Final Project

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Contents

Biofilm Data Analysis

Data Manipulation

```
#Loading all necessary libraries
library(readxl)
library(tidyverse)
```

```
## -- Attaching core tidyverse packages ------------------------ tidyverse 2.0.0 --
## v dplyr     1.1.4     v readr     2.1.5
## v forcats   1.0.0     v stringr   1.5.1
## v ggplot2   3.5.1     v tibble    3.2.1
## v lubridate 1.9.3     v tidyr     1.3.1
## v purrr     1.0.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
## i Use the conflicted package (<http://conflicted.r-lib.org/>) to force all conflicts to become errors
```

```
library(dplyr)
library(tidyr)
library(ggpubr)

# Reading excel files and sheets.
BiofilmFile <- "Data/RawData/Biofilm.xlsx"
```

```r
sheet_names <- excel_sheets(BiofilmFile)

# Initializing list
combined_data <- list()

# Reading and processing each sheets
for (sheet in sheet_names) {
  data1 <- read_excel(BiofilmFile, sheet = sheet, range = "B24:N31") %>%
    slice(-1) %>%  # Removing row 25 (second row from all sheets because it doesnot include samples)
    mutate(Sheet = sheet)

  combined_data[[sheet]] <- data1
}
```

```
## New names:
## New names:
## New names:
## New names:
## New names:
## New names:
## New names:
## New names:
## * `` -> `...1`
```

```r
# Combining all the sheets into a single data
data2 <- bind_rows(combined_data)

# Renaming the columns according to names of strains used in each columns
colnames(data2)[1:13] <- c("Media","Control", "B3SN17-2", "IVIA53.87", "IVIA5901", "ESVL", "XF3348", "A
                           "M12", "M23")

# Renaming the media columns according to different media used in different plates (sheets).
data2$Media <- rep(c("PD3","25SAP", "50SAP", "75SAP"), each = 12)

# Subtracting control from each strain column
for (i in 3:13) {
  data2[, i] <- data2[, i] - data2[, 2]
}

# Removing the control column (column 2)
data3 <- data2[, -2]

# Pivoting the data to long format
final_data <- data3 %>%
  pivot_longer(
    cols = -c(Media, Sheet),
    names_to = "Strain",
    values_to = "Biofilm"
  )


final_data <- final_data %>%
  mutate(Rep = rep(c(1, 2), each = 66, length.out = n())) #Adding a column representing replication bas
```

```r
final_data <- final_data[, -2] #Removing second column


#Saving the manipulated and organized file for further analysis
write.csv(final_data, "Data/CleanData/Biofilm.csv", row.names = FALSE)
```

Data Analysis

```r
#loading necessary libraries
library(tidyverse)
library(ggpubr)
library(ggplot2)
library(nlme)
```

```
##
## Attaching package: 'nlme'

## The following object is masked from 'package:dplyr':
##
##     collapse
```

```r
library(emmeans)
```

```
## Welcome to emmeans.
## Caution: You lose important information if you filter this package's results.
## See '? untidy'
```

```r
library(multcomp)
```

```
## Loading required package: mvtnorm

## Loading required package: survival

## Loading required package: TH.data

## Loading required package: MASS

##
## Attaching package: 'MASS'

## The following object is masked from 'package:dplyr':
##
##     select

##
## Attaching package: 'TH.data'

## The following object is masked from 'package:MASS':
##
##     geyser
```

```r
library(multcompView)


#Loading data and displaying first six rows.
Data_Biofilm <- read.csv("Data/CleanData/Biofilm.csv", na.strings = "na")
head(Data_Biofilm)
```

```
##   Media    Strain Biofilm Rep
## 1   PD3  B3SN17-2   0.157   1
## 2   PD3 IVIA53.87   0.043   1
## 3   PD3  IVIA5901   0.276   1
## 4   PD3      ESVL   0.425   1
## 5   PD3    XF3348   0.573   1
## 6   PD3      ALS6   0.385   1
```

```r
#Setting categorical variables as factor.
