Assessment 4

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Part 1: Importing files, data wrangling, mathematical operations, plots and saving code on GitHub.

Introduction

This report analyzes RNA-seq count data for gene expression and tree circumference measurements at two different sites over a 20-year period.

Task 1: RNA-seq count data for gene expression, high and low expression of 3 genes.

1.1 Read in the file "gene_expression.tsv", making the gene identifiers the row names. Show a table of values for the first six genes.

1) Load libraries

```
#RNA-seq Count Data Analysis
#Load necessary libraries
library(R.utils)
#use BiocManager::install("Biostrings") if it is not already installed in your Rstudio
library(Biostrings)
library(seqinr)
library(dplyr)
library(ggplot2)
library(readr)
library(tidyr)
library(knitr)
```

2) Read in the gene expression data

```
#Download the data from the github link provided
URL = "https://raw.githubusercontent.com/ghazkha/Assessment4/refs/heads/main/gene_expression.tsv"
download.file(URL, destfile = "gene_expression.tsv")

# Read the downloaded TSV file into R
gene_expression <- read.table("gene_expression.tsv", header = TRUE, sep = "\t", row.names = 1)
```

3) 1st First 6 rows of the gene_expression data

```
## Data 1:GTEX.1117F.0226.SM.5GZZ7

## Data 2:GTEX.1117F.0426.SM.5EGHI

## Data 3:GTEX.1117F.0526.SM.5EGHJ
```

Table 1: First 6 genes of gene_expression file

	Data 1	Data 2	Data 3
ENSG00000223972.5_DDX11L1	0	0	0
ENSG00000227232.5_WASH7P	187	109	143
ENSG00000278267.1_MIR6859-1	0	0	1
ENSG00000243485.5_MIR1302-2HG	1	0	0
ENSG00000237613.2_FAM138A	0	0	0
ENSG00000268020.3_OR4G4P	0	1	0

1.2 Make a new column which is the mean of the other columns. Show a table of values for the first six genes.

Calculate Mean Expression

Data 1:GTEX.1117F.0226.SM.5GZZ7

Data 2:GTEX.1117F.0426.SM.5EGHI

Data 3:GTEX.1117F.0526.SM.5EGHJ

Table 2: First 6 genes of gene_expression file

	Data 1	Data 2	Data 3	Mean Expression
ENSG00000223972.5_DDX11L1	0	0	0	0.0000000
ENSG00000227232.5_WASH7P	187	109	143	146.3333333
ENSG00000278267.1_MIR6859-1	0	0	1	0.3333333
ENSG00000243485.5_MIR1302-2HG	1	0	0	0.3333333
ENSG00000237613.2_FAM138A	0	0	0	0.0000000
ENSG00000268020.3_OR4G4P	0	1	0	0.3333333

1.3 List the 10 genes with the highest mean expression.

Identify Top 10 Genes

Data 1:GTEX.1117F.0226.SM.5GZZ7

Data 2:GTEX.1117F.0426.SM.5EGHI

Data 3:GTEX.1117F.0526.SM.5EGHJ

Table 3: Top 10 expressed genes

|	Data 1	Data 2	Data 3	Mean Expression
ENSG00000198804.2_MT-CO1	267250	1101779	218923	529317.3
ENSG00000198886.2_MT-ND4	273188	991891	277628	514235.7
ENSG00000198938.2_MT-CO3	250277	1041376	223178	504943.7
ENSG00000198888.2_MT-ND1	243853	772966	194032	403617.0
ENSG00000198899.2_MT-ATP6	141374	696715	151166	329751.7
ENSG00000198727.2_MT-CYB	127194	638209	141359	302254.0
ENSG00000198763.3_MT-ND2	159303	543786	149564	284217.7
ENSG00000211445.11_GPX3	464959	39396	306070	270141.7
ENSG00000198712.1_MT-CO2	128858	545360	122816	265678.0
ENSG00000156508.17_EEF1A1	317642	39573	339347	232187.3

1.4 Determine the number of genes with a mean <10.

Count Genes with Low Expression (Mean < 10)

```
## 35988
```

1.5 Make a histogram plot of the mean values and include it into your report.

Histogram of <10, Mean Values

Histogram of Mean Low Gene Expression
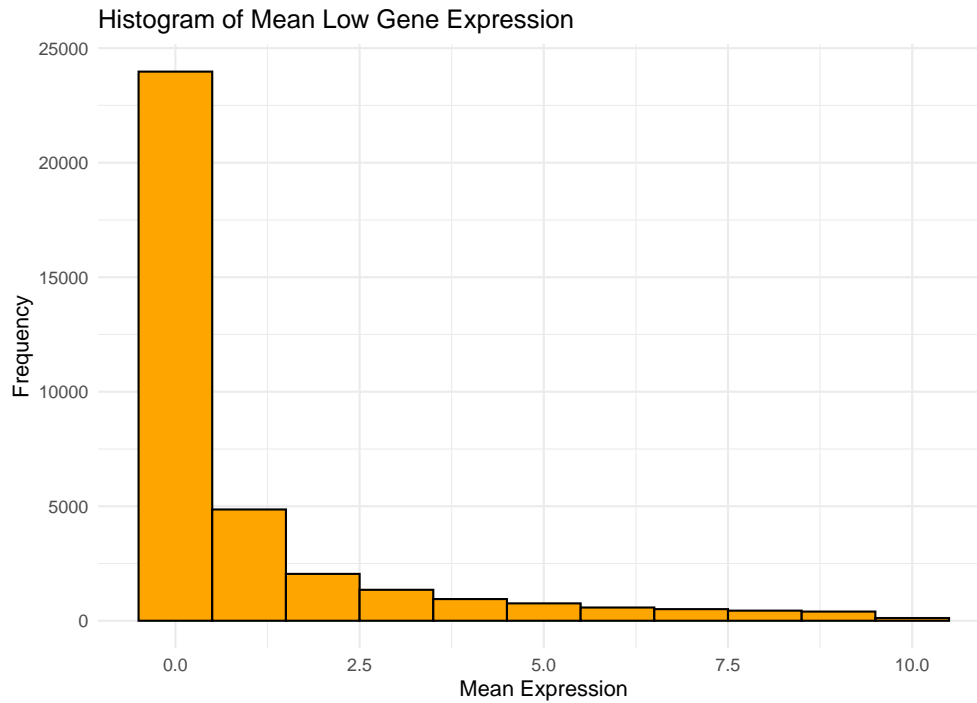
Task 2: Tree circumference measurements over 20 years.

