74151436   0.022370650      1704B         6
##      previous_work species_complex      species_match
## 1      new      P. fluorescens      P. paralactis
## 2      new      P. fluorescens      P. fluorescens
## 3      new      P. fluorescens      P. rhodesiae
## 4      new      P. fluorescens      P. orientalis
## 5      new      P. syringae      P. syringae
## 6      new      P. putida      P. putida

```

```

## 7          new P. fluorescens          P. pergaminensis
## 8          new P. fluorescens          P. fluorescens
## 9          new P. fluorescens          P. pergaminensis
## 10         new P. syringae            P. lijiangensis
## 11         old P. syringae P. syringae pv. Syringae
## 12         old P. syringae            P. syringae
## 13         old Control                Control
## 14         old P. syringae            P. syringae
##           collector                source      ANI
## 1          Kathryn L Herr           Pisum sativum  98.44
## 2          Kathryn L Herr           Pisum sativum  <NA>
## 3          Kathryn L Herr           Pisum sativum  98.92
## 4          Kathryn L Herr           Pisum sativum  95.78
## 5          Kathryn L Herr           Pisum sativum  95.57
## 6          Kathryn L Herr           Pisum sativum  95.42
## 7          Kathryn L Herr           Pisum sativum  98.64
## 8          Kathryn L Herr           Pisum sativum  <NA>
## 9          Kathryn L Herr           Pisum sativum  98.22
## 10         Kathryn L Herr           Pisum sativum  96.6
## 11 Alan Collmer/J. Lindemann? Phaseolus vulgaris  <NA>
## 12          <NA>                    Pisum sativum  <NA>
## 13          Control                Control Control
## 14         David Baltrus Citrus leaf surface  <NA>
##           genome_match      Reseq/Genbank
## 1 Pseudomonas paralactis DSM 29164 GCA_001439735.1
## 2          <NA>                <NA>
## 3 Pseudomonas rhodesiae CIP104664 GCF_024169765.1
## 4 Pseudomonas orientalis DSM 17489 GCF_001439815.1
## 5 Pseudomonas syringae DSM 10604 GCA_000597765.1
## 6 Pseudomonas putida S13.1.2 GCF_000498395.2
## 7 Pseudomonas pergaminensis 1008 GCF_024112395.1
## 8          <NA>                <NA>
## 9 Pseudomonas pergaminensis 1008 GCF_024112395.1
## 10 Pseudomonas lijiangensis LJ2 GCF_018968705.1
## 11          <NA>                CP0000075
## 12          <NA>                AEAJ 00000000
## 13          Control                Control
## 14          <NA>                AEAJ 00000000
##
## 1
## 2
## 3
## 4
## 5
## 6
## 7
## 8
## 9
## 10
## 11
177. https://doi.org/10.1146/annurev.py.28.090190.001103
## 12
177. https://doi.org/10.1146/annurev.py.28.090190.001103
## 13

```

```
## 14 Baltrus, D. A., Nishimura, M. T., Romanchuk, A., Chang, J. H., Mukhtar, M. S., Cherkis, K., Roach
```

Post-hoc test for survival

```
# Perform pairwise log-rank tests between treatment groups and the control
# group
pairwise_tests_treatment <- pairwise_survdif(Surv(time, censored) ~ treatment, data = aphid_virulence_

# Print the pairwise tests
print(pairwise_tests_treatment)
```

```
##
## Pairwise comparisons using Log-Rank test
##
## data: aphid_virulence_data and treatment
##
##      194      200      204      205      215      216      220      221      227
## 200    2.3e-12 -          -          -          -          -          -          -
## 204    1.6e-08 0.26489 -          -          -          -          -          -
## 205    3.7e-06 0.04009 0.38289 -          -          -          -          -
## 215    4.6e-05 < 2e-16 < 2e-16 7.8e-16 -          -          -          -
## 216    0.20589 3.0e-06 0.00054 0.00915 1.3e-05 -          -          -
## 220    1.2e-05 0.34987 0.95899 0.61067 7.5e-10 0.04787 -          -
## 221    0.06597 1.7e-06 0.00063 0.01560 3.0e-08 0.57084 0.05899 -          -
## 227    0.00048 0.05275 0.34910 0.75895 1.7e-08 0.14997 0.65606 0.23547 -
## 228    0.02457 2.3e-05 0.00265 0.03730 5.6e-09 0.52684 0.02668 0.75895 0.15756
## B728a  0.30241 3.8e-15 2.0e-10 1.5e-07 0.00193 0.15635 0.00089 0.00913 0.00558
## Cit7   0.97833 6.8e-10 1.0e-06 8.4e-05 0.00044 0.38289 0.00217 0.11439 0.01459
## Control < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16
## pisi   < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16 < 2e-16
##      228      B728a      Cit7      Control
## 200    -          -          -          -
## 204    -          -          -          -
## 205    -          -          -          -
## 215    -          -          -          -
## 216    -          -          -          -
## 220    -          -          -          -
## 221    -          -          -          -
## 227    -          -          -          -
## 228    -          -          -          -
## B728a  0.00265 -          -          -
## Cit7   0.05646 0.46912 -          -
## Control < 2e-16 < 2e-16 < 2e-16 -
## pisi   < 2e-16 < 2e-16 < 2e-16 0.00042
##
## P value adjustment method: BH
```

```
# Perform pairwise log-rank tests between species_complex groups and the
# control group
pairwise_tests_species <- pairwise_survdif(Surv(time, censored) ~ species_complex,
      data = aphid_virulence_data)
```

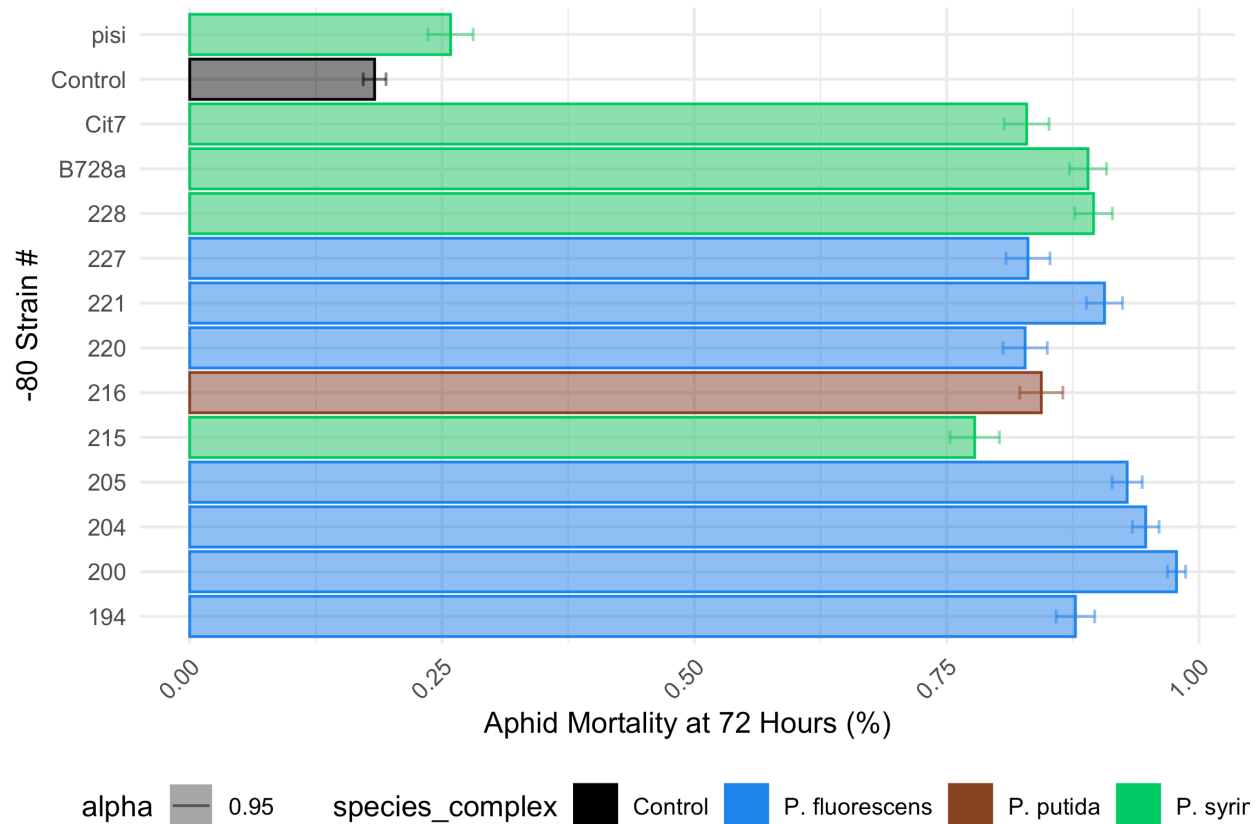
```
# Print the pairwise tests
print(pairwise_tests_species)
```