Data_Biofilm$Media = as.factor(Data_Biofilm$Media)
Data_Biofilm$Strain = as.factor(Data_Biofilm$Strain)

#Loading color blind palette
cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2", "#D55E00", "#CC79A7")

# Creating a vector to loop over all the strains
unique_strains <- unique(Data_Biofilm$Strain)

# Creating a list to store all the plots
plots <- list()

# Loop through each strain
for (strain_name in unique_strains) {
  sub_data <- Data_Biofilm %>% filter(Strain == strain_name) #subsetting the data
  sub_data <- sub_data %>% mutate(logBiofilm = log(Biofilm + 1)) #adding a column with log transformati
  cat("\n===== Analyzing Strain:", strain_name, "=====\n") #to display the name of strain while analyzi
  sub_data$Media <- relevel(sub_data$Media, ref = "PD3")  # Setting reference
  results <- lme(logBiofilm ~ Media, data = sub_data, random = ~1 | Rep) #fitting mixed effect model wi
  print(summary(results))
  print(intervals(results, which = "fixed"))
  sub_data$Media <- factor(sub_data$Media, levels = c("PD3", "25SAP", "50SAP", "75SAP")) #changing the
  lsmeans <- emmeans(results, ~Media) #estimate lsmeans of strain within media

  # Compact letter display (uses default comparison, no Tukey)
  results_lsmeans <- cld(lsmeans, alpha = 0.05, Letters = letters,  sort = FALSE)
  results_lsmeans_df <- as.data.frame(results_lsmeans)

  # Get y position for letter labels
  summary_df <- sub_data %>%
    group_by(Media) %>%
    summarise(y_max = max(logBiofilm) + 0.1)

  label_df <- merge(summary_df, results_lsmeans_df, by.x = "Media", by.y = "Media")

  boxplot <- ggplot(data = sub_data, aes(x = Media, y = logBiofilm, fill = Media)) +  #defining aesthet
    geom_boxplot(position = position_dodge(), outlier.shape = NA) + #creating box plot without overlap
```

```
    geom_point(position = position_jitterdodge(dodge.width = 0.8), aes(fill=Media),alpha = 0.6, shape=2
    scale_fill_manual(values = c(cbbPalette[[2]], cbbPalette[[3]],cbbPalette[[4]], cbbPalette[[7]])) +
    xlab("Media") + #labelling x axis
    ylab("log(Biofilm+1)") + #labelling y axis
    scale_y_continuous(limits = c(0,1.7)) +
    theme_classic()+ #setting theme classic to make plain white background
    ggtitle(paste(strain_name))+ #giving title to boxplot
    geom_text(data = label_df, aes(x = Media, y = y_max, label = .group), vjust = 0)

  plots[[strain_name]] <- boxplot
}
```

```
##
## ===== Analyzing Strain: B3SN17-2 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -86.73617 -76.03104 49.36809
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)   Residual
## StdDev:  0.02846273 0.06909002
##
## Fixed effects:  logBiofilm ~ Media
##                 Value  Std.Error DF  t-value p-value
## (Intercept) 0.1843994 0.02833460 43  6.50792       0
## Media25SAP  0.7138631 0.02820588 43 25.30902       0
## Media50SAP  1.0032091 0.02820588 43 35.56737       0
## Media75SAP  1.2934669 0.02820588 43 45.85805       0
##  Correlation:
##             (Intr) M25SAP M50SAP
## Media25SAP -0.498
## Media50SAP -0.498  0.500
## Media75SAP -0.498  0.500  0.500
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.46526126 -0.53130153 -0.05376473  0.68030117  2.26683096