2.1 Import "growth_data.csv" file into an R object. What are the column names?

Read Data to perform a Tree Circumference Data Analysis

```
# Read in the growth data
#Download the data from the github link provided
URL = "https://raw.githubusercontent.com/ghazkha/Assessment4/refs/heads/main/growth_data.csv"
download.file(URL, destfile = "growth_data.csv")

# Read the downloaded TSV file into R
growth_data <- read.csv("growth_data.csv")

#print the table with the 6 first rows
growth_data_show <- head(growth_data)
kable (growth_data_show, align = 'c', caption = "First 6 rows of growth_data file")
```

Table 4: First 6 rows of growth_data file

Site	TreeID	Circumf_2005_cm	Circumf_2010_cm	Circumf_2015_cm	Circumf_2020_cm
northeast	A012	5.2	10.1	19.9	38.9
southwest	A039	4.9	9.6	18.9	37.0

Site	TreeID	Circumf_2005_cm	Circumf_2010_cm	Circumf_2015_cm	Circumf_2020_cm
southwest	A010	3.7	7.3	14.3	28.1
northeast	A087	3.8	6.5	10.9	18.5
southwest	A074	3.8	6.4	10.9	18.4
northeast	A008	5.9	10.0	16.8	28.4

```
## The column names are:

## [1] "Site"            "TreeID"          "Circumf_2005_cm" "Circumf_2010_cm"
## [5] "Circumf_2015_cm" "Circumf_2020_cm"
```

2.2 Calculate the mean and standard deviation of tree circumference at the start and end of the study at both sites.

Statistics

```
##   mean_start_2005_southwest sd_start_2005_southwest mean_start_2005_northeast
## 1                     4.862                1.147471                     5.292
##   sd_start_2005_northeast mean_end_2020_southwest sd_end_2020_southwest
## 1               0.9140267                  45.596              17.87345
##   mean_end_2020_northeast sd_end_2020_northeast
## 1                  54.228              25.22795
```

2.3 Make a box plot of tree circumference at the start and end of the study at both sites.

Boxplot of circumferences

Tree Circumference at Start (2005) and End (2020) of Study
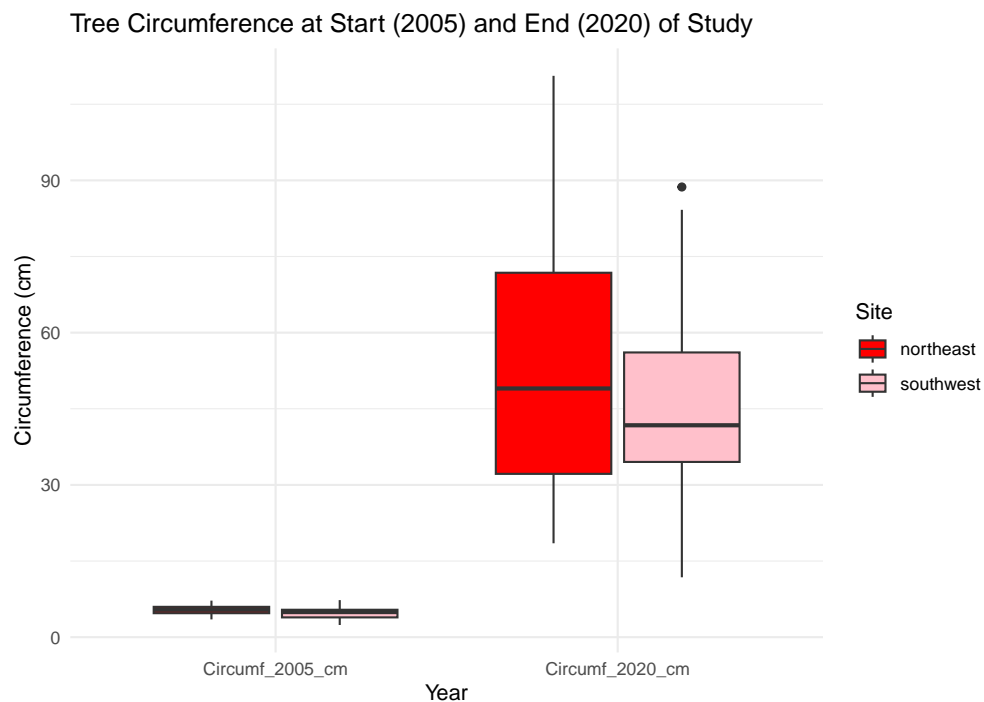
2.4 Calculate the mean growth over the last 10 years at each site.

4

Table 5: Mean Growth Calculation

Site	mean_growth
northeast	42.94
southwest	35.49

2.5 Use the t.test to estimate the p-value that the 10 year growth is different at the two sites.

T-Test Results for Growth Difference

```
##
##  Welch Two Sample t-test
##
## data:  Growth_10_years by Site
## t = 1.8882, df = 87.978, p-value = 0.06229
## alternative hypothesis: true difference in means between group northeast and group southwest is not
## 95 percent confidence interval:
##  -0.3909251 15.2909251
## sample estimates:
## mean in group northeast mean in group southwest
##                   42.94                   35.49
```

Interpretation: p-value: The p-value of 0.06229 suggests that the difference in mean growth between the two sites is not statistically significant at the conventional alpha level of 0.05. However, it is close to this threshold, indicating a potential trend toward significance.

Mean Comparison: The mean growth in the northeast (42.94 cm) is higher than that in the southwest (35.49 cm). This suggests that trees in the northeast experienced greater growth compared to those in the southwest over the last 10 years.

Confidence Interval: The confidence interval includes zero, which means we cannot conclusively say that there is a true difference in growth between the two sites. The upper limit (15.29 cm) indicates that, while the northeast shows higher growth, it is possible that the actual difference might be minimal or even negative.

Part 2: Examining biological sequence diversity

Introduction

This report compares the sequence features of *Streptacidiphilus jiangxiensis* (GCA_900109465) with *Escherichia coli*. *Escherichia coli* is a Gram-negative, rod-shaped bacterium commonly found in the intestines of warm-blooded organisms, playing a vital role in gut health. While some strains of *Escherichia coli* can cause illnesses, the bacterium is extensively used as a model organism in molecular biology due to its relatively simple genome and well-studied genetics. In contrast, *Streptacidiphilus jiangxiensis* is a Gram-positive bacterium isolated from acidic environments (Huang et al., 2004). This species is characterized by its unique metabolic pathways and adaptations to specific ecological niches, which may hold potential for applications in bioremediation or antibiotic development.

Questions:

1) Download the whole set of coding DNA sequences for E. coli and your organism of interest. How many coding sequences are present in these organisms? Present this in the form of a table. Describe any differences between the two organisms.