```
##
## Pairwise comparisons using Log-Rank test
##
## data: aphid_virulence_data and species_complex
##
##           Control P. fluorescens P. putida
## P. fluorescens < 2e-16 - -
## P. putida      < 2e-16 0.0084 -
## P. syringae    < 2e-16 < 2e-16 2.6e-10
##
## P value adjustment method: BH
```

Plot Survival Dataframe at different times

```
surv_plot <- ggplot(data = surv_probs, aes(x = treatment, y = (1-surv_prob), color = species_complex, fill = species_complex)) +
  geom_col() +
  geom_errorbar(aes(ymin = (1-surv_prob) - std_error_data, ymax = (1-surv_prob) + std_error_data),
    width = 0.3, # Width of error bars
    position = position_dodge(width = 0.9)) + # Dodge bars slightly
  labs(x = "-80 Strain #",
    y = "Aphid Mortality at 72 Hours (%)") +
  theme_minimal() +
  coord_flip() +
  scale_fill_manual(values = species_colors) +
  scale_color_manual(values = species_colors) +
  theme(plot.title = element_text(hjust = 0.5),
    axis.text.x = element_text(angle = 45, hjust = 1), legend.position = "bottom")

surv_plot
```



Make Kaplan-Meier Plot

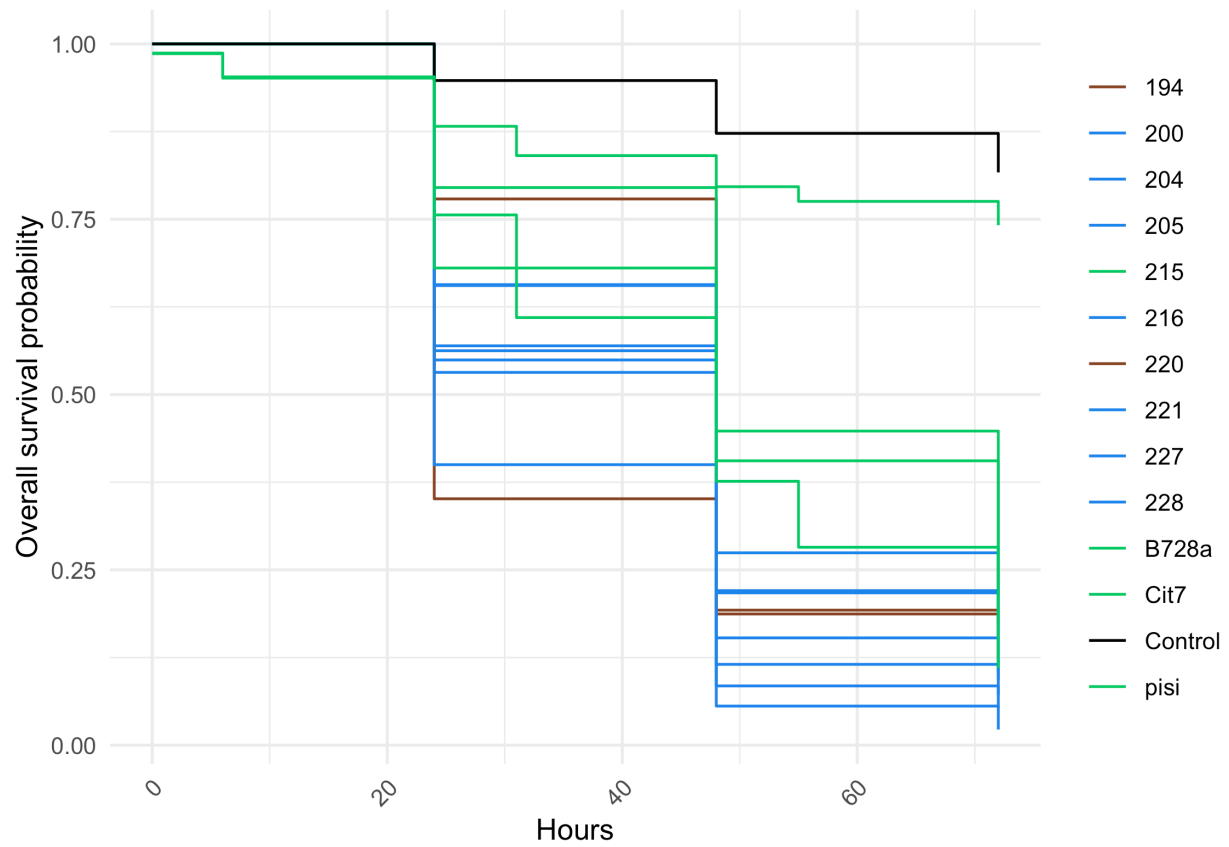
Cohort Survival Curve

```
# Plot the Kaplan-Meier survival curves by treatment
surv_curv_treatment <- survfit2(Surv(time, censored) ~ treatment, data = aphid_virulence_data) %>%
  ggsvfit(type = "survival") + labs(x = "Hours", y = "Overall survival probability") +
  theme_minimal() + scale_fill_manual(values = species_colors_manual) + scale_color_manual(values = species_colors_manual) +
  theme(plot.title = element_text(hjust = 0.5), axis.text.x = element_text(angle = 45, hjust = 1), legend.position = "right")

# Plot the Kaplan-Meier survival curves by species complex
surv_curv_species <- survfit2(Surv(time, censored) ~ species_complex, data = aphid_virulence_data) %>%
  ggsvfit(type = "survival") + labs(x = "Hours", y = "Overall survival probability") +
  theme_minimal() + scale_fill_manual(values = species_colors) + scale_color_manual(values = species_colors) +
  theme(plot.title = element_text(hjust = 0.5), axis.text.x = element_text(angle = 45, hjust = 1), legend.position = "right")

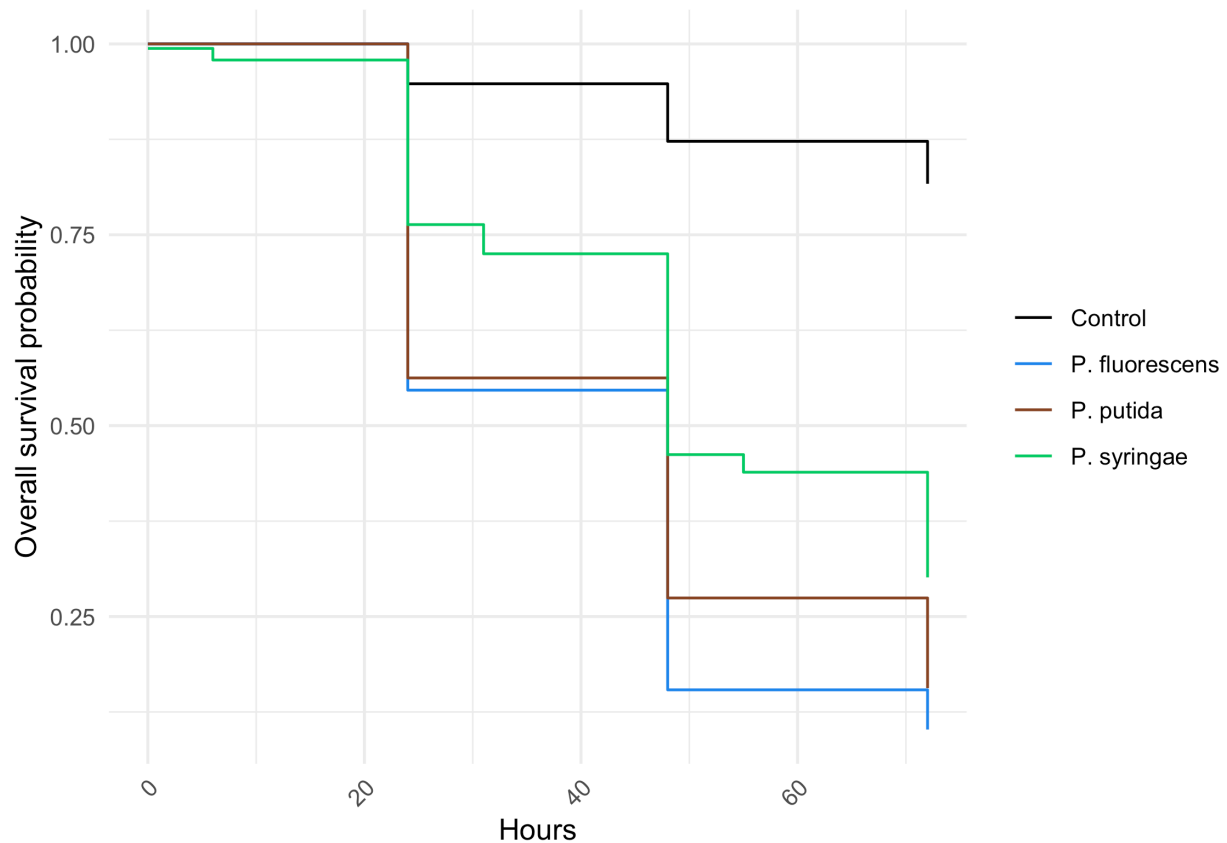
# show plots
surv_curv_treatment
```

```
## Warning: No shared levels found between `names(values)` of the manual scale and the
## data's fill values.
```