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                 lower      est.     upper
## (Intercept) 0.1272572 0.1843994 0.2415415
## Media25SAP  0.6569805 0.7138631 0.7707457
## Media50SAP  0.9463265 1.0032091 1.0600917
## Media75SAP  1.2365843 1.2934669 1.3503495
##
## ===== Analyzing Strain: IVIA53.87 =====
## Linear mixed-effects model fit by REML
```

```
##   Data: sub_data
##        AIC       BIC   logLik
##   -154.6833 -143.9782 83.34167
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)  Residual
## StdDev:  0.02012101 0.0316521
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF  t-value p-value
## (Intercept) 0.03869822 0.01690904 43 2.288612  0.0271
## Media25SAP  0.00292832 0.01292192 43 0.226616  0.8218
## Media50SAP  0.08281719 0.01292192 43 6.409048  0.0000
## Media75SAP  0.10471245 0.01292192 43 8.103477  0.0000
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.382
## Media50SAP -0.382  0.500
## Media75SAP -0.382  0.500  0.500
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.23735049 -0.27227936  0.01610598  0.36049909  3.03454885
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                     lower        est.      upper
## (Intercept)  0.004597901 0.038698222 0.07279854
## Media25SAP  -0.023131212 0.002928316 0.02898784
## Media50SAP   0.056757660 0.082817188 0.10887672
## Media75SAP   0.078652921 0.104712449 0.13077198
##
## ===== Analyzing Strain: IVIA5901 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##        AIC       BIC   logLik
##   -113.2804 -102.5753 62.64021
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)   Residual
## StdDev: 0.004190171 0.05196537
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF   t-value p-value
## (Intercept) 0.26532388 0.01529091 43 17.351735  0.0000
## Media25SAP  0.06561453 0.02121477 43  3.092870  0.0035
## Media50SAP  0.00459823 0.02121477 43  0.216747  0.8294
## Media75SAP  0.07149019 0.02121477 43  3.369831  0.0016
##  Correlation:
```

```
##            (Intr) M25SAP M50SAP
## Media25SAP -0.694
## Media50SAP -0.694  0.500
## Media75SAP -0.694  0.500  0.500
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.64973906 -0.56891295 -0.09592893  0.52878216  2.58904748
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                   lower        est.     upper
## (Intercept)  0.23448681 0.265323881 0.2961609
## Media25SAP   0.02283087 0.065614534 0.1083982
## Media50SAP  -0.03818543 0.004598232 0.0473819
## Media75SAP   0.02870653 0.071490192 0.1142739
##
## ===== Analyzing Strain: ESVL =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -163.6443 -152.9391 87.82214
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)   Residual
## StdDev:  0.01288387 0.02878685
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF   t-value p-value
## (Intercept)  0.3984935 0.01233101 43  32.31636       0
## Media25SAP  -0.2993754 0.01175218 43 -25.47403       0
## Media50SAP  -0.2779317 0.01175218 43 -23.64938       0
## Media75SAP  -0.1138627 0.01175218 43  -9.68864       0
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.477
## Media50SAP -0.477  0.500
## Media75SAP -0.477  0.500  0.500
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -3.295047892 -0.360270413 -0.001036088  0.554906896  2.114543687