Sequences

```
# URLs for the coding DNA sequences
URL_Ecoli <- "https://ftp.ensemblgenomes.ebi.ac.uk/pub/bacteria/release-59/fasta/bacteria_117_collection
URL_Streptacidiphilus <- "https://ftp.ensemblgenomes.ebi.ac.uk/pub/bacteria/release-59/fasta/bacteria_5

# Downloading the sequences
download.file(URL_Ecoli, destfile = "e_coli_cds.fa.gz")
download.file(URL_Streptacidiphilus, destfile = "streptacidiphilus_cds.fa.gz")

#Decompress the files

gunzip("e_coli_cds.fa.gz")
gunzip("streptacidiphilus_cds.fa.gz")

# Reading the sequences
ecoli_seqs <- seqinr::read.fasta ("e_coli_cds.fa")
streptacidiphilus_seqs <- seqinr::read.fasta ("streptacidiphilus_cds.fa")
```

CDS count

Table 6: Coding Sequences of each organism

Organism	Coding_Sequences
Escherichia coli	4931
Streptacidiphilus jiangxiensis	8650

Answer: *Escherichia coli* contains 4,931 coding sequences while *Streptacidiphilus jiangxiensis* has a substantially higher count (8,650) of coding sequences, points to a significant disparity in genetic diversity between the two bacterial species. This greater number of coding sequences in *Streptacidiphilus jiangxiensis* is indicative of a more complex genetic framework, which may translate into enhanced functional capabilities and a broader range of physiological adaptations (Wright, 1990, Malik et al., 2020).

The expanded repertoire of genes in *Streptacidiphilus jiangxiensis* likely contributes to its metabolic versatility, enabling it to thrive in diverse environments. This versatility allows *Streptacidiphilus jiangxiensis* to exploit various substrates, potentially including organic compounds found in soil or plant matter, that *Escherichia coli* might not utilize as efficiently. For example, *Streptacidiphilus jiangxiensis* may possess unique enzymes or metabolic pathways that allow it to break down complex carbohydrates, synthesize essential nutrients, or produce secondary metabolites, such as antimicrobial compounds, which can offer competitive advantages in its ecological niche (Wright, 1990).

Moreover, the increased number of coding sequences may also reflect evolutionary adaptations to environmental pressures. In soil ecosystems, where nutrient availability can fluctuate, the ability to produce a wide range of enzymes and metabolites could enhance survival and reproduction (Harman and Uphoff, 2019). *Streptacidiphilus jiangxiensis* may engage in symbiotic relationships with plants or other soil microorganisms, leveraging its genetic diversity to facilitate nutrient exchange or improve soil health (Cong et al., 2021). In contrast, *Escherichia coli*, while highly adaptable and successful in its own right, is often more specialized for life in nutrient-rich environments, such as the gastrointestinal tract of mammals. Thus, the greater coding sequence count in *Streptacidiphilus jiangxiensis* underscores its potential for metabolic innovation and ecological resilience, reflecting a sophisticated evolutionary response to its environment (Wright, 1990; Malik et al., 2020).

2) How much coding DNA is there in total for these two organisms? Present this in the form of a table. Describe any differences between the two organisms.

Total Coding DNA Length

Table 7: Total length of DNA sequences from each organism

Organism	Total_Length
Escherichia coli	4593474
Streptacidiphilus jiangxiensis	8422779

Answer: The genomic analysis reveals that *Escherichia coli* comprises approximately 4,593,474 base pairs of coding DNA, whereas *Streptacidiphilus jiangxiensis* possesses a significantly larger genomic footprint of about 8,422,779 base pairs. This substantial difference in coding DNA indicates a more complex genome in *Streptacidiphilus*, which is often associated with greater metabolic diversity. A larger genomic content can provide a broader array of genes, facilitating the synthesis of diverse proteins that are crucial for various metabolic pathways and biochemical processes (Bentley, 2009).

The expanded coding capacity of *Streptacidiphilus* likely equips it with the ability to thrive in complex and potentially harsh environmental conditions. For instance, the organism might possess genes that allow it to metabolize a wider range of substrates, adapt to changes in nutrient availability, or withstand environmental stresses, such as acidity or high salinity (Rasko et al., 2008). Such metabolic versatility could enable *Streptacidiphilus* to exploit ecological niches that are less accessible to simpler organisms like *E. coli*, which tends to thrive in nutrient-rich environments typical of the mammalian gut (Lozica et al., 2022).

Furthermore, the increased genetic content in *Streptacidiphilus* may include genes responsible for specialized functions, such as biosynthesis of secondary metabolites, antibiotic resistance, or symbiotic interactions with other organisms (Jarocki et al., 2019). These attributes are particularly valuable for survival in competitive ecosystems where resource availability can be unpredictable. In essence, the larger coding DNA in *Streptacidiphilus jiangxiensis* reflects an evolutionary strategy that enhances its adaptability and functional repertoire, illustrating how genomic complexity can influence ecological success and resilience (Huang et al., 2004).

3) Calculate the length of all coding sequences in these two organisms. Make a boxplot of coding sequence length in these organisms. What is the mean and median coding sequence length of these two organisms? Describe any differences between the two organisms.
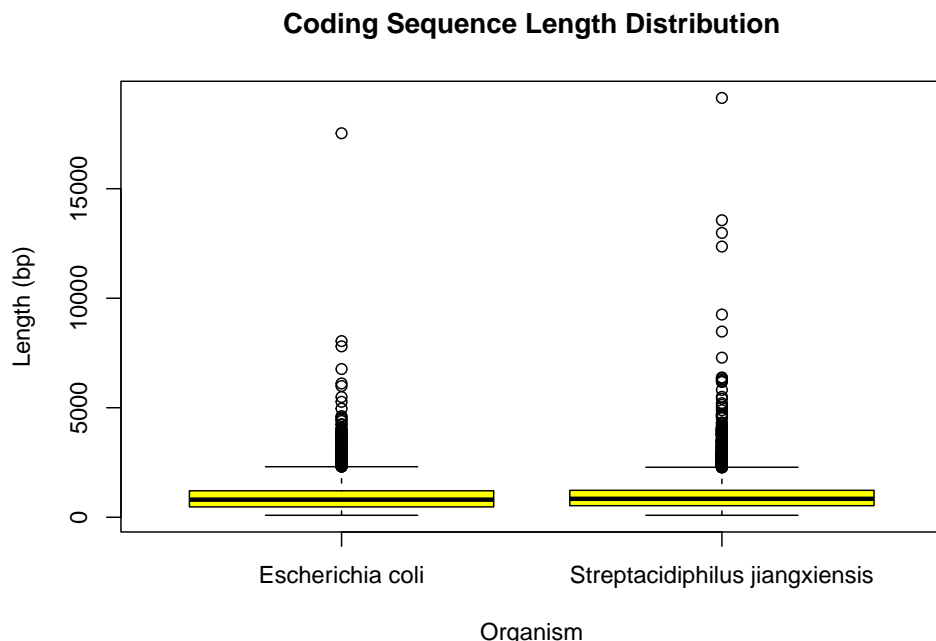
Coding Sequence Length Distribution