```
surv_curv_species
```

```
## Warning: No shared levels found between `names(values)` of the manual scale and the
## data's fill values.
```



Epiphytic Growth Analysis

Calculate mean/variance epiphytic growth ability

```
# Replace NA with a lower value or remove them for visualization Remove rows
# with NA in CFU_per_10_leafdiscs_log_10
epi_growth_clean <- epi_growth_data %>%
  filter(!is.na(CFU_per_10_leafdiscs_log_10))

# Convert CFU_per_10_leafdiscs_log_10 to numeric, handling scientific notation
epi_growth_clean$CFU_per_10_leafdiscs_log_10 <- as.numeric(gsub("<", "", epi_growth_clean$CFU_per_10_lea

## Warning: NAs introduced by coercion

# Subset data to remove unfinished strains and extract the letter part from the
# 'plant_rep' column
epi_growth_clean <- epi_growth_clean %>%
  mutate(plant_rep_letter = substr(plant_rep, 1, 1))

# Calculate the mean and SD CFU per strain for each plant rep letter and retain
# the specified columns
epi_growth_mean_per_rep <- epi_growth_clean %>%
```

```

group_by(treatment, plant_rep_letter) %>%
summarise(CFU_per_10_leafdiscs_log_10 = mean(CFU_per_10_leafdiscs_log_10, na.rm = TRUE),
  sd_CFU = sd(CFU_per_10_leafdiscs_log_10, na.rm = TRUE), sample = first(sample),
  person = first(person), species_complex = first(species_complex), block = first(block),
  treatment = first(treatment), plant_rep = first(plant_rep), .groups = "drop")

# Calculate the mean and SD CFU per strain for each plant rep letter and retain
# the specified columns
epi_growth_mean_per_strain <- epi_growth_clean %>%
  group_by(treatment) %>%
  summarise(CFU_per_10_leafdiscs_log_10 = mean(CFU_per_10_leafdiscs_log_10, na.rm = TRUE),
    sd_CFU = sd(CFU_per_10_leafdiscs_log_10, na.rm = TRUE), sample = first(sample),
    person = first(person), species_complex = first(species_complex), block = first(block),
    treatment = first(treatment), plant_rep = first(plant_rep), .groups = "drop")

epi_growth_mean_per_strain

```

```

## # A tibble: 13 x 8
##   treatment CFU_per_10_leafdiscs_l~1 sd_CFU sample person species_complex block
##   <chr>          <dbl>    <dbl> <chr>  <chr>  <chr>      <chr>
## 1 194          5.07      NA 194-A1 Havi   P. fluorescens 2-1
## 2 200          5.26      NA 200-A1 Havi   P. fluorescens 2-6
## 3 204          4.93      NA 204-A1 Havi   P. fluorescens 2-3
## 4 205          4.87      NA 205-A1 Sara  P. fluorescens 3
## 5 215          3         NA 215-A1 Sara  P. syringae    3
## 6 216          3.59      NA 216-A1 Sara  P. putida      3
## 7 220          4.82      NA 220-A1 Sara  P. fluorescens 5
## 8 221          5.25      NA 221-A1 Havi   P. fluorescens 2-7
## 9 227          4.16      NA 227-A1 Sara  P. fluorescens 5
## 10 228          3.83      NA 228-A1 Sara  P. syringae    5
## 11 B728a        3.72      NA B728a~ Sara  P. syringae    4
## 12 Cit7         4.28      NA Cit7~~ Havi   P. syringae    2-3
## 13 pisi         3.32      NA pisi~~ Havi   P. syringae    2-4
## # i abbreviated name: 1: CFU_per_10_leafdiscs_log_10
## # i 1 more variable: plant_rep <chr>

```

Plot Epiphytic Growth

```

# plot by treatment
epi_growth_treatment <- ggplot(data = epi_growth_mean_per_rep, aes(x = treatment,
  y = CFU_per_10_leafdiscs_log_10, color = species_complex, fill = species_complex)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3) + geom_beeswarm(stroke = 0.5, size = 0.8,
  alpha = 0.8) + theme_minimal() + coord_flip() + scale_fill_manual(values = species_colors) +
  scale_color_manual(values = species_colors) + scale_y_continuous(limits = c(2.5,
  5.5)) + theme(plot.title = element_text(hjust = 0.5), axis.text.x = element_text(angle = 45,
  hjust = 1), legend.position = "bottom")

# plot by species complex
epi_growth_species <- ggplot(data = epi_growth_mean_per_rep, aes(x = species_complex,
  y = CFU_per_10_leafdiscs_log_10, color = species_complex, fill = species_complex)) +
  geom_boxplot(outlier.shape = NA, alpha = 0.3) + geom_beeswarm(stroke = 0.5, size = 0.8,

```

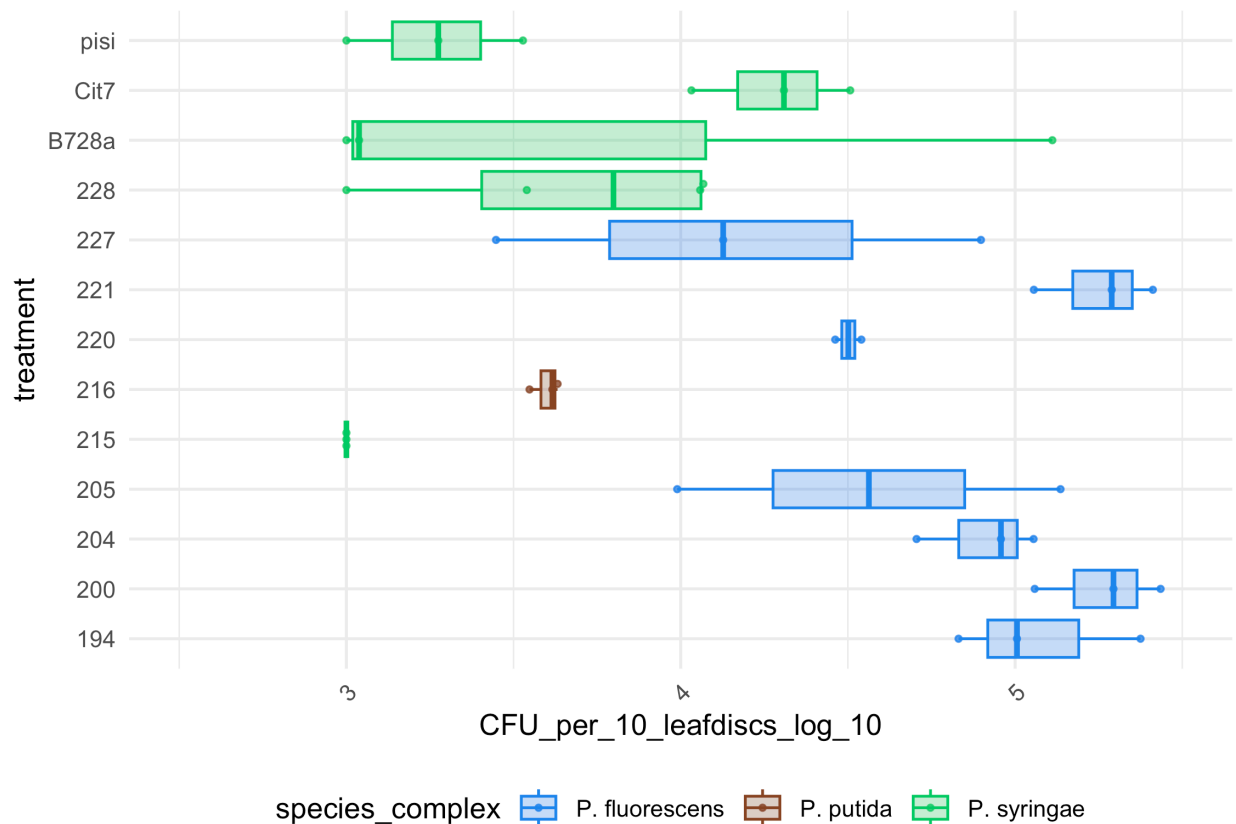
```
alpha = 0.8) + theme_minimal() + scale_fill_manual(values = species_colors) +
scale_color_manual(values = species_colors) + scale_y_continuous(limits = c(2.5,
5.5)) + theme(plot.title = element_text(hjust = 0.5), axis.text.x = element_text(angle = 45,
hjust = 1), legend.position = "bottom")
```