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                  lower       est.       upper
## (Intercept)  0.3736256  0.3984935  0.42336133
```

```
## Media25SAP  -0.3230759 -0.2993754 -0.27567485
## Media50SAP  -0.3016323 -0.2779317 -0.25423122
## Media75SAP  -0.1375632 -0.1138627 -0.09016214
##
## ===== Analyzing Strain: XF3348 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -43.91158 -33.20645 27.95579
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)  Residual
## StdDev:  0.06238557 0.1117445
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF   t-value p-value
## (Intercept)  0.4992416 0.05464932 43  9.135367  0.0000
## Media25SAP  -0.0255990 0.04561949 43 -0.561141  0.5776
## Media50SAP  -0.1121791 0.04561949 43 -2.459017  0.0180
## Media75SAP   0.0428393 0.04561949 43  0.939058  0.3529
##  Correlation:
##             (Intr) M25SAP M50SAP
## Media25SAP -0.417
## Media50SAP -0.417  0.500
## Media75SAP -0.417  0.500  0.500
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.92226264 -0.38299841  0.05260412  0.69178913  1.74499832
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                   lower        est.       upper
## (Intercept)  0.38903076  0.49924163  0.60945249
## Media25SAP  -0.11759943 -0.02559896  0.06640151
## Media50SAP  -0.20417959 -0.11217912 -0.02017865
## Media75SAP  -0.04916113  0.04283934  0.13483981
##
## ===== Analyzing Strain: ALS6 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -139.4415 -128.7363 75.72073
##
## Random effects:
##  Formula: ~1 | Rep
##          (Intercept)   Residual
## StdDev: 1.741197e-06 0.03866523
##
## Fixed effects:  logBiofilm ~ Media
```

```
##                  Value  Std.Error DF    t-value p-value
## (Intercept)  0.3025161 0.01116169 43  27.103069       0
## Media25SAP  -0.2441547 0.01578501 43 -15.467500       0
## Media50SAP  -0.2573058 0.01578501 43 -16.300635       0
## Media75SAP  -0.1429321 0.01578501 43  -9.054922       0
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.707
## Media50SAP -0.707  0.500
## Media75SAP -0.707  0.500  0.500
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -2.91477413 -0.31129379 -0.05360676  0.51693159  2.61079574
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                  lower       est.      upper
## (Intercept)  0.2800064  0.3025161  0.3250258
## Media25SAP  -0.2759882 -0.2441547 -0.2123212
## Media50SAP  -0.2891393 -0.2573058 -0.2254723
## Media75SAP  -0.1747656 -0.1429321 -0.1110986
##
## ===== Analyzing Strain: ALS17T10 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -98.45176 -87.74662 55.22588
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)   Residual
## StdDev:  0.02477814 0.06048412
##
## Fixed effects:  logBiofilm ~ Media
##                 Value  Std.Error DF  t-value p-value
## (Intercept) 0.6405212 0.02473538 43 25.89495       0
## Media25SAP  0.8327521 0.02469254 43 33.72485       0
## Media50SAP  0.9067596 0.02469254 43 36.72201       0
## Media75SAP  0.8596393 0.02469254 43 34.81373       0
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.499
## Media50SAP -0.499  0.500
## Media75SAP -0.499  0.500  0.500
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.1541508 -0.3227502  0.1005042  0.6787080  1.5295354