Table 8: Mean and Median of the CDS length each organism

Organism	Mean_Length	Median_Length
Escherichia coli	931.5502	804
Streptacidiphilus jiangxiensis	973.7317	843

Answer: The comparison of coding sequence lengths between *Escherichia coli* and *Streptacidiphilus jiangxiensis* reveals significant insights into their genomic architectures and evolutionary adaptations. *Escherichia coli*, with a mean coding sequence length of 931.55 bp and a median of 804 bp, is known for its streamlined genome, which is optimized for rapid growth and efficient protein production in the nutrient-rich environments of mammalian intestines (Koonin, 2009). This bacterium's genetic simplicity has made it a model organism for studying fundamental biological processes, allowing researchers to explore genetic functions and interactions in a relatively uncomplicated context (Lizana and Schwartz, 2024).
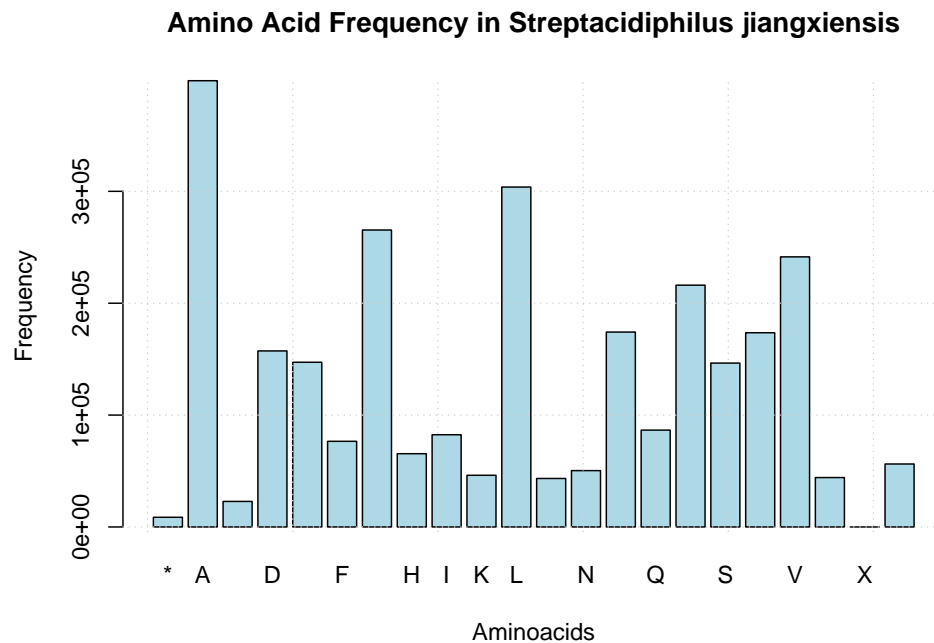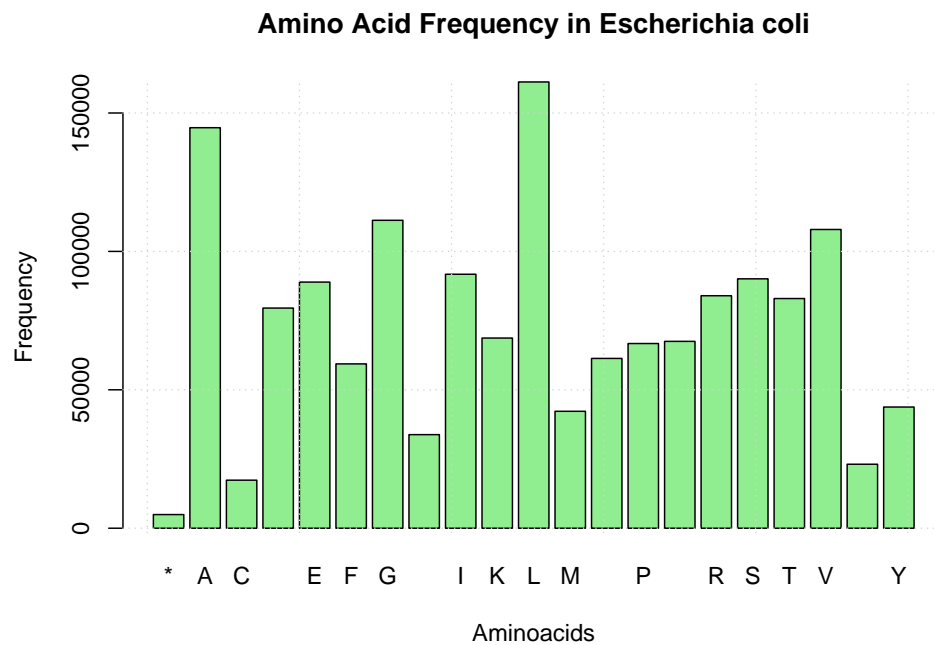
In contrast, *Streptacidiphilus jiangxiensis* presents a longer mean coding sequence of 973.73 bp and a median of 843 bp, suggesting a more intricate gene structure. This complexity may be indicative of specialized adaptations to its unique ecological niche, which includes survival in acidic environments (Malik et al., 2020). Longer coding sequences can allow for the encoding of more extensive and functionally diverse proteins, potentially enabling the bacterium to exploit a broader range of substrates or cope with environmental stresses (Maharjan and Ferenci, 2014). The differences in coding sequence length may reflect distinct evolutionary pressures faced by these organisms, with *Escherichia coli* evolving for rapid proliferation and *Streptacidiphilus jiangxiensis* potentially developing complex metabolic pathways or interactions to thrive in less hospitable conditions (Wan et al., 2022). This variation underscores the diverse functional requirements and ecological strategies that shape the genomes of bacteria, highlighting how environmental factors influence genetic structure and complexity (Chan et al., 2018).

4) Calculate the frequency of DNA bases in the total coding sequences for both organisms. Perform the same calculation for the total protein sequence. Create bar plots for nucleotide and amino acid frequency. Describe any differences between the two organisms.