```
# show plots
```

```
epi_growth_treatment
```

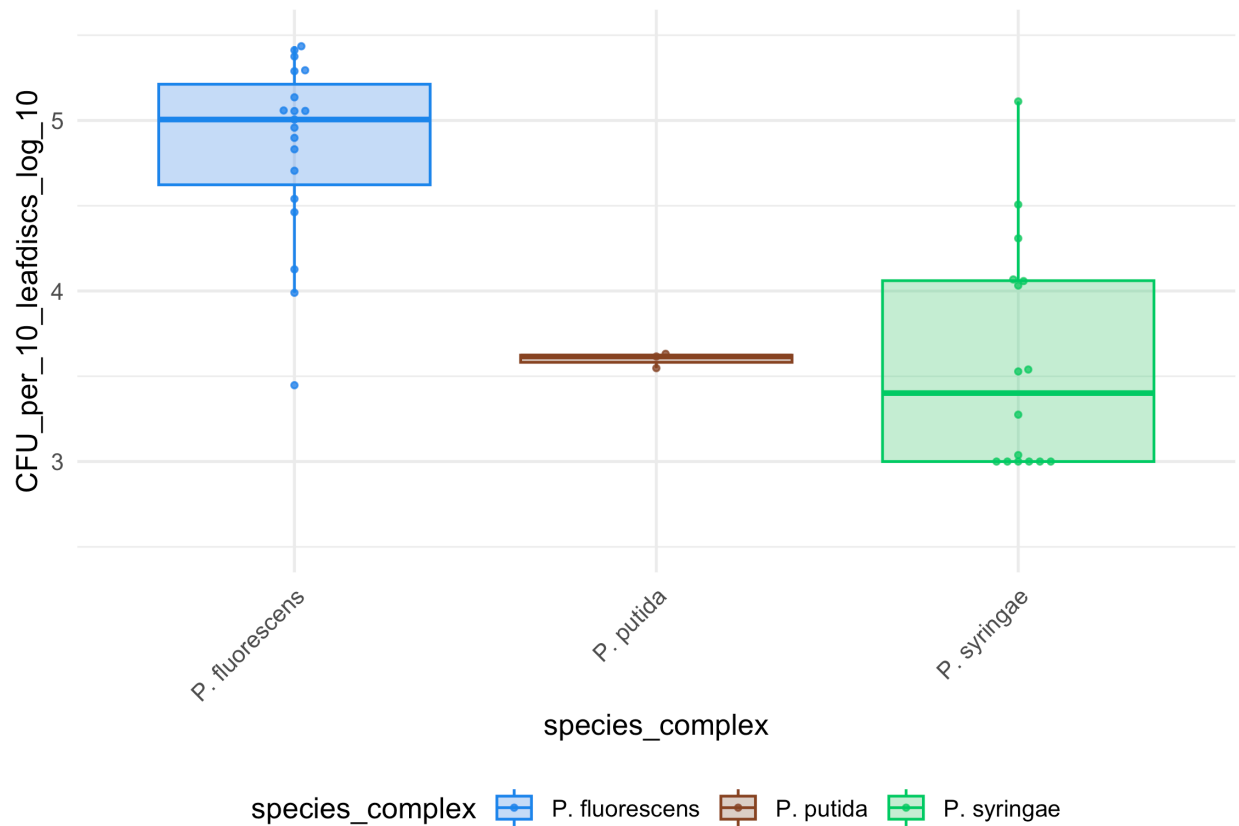
```
## Warning: Removed 2 rows containing non-finite outside the scale range
## (`stat_boxplot()`).
```

```
## Warning: Removed 2 rows containing missing values or values outside the scale range
## (`geom_point()`).
```



```
epi_growth_species
```

```
## Warning: Removed 2 rows containing non-finite outside the scale range
## (`stat_boxplot()`).
## Removed 2 rows containing missing values or values outside the scale range
## (`geom_point()`).
```



Epiphytic growth stats

```
# stats per treatment
epi_growth_mod_treatment = lm(CFU_per_10_leafdiscs_log_10 ~ treatment, data = epi_growth_mean_per_rep)

emmeans(epi_growth_mod_treatment, pairwise ~ treatment)
```

```
## $emmeans
## treatment emmean SE df lower.CL upper.CL
## 194 5.07 0.305 27 4.45 5.70
## 200 5.26 0.305 27 4.64 5.89
## 204 4.91 0.305 27 4.28 5.53
## 205 4.90 0.305 27 4.28 5.53
## 215 3.00 0.305 27 2.37 3.63
## 216 3.60 0.305 27 2.97 4.22
## 220 4.87 0.305 27 4.24 5.49
## 221 5.25 0.305 27 4.63 5.88
## 227 4.16 0.305 27 3.53 4.78
## 228 3.67 0.264 27 3.13 4.21
## B728a 3.72 0.305 27 3.09 4.34
## Cit7 4.28 0.305 27 3.66 4.91
## pisi 3.27 0.305 27 2.64 3.89
##
```