##
## Number of Observations: 48
```

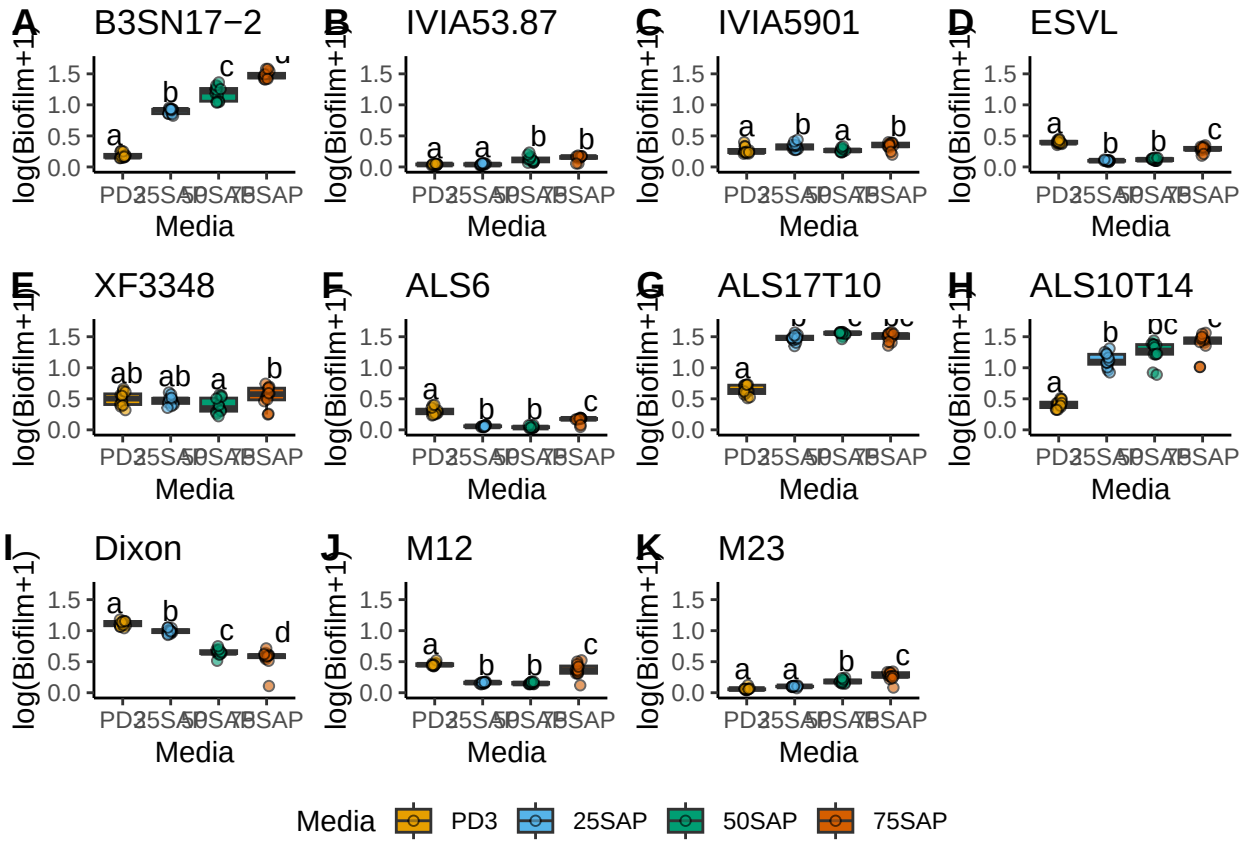
```
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                lower      est.     upper
## (Intercept) 0.5906376 0.6405212 0.6904049
## Media25SAP  0.7829548 0.8327521 0.8825493
## Media50SAP  0.8569623 0.9067596 0.9565568
## Media75SAP  0.8098420 0.8596393 0.9094365
##
## ===== Analyzing Strain: ALS10T14 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -23.06788 -12.36274 17.53394
##
## Random effects:
##  Formula: ~1 | Rep
##          (Intercept) Residual
## StdDev: 7.227924e-07 0.145092
##
## Fixed effects:  logBiofilm ~ Media
##                Value  Std.Error DF   t-value p-value
## (Intercept) 0.4091363 0.04188444 43  9.768216       0
## Media25SAP  0.7088708 0.05923355 43 11.967388       0
## Media50SAP  0.8372356 0.05923355 43 14.134483       0
## Media75SAP  0.9789201 0.05923355 43 16.526448       0
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.707
## Media50SAP -0.707  0.500
## Media75SAP -0.707  0.500  0.500
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -2.5920931 -0.2548798  0.1493646  0.6677695  1.3229269
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                lower      est.     upper
## (Intercept) 0.3246682 0.4091363 0.4936043
## Media25SAP  0.5894150 0.7088708 0.8283266
## Media50SAP  0.7177797 0.8372356 0.9566914
## Media75SAP  0.8594643 0.9789201 1.0983759
##
## ===== Analyzing Strain: Dixon =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC  logLik
##   -72.99841 -62.29327 42.4992
##
```

```
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)   Residual
## StdDev:   0.0391003 0.08051378
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF    t-value p-value
## (Intercept)  1.1117552 0.03611956 43  30.779867   0e+00
## Media25SAP  -0.1188104 0.03286961 43  -3.614596   8e-04
## Media50SAP  -0.4639781 0.03286961 43 -14.115717   0e+00
## Media75SAP  -0.5534669 0.03286961 43 -16.838256   0e+00
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.455
## Media50SAP -0.455  0.500
## Media75SAP -0.455  0.500  0.500
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -5.2766502 -0.2203479  0.1347590  0.3244706  1.6226158
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                  lower       est.       upper
## (Intercept)  1.0389132  1.1117552  1.18459726
## Media25SAP  -0.1850983 -0.1188104 -0.05252249
## Media50SAP  -0.5302660 -0.4639781 -0.39769026
## Media75SAP  -0.6197548 -0.5534669 -0.48717905
##
## ===== Analyzing Strain: M12 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC       BIC   logLik
##   -107.3202 -96.61509 59.66012
##
## Random effects:
##  Formula: ~1 | Rep
##         (Intercept)  Residual
## StdDev:  0.01549152 0.0550283
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF   t-value p-value
## (Intercept)  0.4551107 0.01929602 43  23.58572   0e+00
## Media25SAP  -0.2951976 0.02246521 43 -13.14021   0e+00
## Media50SAP  -0.3010783 0.02246521 43 -13.40198   0e+00
## Media75SAP  -0.0851515 0.02246521 43  -3.79037   5e-04
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.582
## Media50SAP -0.582  0.500
## Media75SAP -0.582  0.500  0.500
```