Frequency of DNA Bases

E. coli CDS composition

S. jiangxiensis CDS composition

Amino Acid Frequency

Amino Acid Frequency in Escherichia coli

Amino Acid Frequency in Streptacidiphilus jiangxiensis
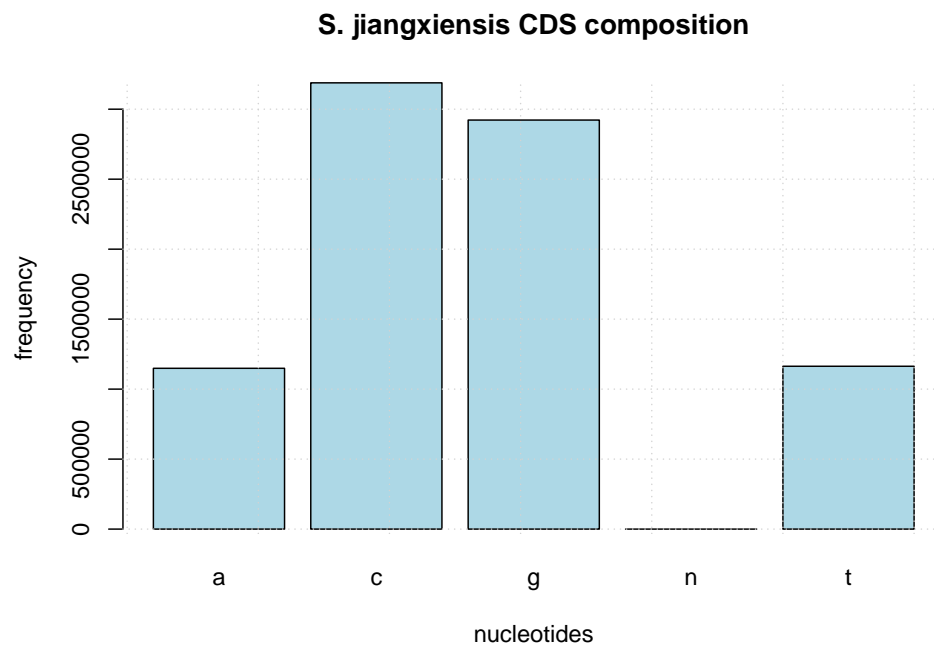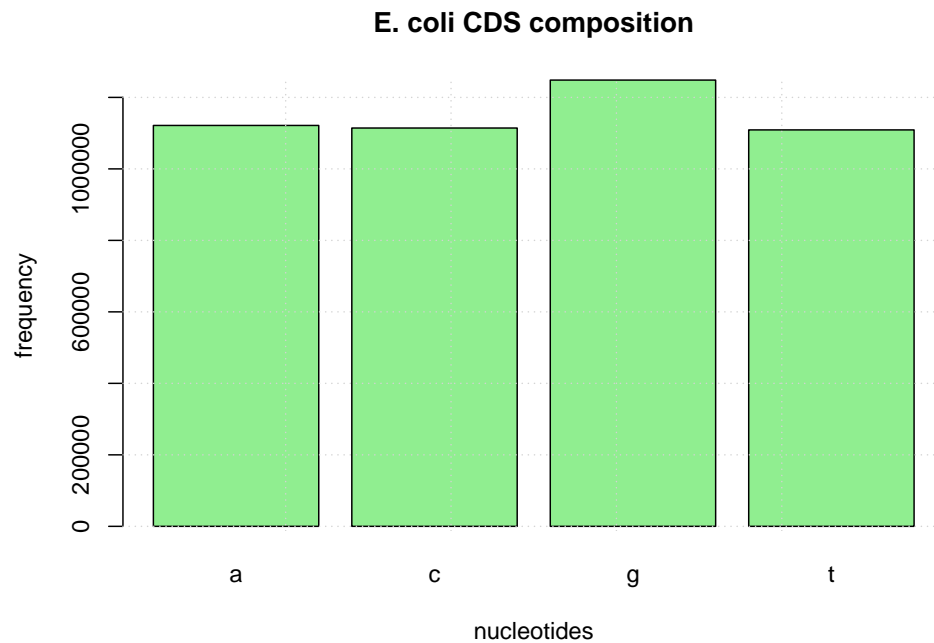
Answer: The observation that *Escherichia coli* exhibits a relatively even distribution of nucleotides—where adenine (A), thymine (T), cytosine (C), and guanine (G) are present in comparable proportions—contrasts sharply with the significantly higher CG content observed in *Streptacidiphilus jiangxiensis*. This difference in nucleotide composition can have profound implications for the evolutionary strategies and ecological niches occupied by these two bacteria (Malik et al., 2020). The balanced nucleotide composition in *E. coli* reflects its adaptation to a diverse range of environments, particularly in the nutrient-rich gastrointestinal tracts of mammals, where rapid replication and metabolic efficiency are advantageous (Alteri and Mobley, 2012). In

contrast, the elevated CG content in *Streptacidiphilus* may indicate specialized adaptations to more extreme or variable environmental conditions, such as those found in soil ecosystems. Higher GC content can contribute to increased stability of the DNA helix and may be linked to resistance against environmental stresses, such as desiccation or temperature fluctuations (Šmarda et al., 2014). Moreover, elevated CG content can affect gene expression and regulation, allowing *Streptacidiphilus* to potentially encode proteins that facilitate survival in nutrient-poor or competitive environments. Thus, the differences in nucleotide composition not only reflect the evolutionary histories of these bacteria but also underscore their distinct ecological strategies and adaptations to their respective habitats (Hu et al., 2022).

The differences in amino acid frequency between *Escherichia coli* and *Streptacidiphilus jiangxiensis* not only reveal evolutionary adaptations but also highlight functional specializations that may be crucial for each organism's survival in their respective environments. In *E. coli*, the five most frequent amino acids—leucine (L), alanine (A), glycine (G), valine (V), and isoleucine (I)—suggest a protein composition that supports its versatile metabolic functions and adaptability. Leucine, being the most abundant, is known for its role in protein synthesis and cellular signaling, indicating a potential emphasis on growth and reproduction (Maser et al., 2020).

Conversely, *Streptacidiphilus jiangxiensis* shows a slightly different pattern, with alanine (A), leucine (L), glycine (G), valine (V), and arginine (R) as the five most frequent amino acids. The presence of arginine in this top five may indicate specific adaptations related to stress responses or metabolic processes unique to its ecological niche. The dominance of alanine, along with the other branched-chain amino acids, suggests that *Streptacidiphilus* might be optimized for particular biochemical pathways that enable it to thrive in potentially nutrient-poor or competitive environments (Koonin, 2009).