```

## Confidence level used: 0.95
##
## $contrasts
## contrast      estimate      SE df t.ratio p.value
## 194 - 200      -0.19212 0.431 27  -0.446  1.0000
## 194 - 204       0.16452 0.431 27   0.382  1.0000
## 194 - 205       0.16752 0.431 27   0.389  1.0000
## 194 - 215       2.07066 0.431 27   4.807  0.0028
## 194 - 216       1.47217 0.431 27   3.417  0.0782
## 194 - 220       0.20476 0.431 27   0.475  1.0000
## 194 - 221      -0.18180 0.431 27  -0.422  1.0000
## 194 - 227       0.91321 0.431 27   2.120  0.6504
## 194 - 228       1.40429 0.403 27   3.485  0.0676
## 194 - B728a     1.35417 0.431 27   3.143  0.1373
## 194 - Cit7      0.78812 0.431 27   1.829  0.8188
## 194 - pisi      1.80298 0.431 27   4.185  0.0134
## 200 - 204       0.35664 0.431 27   0.828  0.9996
## 200 - 205       0.35964 0.431 27   0.835  0.9996
## 200 - 215       2.26278 0.431 27   5.253  0.0009
## 200 - 216       1.66429 0.431 27   3.863  0.0288
## 200 - 220       0.39688 0.431 27   0.921  0.9990
## 200 - 221       0.01032 0.431 27   0.024  1.0000
## 200 - 227       1.10533 0.431 27   2.566  0.3752
## 200 - 228       1.59641 0.403 27   3.962  0.0228
## 200 - B728a     1.54629 0.431 27   3.589  0.0537
## 200 - Cit7      0.98024 0.431 27   2.275  0.5509
## 200 - pisi      1.99510 0.431 27   4.631  0.0044
## 204 - 205       0.00299 0.431 27   0.007  1.0000
## 204 - 215       1.90614 0.431 27   4.425  0.0074
## 204 - 216       1.30765 0.431 27   3.035  0.1691
## 204 - 220       0.04024 0.431 27   0.093  1.0000
## 204 - 221      -0.34632 0.431 27  -0.804  0.9997
## 204 - 227       0.74869 0.431 27   1.738  0.8620
## 204 - 228       1.23976 0.403 27   3.077  0.1563
## 204 - B728a     1.18965 0.431 27   2.762  0.2756
## 204 - Cit7      0.62360 0.431 27   1.448  0.9559
## 204 - pisi      1.63845 0.431 27   3.803  0.0331
## 205 - 215       1.90314 0.431 27   4.418  0.0075
## 205 - 216       1.30465 0.431 27   3.029  0.1713
## 205 - 220       0.03724 0.431 27   0.086  1.0000
## 205 - 221      -0.34931 0.431 27  -0.811  0.9997
## 205 - 227       0.74569 0.431 27   1.731  0.8650
## 205 - 228       1.23677 0.403 27   3.069  0.1586
## 205 - B728a     1.18665 0.431 27   2.755  0.2788
## 205 - Cit7      0.62060 0.431 27   1.441  0.9574
## 205 - pisi      1.63546 0.431 27   3.796  0.0336
## 215 - 216      -0.59849 0.431 27  -1.389  0.9671
## 215 - 220      -1.86590 0.431 27  -4.331  0.0093
## 215 - 221      -2.25246 0.431 27  -5.229  0.0010
## 215 - 227      -1.15745 0.431 27  -2.687  0.3114
## 215 - 228      -0.66637 0.403 27  -1.654  0.8961
## 215 - B728a    -0.71649 0.431 27  -1.663  0.8925
## 215 - Cit7     -1.28254 0.431 27  -2.977  0.1886
## 215 - pisi     -0.26768 0.431 27  -0.621  1.0000

```

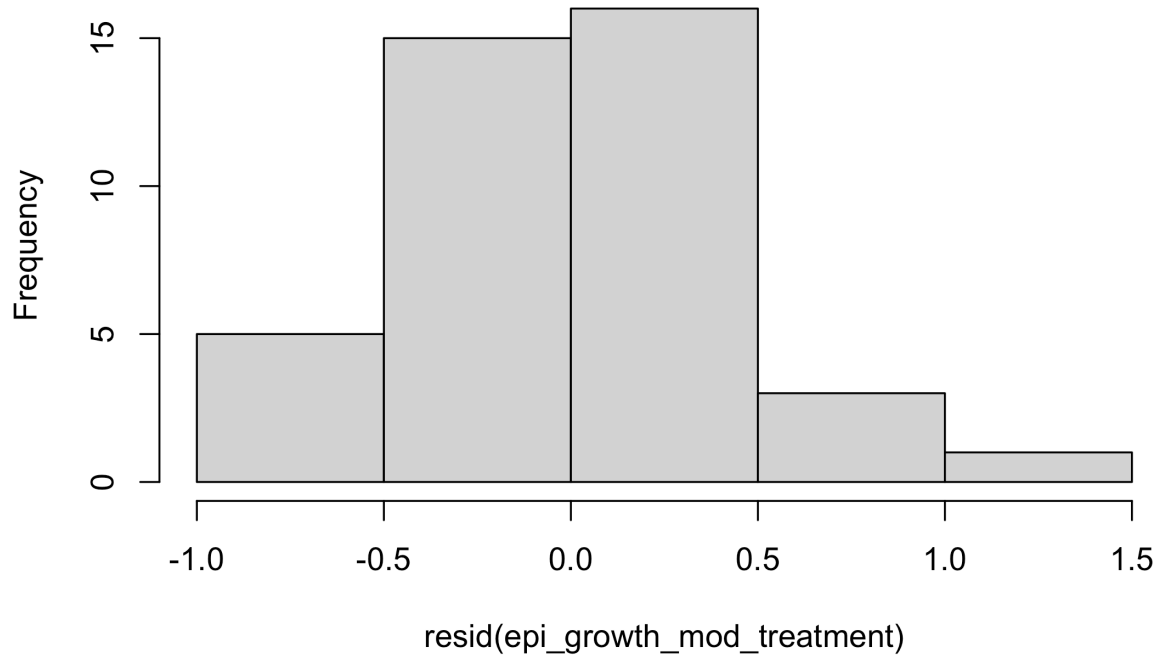
```

## 216 - 220      -1.26741 0.431 27  -2.942 0.2011
## 216 - 221      -1.65397 0.431 27  -3.839 0.0304
## 216 - 227      -0.55896 0.431 27  -1.298 0.9802
## 216 - 228      -0.06788 0.403 27  -0.168 1.0000
## 216 - B728a    -0.11800 0.431 27  -0.274 1.0000
## 216 - Cit7     -0.68405 0.431 27  -1.588 0.9188
## 216 - pisi      0.33081 0.431 27   0.768 0.9998
## 220 - 221      -0.38656 0.431 27  -0.897 0.9992
## 220 - 227       0.70845 0.431 27   1.645 0.8995
## 220 - 228       1.19953 0.403 27   2.977 0.1887
## 220 - B728a    1.14941 0.431 27   2.668 0.3208
## 220 - Cit7      0.58336 0.431 27   1.354 0.9727
## 220 - pisi      1.59822 0.431 27   3.710 0.0410
## 221 - 227       1.09501 0.431 27   2.542 0.3886
## 221 - 228       1.58608 0.403 27   3.936 0.0243
## 221 - B728a    1.53597 0.431 27   3.565 0.0567
## 221 - Cit7      0.96992 0.431 27   2.251 0.5662
## 221 - pisi      1.98477 0.431 27   4.607 0.0047
## 227 - 228       0.49108 0.403 27   1.219 0.9880
## 227 - B728a    0.44096 0.431 27   1.024 0.9973
## 227 - Cit7     -0.12509 0.431 27  -0.290 1.0000
## 227 - pisi      0.88977 0.431 27   2.065 0.6844
## 228 - B728a   -0.05012 0.403 27  -0.124 1.0000
## 228 - Cit7     -0.61617 0.403 27  -1.529 0.9362
## 228 - pisi      0.39869 0.403 27   0.989 0.9980
## B728a - Cit7   -0.56605 0.431 27  -1.314 0.9782
## B728a - pisi    0.44881 0.431 27   1.042 0.9969
## Cit7 - pisi     1.01486 0.431 27   2.356 0.5000
##
## P value adjustment: tukey method for comparing a family of 13 estimates

```

```
hist(resid(epi_growth_mod_treatment))
```

Histogram of resid(epi_growth_mod_treatment)

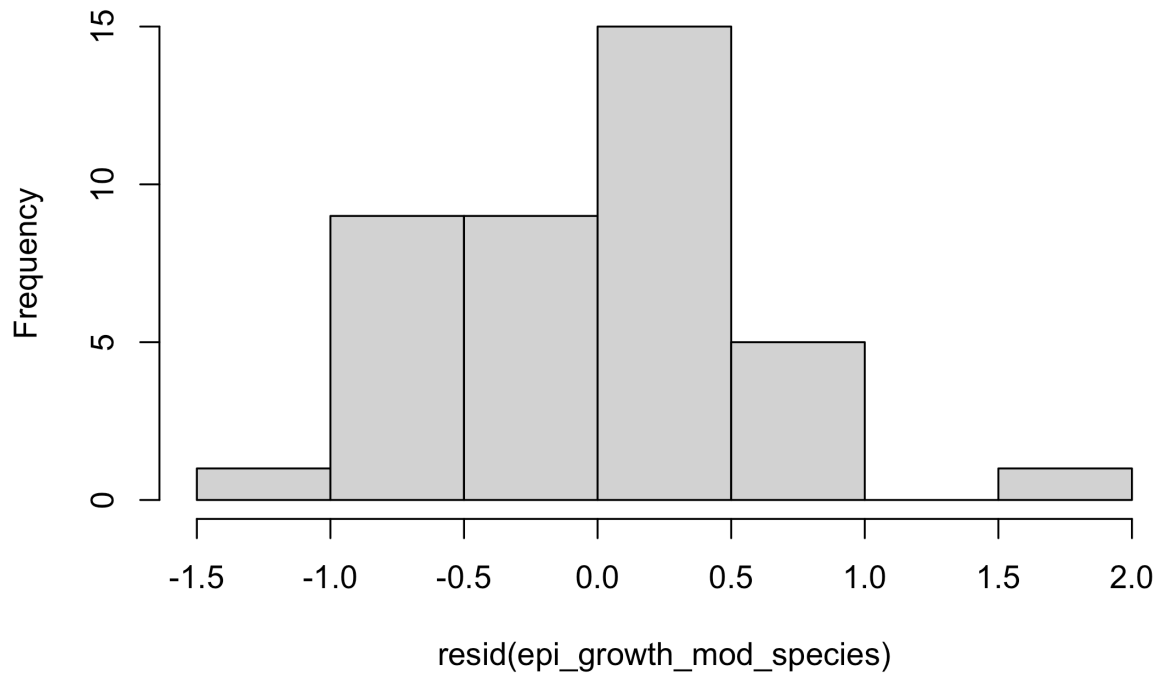