```
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -4.38922714 -0.31231179 -0.05709867  0.25498103  2.93063583
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                  lower        est.       upper
## (Intercept)  0.4161965  0.45511066  0.49402480
## Media25SAP  -0.3405031 -0.29519765 -0.24989223
## Media50SAP  -0.3463837 -0.30107826 -0.25577285
## Media75SAP  -0.1304569 -0.08515145 -0.03984604
##
## ===== Analyzing Strain: M23 =====
## Linear mixed-effects model fit by REML
##   Data: sub_data
##         AIC      BIC   logLik
##   -135.7241 -125.019 73.86207
##
## Random effects:
##  Formula: ~1 | Rep
##          (Intercept)   Residual
## StdDev: 1.025175e-06 0.04033353
##
## Fixed effects:  logBiofilm ~ Media
##                  Value  Std.Error DF   t-value p-value
## (Intercept) 0.06119563 0.01164329 43  5.255872  0.0000
## Media25SAP  0.03942781 0.01646609 43  2.394484  0.0211
## Media50SAP  0.12453204 0.01646609 43  7.562937  0.0000
## Media75SAP  0.20659276 0.01646609 43 12.546555  0.0000
##  Correlation:
##            (Intr) M25SAP M50SAP
## Media25SAP -0.707
## Media50SAP -0.707  0.500
## Media75SAP -0.707  0.500  0.500
##
## Standardized Within-Group Residuals:
##          Min           Q1          Med           Q3          Max
## -4.571054992 -0.280217205  0.009163961  0.213941688  1.808926841
##
## Number of Observations: 48
## Number of Groups: 2
## Approximate 95% confidence intervals
##
##  Fixed effects:
##                   lower       est.      upper
## (Intercept) 0.037714704 0.06119563 0.08467656
## Media25SAP  0.006220761 0.03942781 0.07263485
## Media50SAP  0.091324992 0.12453204 0.15773908
## Media75SAP  0.173385715 0.20659276 0.23979980
```

```
# Combining all plots into one figure with a common legend
combined_biofilm_plot <- ggarrange(plotlist = plots, ncol = 4, nrow = 3, labels = "AUTO", common.legend

# Displaying the combined plot
print(combined_biofilm_plot)
```




```
#Saving the final figure
ggsave("Figures/combined_biofilm_plot.png", plot = combined_biofilm_plot, width = 10, height = 8, dpi =
```

Growth Data Analysis

Data Manipulation

```
library(readxl)
library(dplyr)
library(tidyr)
library(purrr)

# Listing all Day files
GrowthData <- list.files(path = "Data/RawData", pattern = "^Day[0-7]\\.xlsx$", full.names = TRUE)

# Making a list of the all days data
```

```r
all_days_data <- list()

# Looping through each file
for (file_name in GrowthData) {
  day_label <- tools::file_path_sans_ext(file_name)
  sheet_names <- excel_sheets(file_name)

  combined_data <- list()

# Looping through each sheet in the file
  for (sheet in sheet_names) {
    Growth1 <- read_excel(file_name, sheet = sheet, range = "B24:N31") %>%
      slice(-1) %>%  # Remove second row (Excel row 25)
      mutate(Sheet = sheet)

    combined_data[[sheet]] <- Growth1
  }

  # Combining all sheets into a single data
  Growth2 <- bind_rows(combined_data)

  # Renaming the columns according to names of strains used in each columns
  colnames(Growth2)[1:13] <- c("Media", "Control", "B3SN17-2", "IVIA53.87", "IVIA5901", "ESVL",
                               "XF3348", "ALS6", "ALS17T10", "ALS10T14", "Dixon", "M12", "M23")