These variations in amino acid frequencies not only reflect the distinct metabolic capabilities of each organism but also offer insights into how they might respond to environmental challenges. For instance, the higher frequency of arginine in *Streptacidiphilus* may confer advantages in environments requiring enhanced nitrogen metabolism or stress resilience (Chen et al., 2021). Thus, examining the amino acid composition provides a deeper understanding of the functional diversity and ecological adaptations of these two bacteria, shedding light on their evolutionary trajectories in different ecological contexts (Monteiro et al., 2023).

5) Create a codon usage table and quantify the codon usage bias among all coding sequences. Describe any differences between the two organisms with respect to their codon usage bias. Provide charts to support your observations.
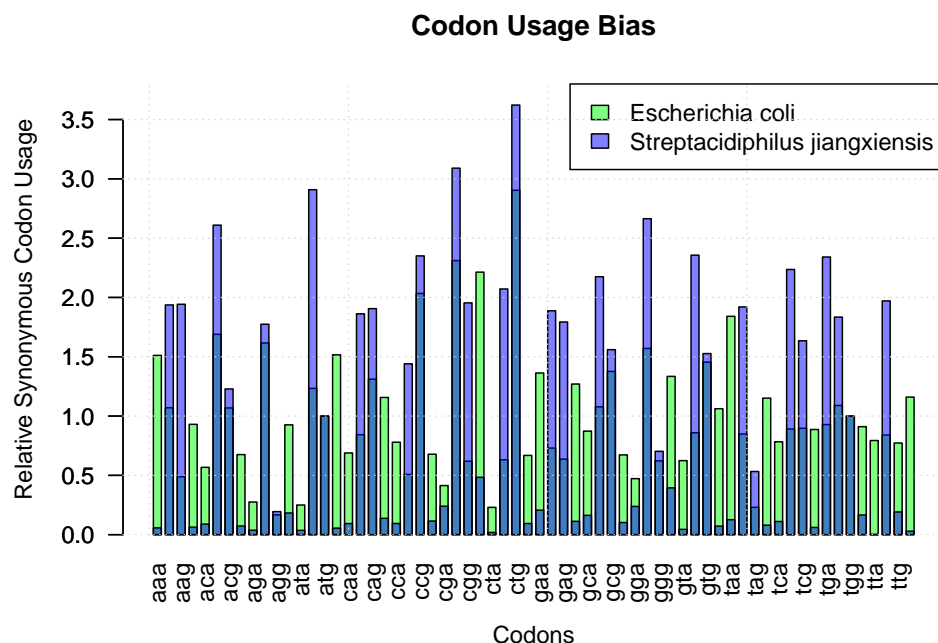
Codon Usage Bias

Table 9: Codon Usage Bias from each from each organism

	AA	codon	eff	freq	E. coli RSCU	S. jiangxiensis RSCU
aaa	Lys	aaa	51923	0.0339109	1.5116526	0.0573964
aac	Asn	aac	32806	0.0214256	1.0696620	1.9369244
aag	Lys	aag	16774	0.0109551	0.4883474	1.9426036
aat	Asn	aat	28533	0.0186349	0.9303380	0.0630756
aca	Thr	aca	11773	0.0076890	0.5675786	0.0887921
acc	Thr	acc	35034	0.0228807	1.6889960	2.6096222

Codon Usage Bias in Escherichia coli

Codon Usage in Streptacidiphilus jiangxiensis
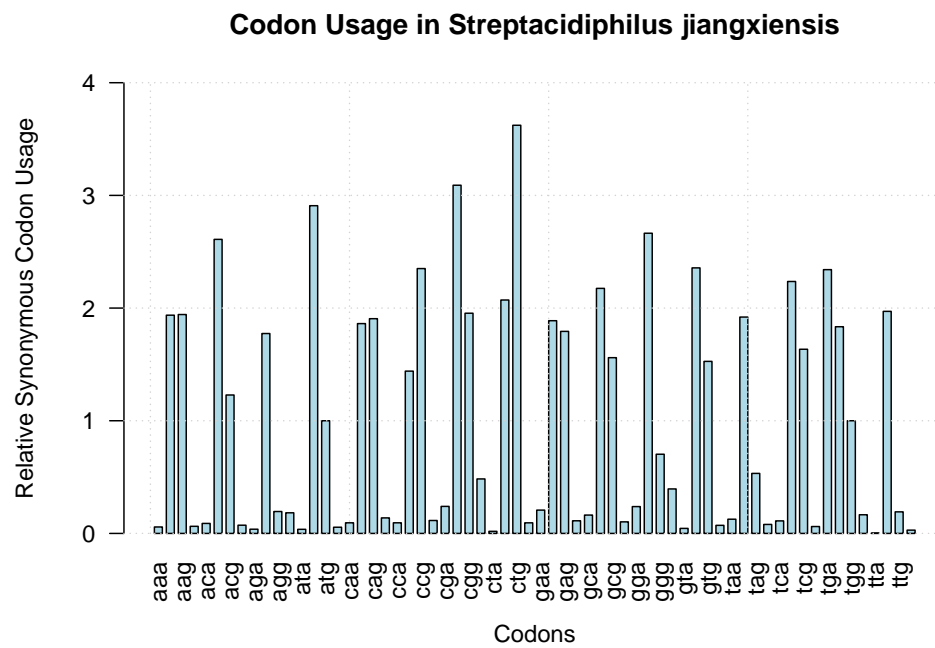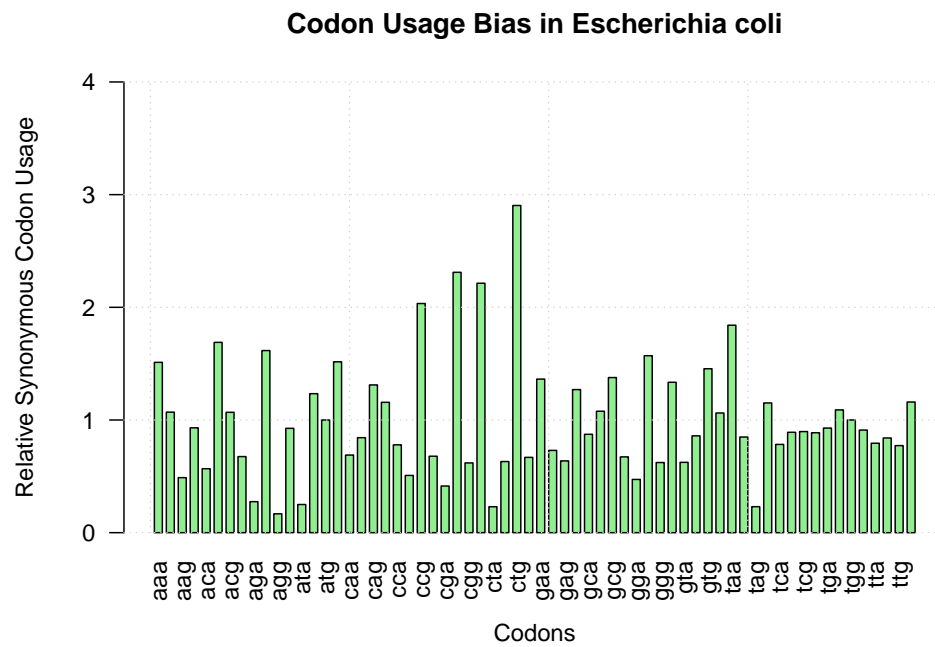
Overlay the two barplots to compare the RSCU between the two organisms.