```
# stats per species
epi_growth_mod_species = lm(CFU_per_10_leafdiscs_log_10 ~ species_complex, data = epi_growth_mean_per_r
emmeans(epi_growth_mod_species, pairwise ~ species_complex)
```

```
## $emmeans
## species_complex emmean SE df lower.CL upper.CL
## P. fluorescens 4.92 0.129 37 4.66 5.18
## P. putida 3.60 0.341 37 2.91 4.29
## P. syringae 3.59 0.148 37 3.29 3.89
##
## Confidence level used: 0.95
##
## $contrasts
## contrast estimate SE df t.ratio p.value
## P. fluorescens - P. putida 1.31844 0.364 37 3.619 0.0025
## P. fluorescens - P. syringae 1.32533 0.196 37 6.767 <.0001
## P. putida - P. syringae 0.00689 0.371 37 0.019 0.9998
##
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
hist(resid(epi_growth_mod_species))
```


Histogram of resid(epi_growth_mod_species)



Combine epiphytic and virulence data

```
# Combine strain_stats and surv_probs using left_join
epi_virulence_data <- left_join(epi_growth_mean_per_strain, surv_probs, by = "treatment")

# Print the combined data
print(epi_virulence_data)
```

```
## # A tibble: 13 x 22
##   treatment CFU_per_10_leafdiscs~1 sd_CFU sample person species_complex.x block
##   <chr>          <dbl>    <dbl> <chr>  <chr>  <chr>          <chr>
## 1 194            5.07      NA 194-A1 Havi    P. fluorescens 2-1
## 2 200            5.26      NA 200-A1 Havi    P. fluorescens 2-6
## 3 204            4.93      NA 204-A1 Havi    P. fluorescens 2-3
## 4 205            4.87      NA 205-A1 Sara    P. fluorescens 3
## 5 215            3        NA 215-A1 Sara    P. syringae    3
## 6 216            3.59      NA 216-A1 Sara    P. putida      3
## 7 220            4.82      NA 220-A1 Sara    P. fluorescens 5
## 8 221            5.25      NA 221-A1 Havi    P. fluorescens 2-7
## 9 227            4.16      NA 227-A1 Sara    P. fluorescens 5
## 10 228            3.83      NA 228-A1 Sara    P. syringae    5
## 11 B728a         3.72      NA B728a~ Sara    P. syringae    4
## 12 Cit7          4.28      NA Cit7~~ Havi    P. syringae    2-3
```

```
## 13 pisi 3.32 NA pisi-- Havi P. syringae 2-4
## # i abbreviated name: 1: CFU_per_10_leafdiscs_log_10
## # i 15 more variables: plant_rep <chr>, time <dbl>, surv_prob <dbl>,
## # std_error_data <dbl>, strain <chr>, `~80` <chr>, previous_work <chr>,
## # species_complex.y <chr>, species_match <chr>, collector <chr>,
## # source <chr>, ANI <chr>, genome_match <chr>, `Reseq/Genbank` <chr>,
## # Paper <chr>
```

```
# Calculate correlation coefficient between survival probability and epiphytic
# growth ability
correlation <- -1 * cor(epi_virulence_data$surv_prob, epi_virulence_data$CFU_per_10_leafdiscs_log_10,
  method = "pearson")
```

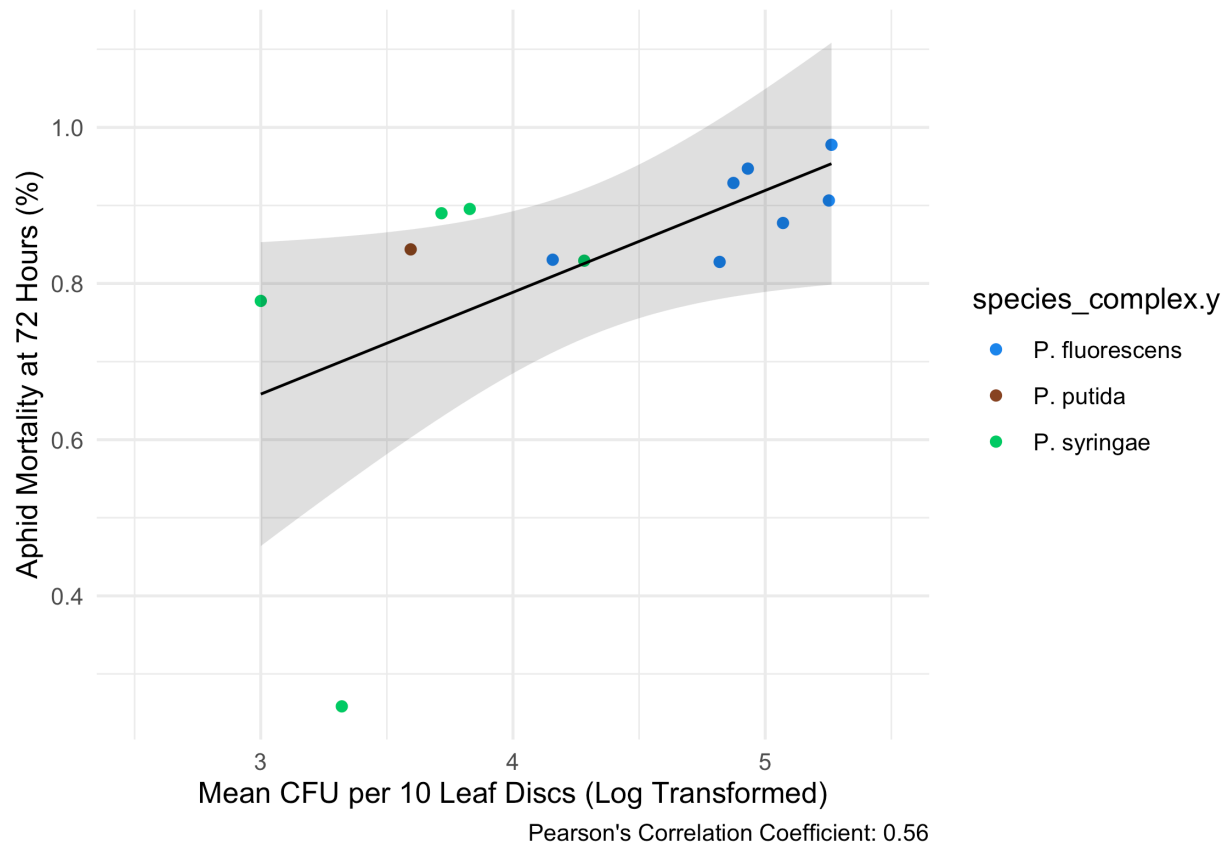
Plot epi virulence plot

```
# Create scatter plot for 72 hours
epi_virulence_plot_strains <- ggplot(epi_virulence_data, aes(x = CFU_per_10_leafdiscs_log_10,
  y = 1 - surv_prob)) + geom_point(aes(color = species_complex.y)) + geom_smooth(method = "lm",
  se = TRUE, color = "black", fill = "black", alpha = 0.15, linewidth = 0.5) +
  labs(x = "Mean CFU per 10 Leaf Discs (Log Transformed)", y = "Aphid Mortality at 72 Hours (%)",
  caption = paste("Pearson's Correlation Coefficient:", round(correlation,
    2))) + scale_x_continuous(limits = c(2.5, 5.5)) + scale_fill_manual(values = species_colors) +
  scale_color_manual(values = species_colors) + theme_minimal()

epi_virulence_plot_strains
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

```
## Warning: No shared levels found between `names(values)` of the manual scale and the
## data's fill values.
```