  # Renaming the media columns according to different media used in different plates (sheets).
  Growth2$Media <- rep(c("PD3","25SAP", "50SAP", "75SAP"), each = 12)

  # Subtracting control from each strain column
  for (i in 3:13) {
    Growth2[, i] <- Growth2[, i] - Growth2[, 2]
  }

  # Removing the control column (column 2)
  Growth3 <- Growth2[, -2]

  # Pivoting the data to long format
  final_data <- Growth3 %>%
    pivot_longer(
      cols = -c(Media, Sheet),
      names_to = "Strain",
      values_to = "OD600"
    )

  # Adding a column representing replication based on number of plates
  final_data <- final_data %>%
    mutate(Rep = rep(c(1, 2), each = 66, length.out = n()))

  # Adding Day column
  final_data$Day <- day_label

  all_days_data[[day_label]] <- final_data
}
```

```
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```
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## * `` -> `...1`
```

```
# Combining all the data into one dataframe
all_data_combined <- bind_rows(all_days_data)

#Replacing negative values with zero
all_data_combined$OD600[all_data_combined$OD600 < 0] <- 0

#Removing column 2
all_data_combined <- all_data_combined[ , -2]

#Saving the manipulated and organized file to CSV for further analysis.
write.csv(all_data_combined, "Data/CleanData/GrowthData.csv", row.names = FALSE)
```

Growth Curves

```
#loading necessary libraries
library(tidyverse)
library(stringr)
library(ggpubr)
library(ggplot2)
library(nlme)
library(emmeans)
library(multcomp)
library(multcompView)


#Reading data to R and displaying first six rows
datum = read.csv("Data/CleanData/GrowthData.csv", )
head(datum)
```

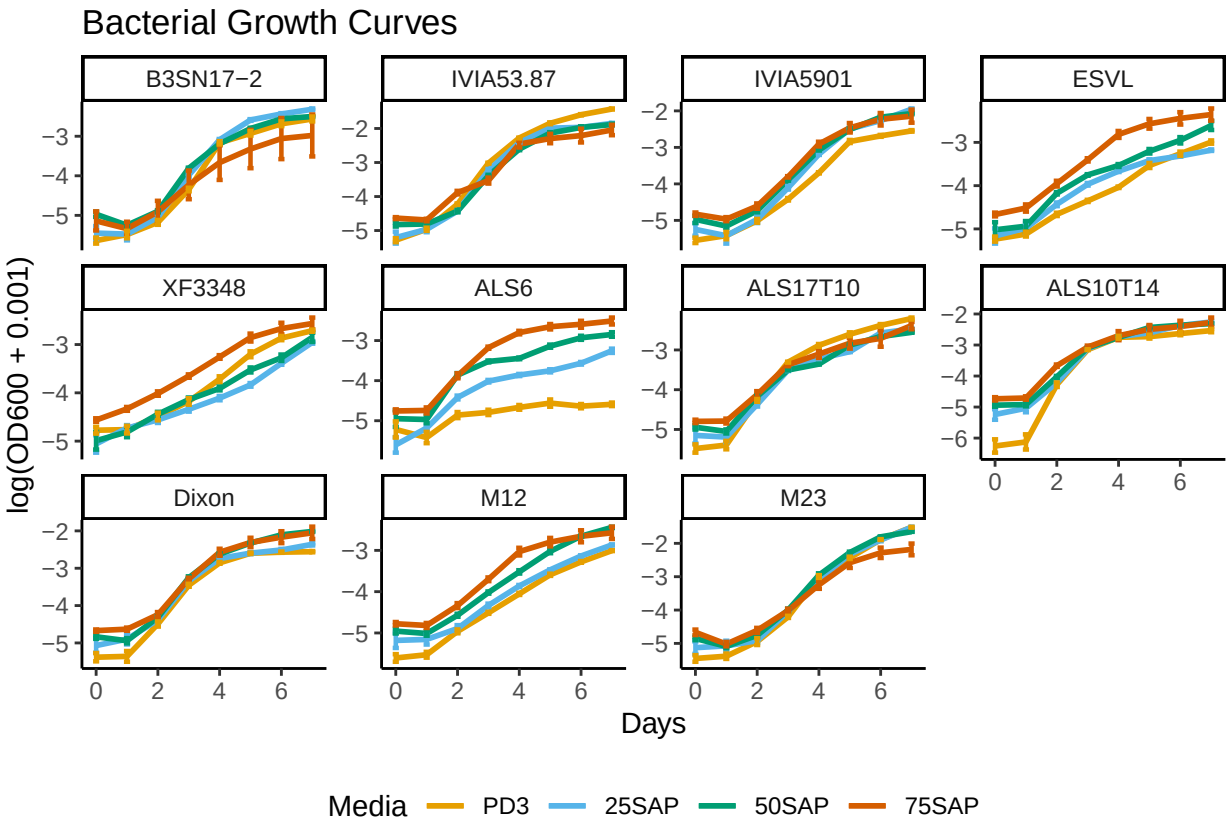
```
##   Media    Strain OD600 Rep               Day
## 1   PD3  B3SN17-2 0.003   1 Data/RawData/Day0
## 2   PD3 IVIA53.87 0.003   1 Data/RawData/Day0
## 3   PD3  IVIA5901 0.003   1 Data/RawData/Day0
## 4   PD3      ESVL 0.005   1 Data/RawData/Day0
## 5   PD3    XF3348 0.009   1 Data/RawData/Day0
## 6   PD3      ALS6 0.006   1 Data/RawData/Day0
```

```
#Setting categorical variables as factor and log transforming the optical density values.