Codon Usage Bias

Answer: The comparison of codon usage bias between *Escherichia coli* and *Streptacidiphilus jiangxiensis* reveals intriguing insights into their evolutionary adaptations and functional capabilities. In *E. coli*, the Relative Synonymous Codon Usage (RSCU) is notably high for codons such as CTG, CGC, CGT, CCG, and TAA, indicating a preference for these codons in highly expressed genes (Stoletzki and Eyre-Walker, 2006). This pattern suggests that *E. coli* has evolved to optimize its translational efficiency and speed in its typical environments, which often include nutrient-rich conditions that favor rapid growth and division (Lipinszki et al., 2018). Conversely, *Streptacidiphilus jiangxiensis* exhibits a distinct codon usage bias with elevated RSCU values for CTG, CGC, ATC, ACC, and GGC. The presence of ATC and ACC among the preferred codons indicates a potential adaptation to a different ecological niche, possibly involving specialized metabolic functions or responses to varying environmental pressures. These differences in codon preference may reflect the organisms' evolutionary histories, metabolic versatility, and ecological roles (Tyagi et al., 2023), with *Streptacidiphilus* possibly relying on a broader array of substrates or exhibiting different growth strategies compared to the more straightforward, rapid proliferation of *E. coli*. Understanding these patterns can provide deeper insights into how these bacteria interact with their environments and adapt to the challenges they face (Chan et al., 2018).

6) In the organism of interest, identify 10 protein sequence k-mers of length 3-5 which are the most over- and under-represented k-mers in your organism of interest. Are these k-mers also over- and under-represented in E. coli to a similar extent? Provide plots to support your observations. Why do you think these sequences are present at different levels in the genomes of these organisms? K-mer Analysis

Table 10: Top 10 most frequent k-mers in E.coli

	Kmer	Frequency
3781	LLA	0.0013499
181	ALA	0.0013044
190	ALL	0.0012807
10	AAL	0.0012174
3601	LAA	0.0011989

13

	Kmer	Frequency
3790	LLL	0.0011600

Table 11: Least 10 frequent k-mers in S.jiangxiensis

	Kmer	Frequency
4362	MWC	7e-07
8039	AACW	7e-07
8219	AAMW	7e-07
8421	ACCA	7e-07
8423	ACCD	7e-07
8432	ACCN	7e-07

```
## Number of E. coli K-mers with 0 frequency:
```
```
## [1] 2297663
```

Table 12: Top 10 most frequent k-mers in S.jiangxiensis

	Kmer	Frequency
1	AAA	0.0039966
3970	LAA	0.0030309
10	AAL	0.0028426
190	ALA	0.0027951
6	AAG	0.0022728
4159	LLA	0.0021164

Table 13: Least 10 frequent k-mers in S.jiangxiensis

	Kmer	Frequency
660	CMK	4e-07
670	CMW	4e-07
2078	FRX	4e-07
2816	HKC	4e-07
4433	MCC	4e-07
6593	RXX	4e-07

```
## Number of S. jiangxiensis K-mers with 0 frequency:
```
```
## [1] 3149491
```