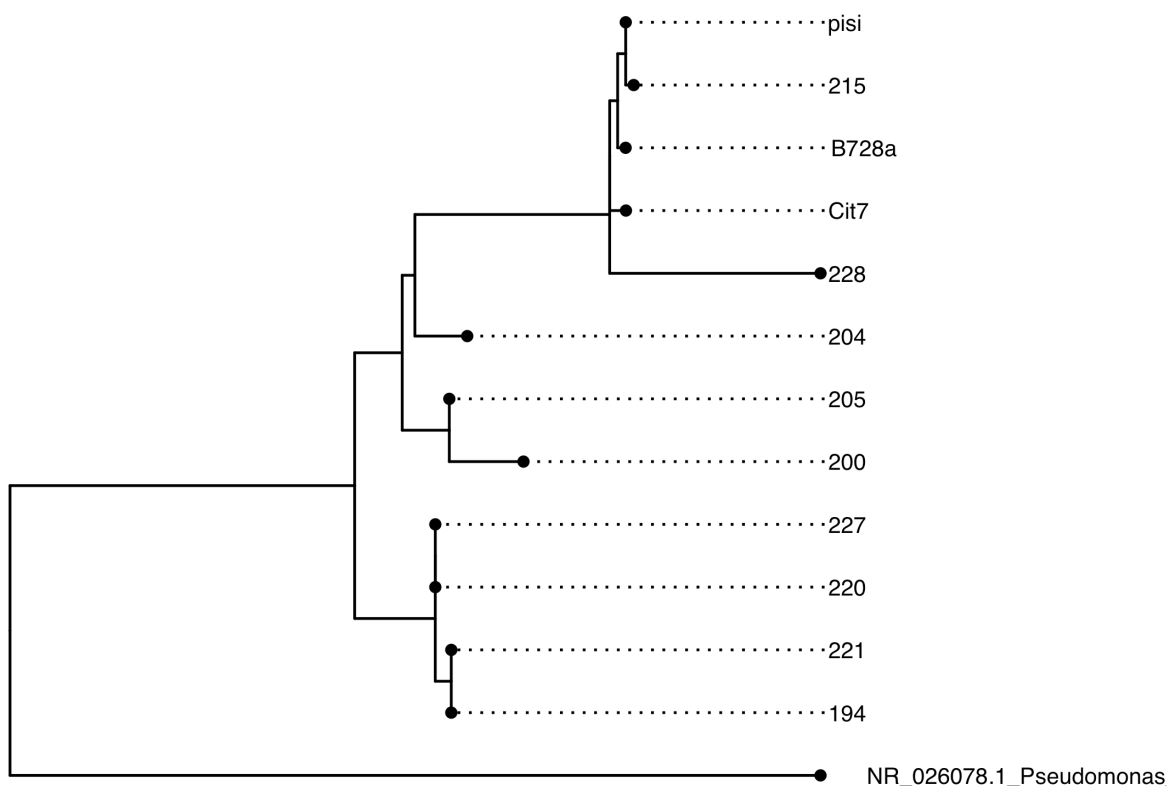
Make Phylogenetic Tree of Strains

```
# Load the tree from the .contree file
tree <- read.tree("tree/EpiVir_IQTree_2024july9/EpiVir_16S.contree")

# Root the tree at the midpoint
midpoint_tree <- midpoint(tree)

# Visualize the tree with customizations
phylo_tree <- ggtree(midpoint_tree) + geom_tiplab(size = 3, hjust = -0.1, align = TRUE) +
  geom_tippoint() + xlim(0, 0.09)
# +
# geom_text(aes(label=node), hjust=-1)
# + geom_cladelabel(node=17, label='Some random clade', color='red', offset=.8)

phylo_tree
```



Combine my tree and my barplot

```
# https://4va.github.io/biodatasci/r-ggtree.html#connecting\_taxa
# https://yulab-smu.top/treedata-book/chapter7.html

surv_plot_no_legend

surv_plot_no_legend %>%
  insert_left(phylo_tree)
# note: the tips are not lining up properly
```

Session Information

```
devtools::session_info()
```

```
## - Session info -----
## setting value
```

```

## version R version 4.4.0 (2024-04-24)
## os      macOS Ventura 13.4
## system  x86_64, darwin20
## ui      X11
## language (EN)
## collate en_US.UTF-8
## ctype   en_US.UTF-8
## tz      America/New_York
## date    2024-07-12
## pandoc  3.1.11 @ /Applications/RStudio.app/Contents/Resources/app/quarto/bin/tools/x86_64/ (via rm
##
## - Packages -----
## ! package      * version    date (UTC) lib source
##   abind          1.4-5      2016-07-21 [1] RSPM (R 4.4.0)
##   ape            * 5.8       2024-04-11 [1] RSPM (R 4.4.0)
##   P aplot        * 0.2.3     2024-06-17 [?] RSPM
##   backports      1.5.0     2024-05-23 [1] RSPM (R 4.4.0)
##   beeswarm       0.4.0     2021-06-01 [1] RSPM (R 4.4.0)
##   P BiocManager  * 1.30.23   2024-05-04 [?] RSPM
##   bit            4.0.5     2022-11-15 [1] RSPM (R 4.4.0)
##   bit64          4.0.5     2020-08-30 [1] RSPM (R 4.4.0)
##   broom          1.0.6     2024-05-17 [1] RSPM (R 4.4.0)
##   broom.helpers  1.15.0    2024-04-05 [1] RSPM (R 4.4.0)
##   P cachem       1.1.0     2024-05-16 [?] RSPM
##   car            3.1-2     2023-03-30 [1] RSPM (R 4.4.0)
##   carData        3.0-5     2022-01-06 [1] RSPM (R 4.4.0)
##   cellranger     1.1.0     2016-07-27 [1] RSPM (R 4.4.0)
##   P cli          3.6.3     2024-06-21 [?] RSPM
##   P clusterGeneration 1.3.8     2023-08-16 [?] RSPM
##   P coda         0.19-4.1  2024-01-31 [?] RSPM
##   P codetools    0.2-20    2024-03-31 [?] CRAN (R 4.4.0)
##   P colorspace   2.1-0     2023-01-23 [?] CRAN (R 4.4.0)
##   P combinat     0.0-8     2012-10-29 [?] RSPM
##   cowplot        * 1.1.3     2024-01-22 [1] RSPM (R 4.4.0)
##   P crayon       1.5.3     2024-06-20 [?] RSPM
##   data.table     1.15.4    2024-03-30 [1] RSPM (R 4.4.0)
##   P DEoptim      2.2-8     2022-11-11 [?] RSPM
##   P devtools     * 2.4.5     2022-10-11 [?] RSPM
##   P digest       0.6.36    2024-06-23 [?] RSPM
##   P doParallel   1.0.17    2022-02-07 [?] RSPM
##   dplyr          * 1.1.4     2023-11-17 [1] RSPM (R 4.4.0)
##   P ellipsis     0.3.2     2021-04-29 [?] RSPM
##   emmeans       * 1.10.3    2024-07-01 [1] RSPM (R 4.4.0)
##   estimability   1.5.1     2024-05-12 [1] RSPM (R 4.4.0)
##   P evaluate     0.24.0    2024-06-10 [?] RSPM
##   P expm         0.999-9   2024-01-11 [?] RSPM
##   P fansi        1.0.6     2023-12-08 [?] CRAN (R 4.4.0)
##   P farver       2.1.2     2024-05-13 [?] CRAN (R 4.4.0)
##   P fastmap      1.2.0     2024-05-15 [?] RSPM
##   P fastmatch    1.1-4     2023-08-18 [?] RSPM
##   forcats       * 1.0.0     2023-01-29 [1] RSPM (R 4.4.0)
##   P foreach      1.5.2     2022-02-02 [?] RSPM
##   formatR       * 1.14      2023-01-17 [1] RSPM (R 4.4.0)
##   P fs           1.6.4     2024-04-25 [?] CRAN (R 4.4.0)