GrowthData<- datum%>%
```

```r
  mutate(
    Strain = factor(Strain),
    Media = factor(Media),
    Rep = factor(Rep),
    Day = as.numeric(str_replace(Day, "Data/RawData/Day", "")),  # Converts "Day0" → 0
    logOD = log(OD600 + 0.001)  # Log-transform while avoiding log(0)
  )

# Color palette
cbbPalette <- c("#000000", "#E69F00", "#56B4E9", "#009E73", "#F0E442", "#0072B2", "#D55E00", "#CC79A7")

# Setting desirable media colors.
media_colors <- c("PD3" = "#E69F00",
                  "25SAP" = "#56B4E9",
                  "50SAP" = "#009E73",
                  "75SAP" = "#D55E00")


# Calculating summary stats
growth_summary <- GrowthData %>%
  group_by(Strain, Media, Day) %>%
  summarise(
    mean_logOD600 = mean(logOD, na.rm = TRUE),
    se_logOD600 = sd(logOD, na.rm = TRUE) / sqrt(n()),
    .groups = "drop"
  )

# Ensuring correct media order and strain order
growth_summary$Media <- factor(growth_summary$Media, levels = names(media_colors))
growth_summary$Strain <- factor(growth_summary$Strain, levels = c("B3SN17-2", "IVIA53.87", "IVIA5901",

# Creating the faceted plot
growth_curve_log <- ggplot(growth_summary, aes(x = Day, y = mean_logOD600, color = Media, group = Media
  geom_line(size = 1) +
  geom_errorbar(aes(ymin = mean_logOD600 - se_logOD600, ymax = mean_logOD600+ se_logOD600),
                width = 0.2, size = 0.8) +
  scale_color_manual(values = media_colors) +
  facet_wrap(~ Strain, scales = "free_y") +
  labs(title = "Bacterial Growth Curves",
       x = "Days",
       y = "log(OD600 + 0.001)",
       color = "Media") +
  theme_classic() +
  theme(legend.position = "bottom")
```

```
## Warning: Using ‘size‘ aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use ‘linewidth‘ instead.
## This warning is displayed once every 8 hours.
## Call ‘lifecycle::last_lifecycle_warnings()‘ to see where this warning was
## generated.
```

```
# Printing the plot
print(growth_curve_log)
```




```
#Saving the plot
ggsave("Figures/GrowthCurves.png", plot = growth_curve_log, width = 10, height = 8, dpi = 300)
```