```

##	generics	0.1.3	2022-07-05	[1]	RSPM (R 4.4.0)
##	ggbeeswarm	* 0.7.2	2023-04-29	[1]	RSPM (R 4.4.0)
##	ggfun	0.1.5	2024-05-28	[1]	RSPM (R 4.4.0)
##	P ggplot2	* 3.5.1	2024-04-23	[?]	CRAN (R 4.4.0)
##	ggplotify	0.1.2	2023-08-09	[1]	RSPM (R 4.4.0)
##	ggpubr	* 0.6.0	2023-02-10	[1]	RSPM (R 4.4.0)
##	ggsignif	0.6.4	2022-10-13	[1]	RSPM (R 4.4.0)
##	ggsurvfit	* 1.1.0	2024-05-08	[1]	RSPM (R 4.4.0)
##	P ggtree	* 3.12.0	2024-04-30	[?]	Bioconduc~
##	P glue	1.7.0	2024-01-09	[?]	CRAN (R 4.4.0)
##	gridExtra	* 2.3	2017-09-09	[1]	RSPM (R 4.4.0)
##	gridGraphics	0.5-1	2020-12-13	[1]	RSPM (R 4.4.0)
##	gt	0.10.1	2024-01-17	[1]	RSPM (R 4.4.0)
##	P gtable	0.3.5	2024-04-22	[?]	CRAN (R 4.4.0)
##	gtsummary	* 1.7.2	2023-07-15	[1]	RSPM (R 4.4.0)
##	here	* 1.0.1	2020-12-13	[1]	RSPM (R 4.4.0)
##	hms	1.1.3	2023-03-21	[1]	RSPM (R 4.4.0)
##	P htmltools	0.5.8.1	2024-04-04	[?]	CRAN (R 4.4.0)
##	P htmlwidgets	1.6.4	2023-12-06	[?]	CRAN (R 4.4.0)
##	P httpuv	1.6.15	2024-03-26	[?]	RSPM
##	P igraph	2.0.3	2024-03-13	[?]	RSPM
##	P iterators	1.0.14	2022-02-05	[?]	RSPM
##	P jsonlite	1.8.8	2023-12-04	[?]	CRAN (R 4.4.0)
##	km.ci	0.5-6	2022-04-06	[1]	RSPM (R 4.4.0)
##	KMsurv	0.1-5	2012-12-03	[1]	RSPM (R 4.4.0)
##	P knitr	* 1.48	2024-07-07	[?]	RSPM
##	P labeling	0.4.3	2023-08-29	[?]	CRAN (R 4.4.0)
##	P later	1.3.2	2023-12-06	[?]	RSPM
##	P lattice	0.22-6	2024-03-20	[?]	CRAN (R 4.4.0)
##	lazyeval	0.2.2	2019-03-15	[1]	RSPM (R 4.4.0)
##	P lifecycle	1.0.4	2023-11-07	[?]	CRAN (R 4.4.0)
##	lubridate	* 1.9.3	2023-09-27	[1]	RSPM (R 4.4.0)
##	P magrittr	2.0.3	2022-03-30	[?]	CRAN (R 4.4.0)
##	P maps	* 3.4.2	2023-12-15	[?]	RSPM
##	P MASS	7.3-61	2024-06-13	[?]	CRAN (R 4.4.0)
##	P Matrix	1.7-0	2024-03-22	[?]	CRAN (R 4.4.0)
##	P memoise	2.0.1	2021-11-26	[?]	CRAN (R 4.4.0)
##	P mgcv	1.9-1	2023-12-21	[?]	CRAN (R 4.4.0)
##	P mime	0.12	2021-09-28	[?]	CRAN (R 4.4.0)
##	P miniUI	0.1.1.1	2018-05-18	[?]	RSPM
##	P mnormt	2.1.1	2022-09-26	[?]	RSPM
##	P munsell	0.5.1	2024-04-01	[?]	CRAN (R 4.4.0)
##	mvtnorm	1.2-5	2024-05-21	[1]	RSPM (R 4.4.0)
##	P nlme	3.1-165	2024-06-06	[?]	CRAN (R 4.4.0)
##	numDeriv	2016.8-1.1	2019-06-06	[1]	RSPM (R 4.4.0)
##	P optimParallel	1.0-2	2021-02-11	[?]	RSPM
##	P pacman	0.5.1	2019-03-11	[?]	CRAN (R 4.4.0)
##	paletteer	* 1.6.0	2024-01-21	[1]	RSPM (R 4.4.0)
##	patchwork	1.2.0	2024-01-08	[1]	RSPM (R 4.4.0)
##	P phangorn	* 2.11.1	2023-01-23	[?]	RSPM
##	P phytools	* 2.3-0	2024-06-13	[?]	RSPM
##	P pillar	1.9.0	2023-03-22	[?]	CRAN (R 4.4.0)
##	P pkgbuild	1.4.4	2024-03-17	[?]	RSPM
##	P pkgconfig	2.0.3	2019-09-22	[?]	CRAN (R 4.4.0)

```

## P pkgload          1.4.0      2024-06-28 [?] RSPM
## P profvis          0.3.8      2023-05-02 [?] RSPM
## P promises         1.3.0      2024-04-05 [?] RSPM
## P purrr            * 1.0.2     2023-08-10 [?] CRAN (R 4.4.0)
## P quadprog         1.5-8      2019-11-20 [?] RSPM
## P R6                2.5.1     2021-08-19 [?] CRAN (R 4.4.0)
## P RColorBrewer     * 1.1-3     2022-04-03 [?] CRAN (R 4.4.0)
## P Rcpp             1.0.12     2024-01-09 [?] CRAN (R 4.4.0)
##   readr            * 2.1.5     2024-01-10 [1] RSPM (R 4.4.0)
##   readxl           * 1.4.3     2023-07-06 [1] RSPM (R 4.4.0)
## P rematch2         2.1.2     2020-05-01 [?] CRAN (R 4.4.0)
## P remotes          2.5.0      2024-03-17 [?] CRAN (R 4.4.0)
## P renv             1.0.7      2024-04-11 [1] RSPM (R 4.4.0)
## P rlang            1.1.4      2024-06-04 [?] RSPM
## P rmarkdown        2.27       2024-05-17 [?] RSPM
## P rprojroot        2.0.4      2023-11-05 [?] CRAN (R 4.4.0)
##   rstatix          0.7.2      2023-02-01 [1] RSPM (R 4.4.0)
## P rstudioapi       0.16.0     2024-03-24 [?] CRAN (R 4.4.0)
## P scales           1.3.0      2023-11-28 [?] CRAN (R 4.4.0)
## P scatterplot3d    0.3-44     2023-05-05 [?] RSPM
## P sessioninfo      1.2.2      2021-12-06 [?] RSPM
## P shiny            1.8.1.1    2024-04-02 [?] RSPM
## P stringi          1.8.4      2024-05-06 [?] RSPM
## P stringr          * 1.5.1     2023-11-14 [?] CRAN (R 4.4.0)
## P survival          * 3.7-0     2024-06-05 [?] CRAN (R 4.4.0)
##   survminer        * 0.4.9     2021-03-09 [1] RSPM (R 4.4.0)
##   survMisc         0.5.6      2022-04-07 [1] RSPM (R 4.4.0)
## P tibble           * 3.2.1     2023-03-20 [?] CRAN (R 4.4.0)
##   tidycmprsk       * 1.0.0     2023-10-30 [1] RSPM (R 4.4.0)
##   tidyr            * 1.3.1     2024-01-24 [1] RSPM (R 4.4.0)
##   tidyselect       1.2.1     2024-03-11 [1] RSPM (R 4.4.0)
##   tidytree         0.4.6     2023-12-12 [1] RSPM (R 4.4.0)
##   tidyverse        * 2.0.0     2023-02-22 [1] RSPM (R 4.4.0)
##   timechange       0.3.0     2024-01-18 [1] RSPM (R 4.4.0)
##   treeio           * 1.28.0    2024-04-30 [1] Bioconductor 3.19 (R 4.4.0)
##   tzdb            0.4.0     2023-05-12 [1] RSPM (R 4.4.0)
## P urlchecker       1.0.1     2021-11-30 [?] RSPM
## P usethis          * 2.2.3     2024-02-19 [?] RSPM
## P utf8             1.2.4     2023-10-22 [?] CRAN (R 4.4.0)
## P vctrs            0.6.5     2023-12-01 [?] CRAN (R 4.4.0)
##   vipor           0.4.7     2023-12-18 [1] RSPM (R 4.4.0)
##   vroom           1.6.5     2023-12-05 [1] RSPM (R 4.4.0)
## P withr           3.0.0     2024-01-16 [?] CRAN (R 4.4.0)
## P xfun            0.45      2024-06-16 [?] RSPM
## P xml2            1.3.6     2023-12-04 [?] CRAN (R 4.4.0)
## P xtable          1.8-4     2019-04-21 [?] RSPM
## P yaml            2.3.9     2024-07-05 [?] RSPM
##   yulab.utils      0.1.4     2024-01-28 [1] RSPM (R 4.4.0)
##   zoo             1.8-12    2023-04-13 [1] RSPM (R 4.4.0)
##
## [1] /Users/zahavahrojer/Desktop/Cornell/Hendry Lab/pseud-epi-growth/renv/library/macos/R-4.4/x86_64
## [2] /Users/zahavahrojer/Library/Caches/org.R-project.R/R/renv/sandbox/macos/R-4.4/x86_64-apple-darw
##
## P -- Loaded and on-disk path mismatch.

```


##