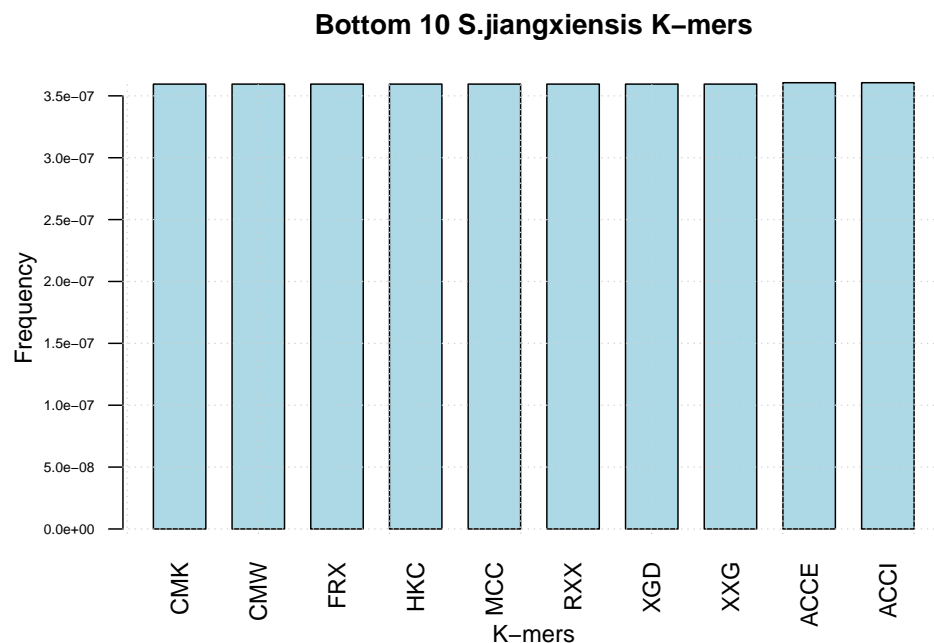## Top 10 E.coli K−mers

Top 10 S.jiangxiensis K−mers

Bottom 10 E.coli K–mers
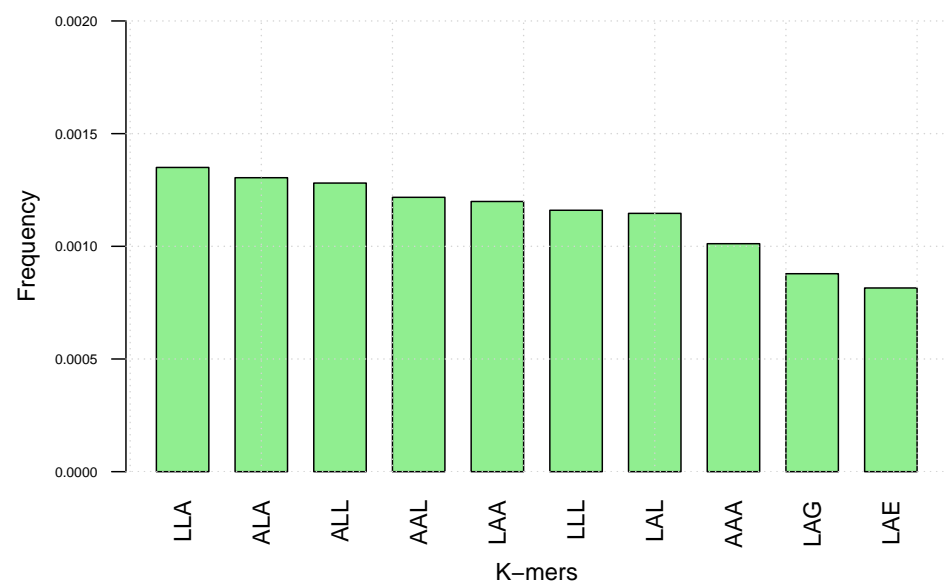
Bottom 10 S.jiangxiensis K–mers
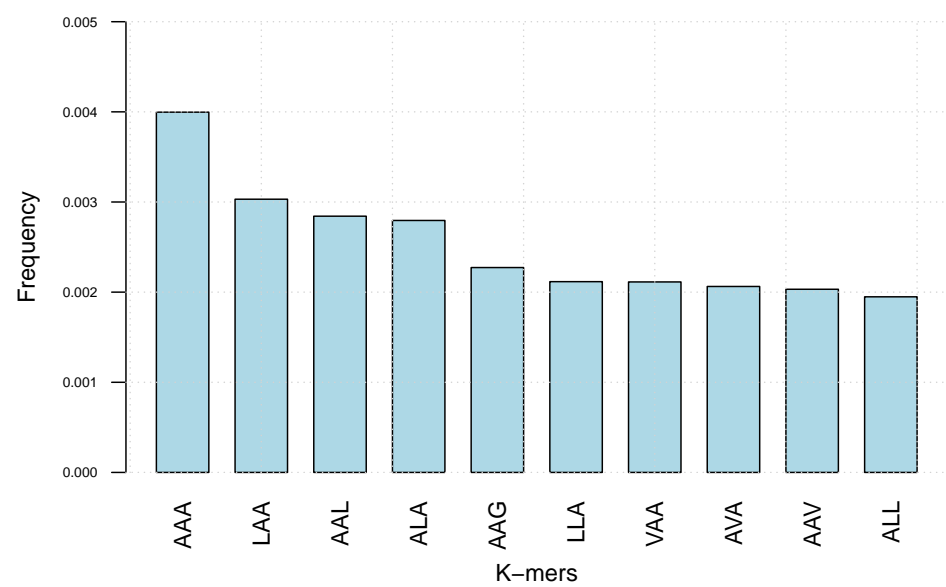
Answer: The analysis of k-mer frequency in proteins offers valuable insights into the evolutionary strategies and functional adaptations of *Escherichia coli* and *Streptacidiphilus jiangxiensis*. The top 10 k-mers in *E. coli*, which include sequences like LLA, ALA, and ALL, predominantly feature leucine (L) and alanine (A). This amino acid composition suggests a potential focus on proteins that require flexibility and structural stability, characteristics that are essential for E. coli's rapid growth and metabolic efficiency in nutrient-rich environments, such as the intestines of warm-blooded animals (Yang et al., 2020). The abundance of leucine, known for its role in protein synthesis and cellular metabolism, may enhance the organism's ability to adapt

quickly to varying nutrient availability, providing a competitive advantage in diverse ecological niches (Tian et al., 2017).

In contrast, *Streptacidiphilus jiangxiensis* presents a different k-mer profile, with its top sequences such as AAA and AAG indicating a higher representation of alanine and a notable presence of arginine (R). The inclusion of arginine in its protein repertoire may enable *Streptacidiphilus* to engage in a wider range of metabolic pathways, including nitrogen metabolism and stress responses, which are critical for survival in the variable soil environments it inhabits. This greater diversity in k-mers suggests a more complex protein structure that may facilitate specialized functions, such as the synthesis of secondary metabolites that help in nutrient acquisition and competition with other soil microorganisms (Yang et al., 2020).

The contrasting least frequent k-mers further illuminate these differences. For example, *E. coli* contains k-mers like MWC and AACW, which may correspond to rare or less-utilized protein motifs, possibly limiting its capacity to produce certain proteins under specific environmental conditions. Meanwhile, *Streptacidiphilus* features k-mers such as CMK and FRX, which may represent unique adaptations or niche-specific proteins that enhance its survival in less competitive environments. The presence of such rare sequences could confer the ability to utilize specific substrates or produce bioactive compounds that aid in survival and competitive advantage in its soil habitat (Bussi et al., 2021).

Overall, the differences in k-mer frequency between these two organisms reveal not only their distinct evolutionary paths but also their strategies for thriving in their respective environments. E. coli's k-mer composition supports rapid growth and adaptability in rich environments, while Streptacidiphilus's diverse k-mer patterns likely enhance its metabolic versatility and ecological resilience in variable soil ecosystems.

References

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```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 22.04.4 LTS
##
## Matrix products: default
## BLAS:   /usr/lib/x86_64-linux-gnu/blas/libblas.so.3.10.0
## LAPACK: /usr/lib/x86_64-linux-gnu/lapack/liblapack.so.3.10.0
##
## locale:
##  [1] LC_CTYPE=C.UTF-8       LC_NUMERIC=C           LC_TIME=C.UTF-8
##  [4] LC_COLLATE=C.UTF-8     LC_MONETARY=C.UTF-8    LC_MESSAGES=C.UTF-8
##  [7] LC_PAPER=C.UTF-8       LC_NAME=C              LC_ADDRESS=C
```

```
## [10] LC_TELEPHONE=C           LC_MEASUREMENT=C.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] knitr_1.48         tidyr_1.3.1        readr_2.1.5
##  [4] ggplot2_3.5.1      dplyr_1.1.4        seqinr_4.2-36
##  [7] Biostrings_2.62.0  GenomeInfoDb_1.30.1 XVector_0.34.0
## [10] IRanges_2.28.0     S4Vectors_0.32.4  BiocGenerics_0.40.0
## [13] R.utils_2.12.3     R.oo_1.26.0       R.methodsS3_1.8.2
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.13        compiler_4.1.2    pillar_1.9.0
##  [4] bitops_1.0-9       tools_4.1.2       zlibbioc_1.40.0
##  [7] digest_0.6.37      gtable_0.3.5      evaluate_1.0.0
## [10] lifecycle_1.0.4    tibble_3.2.1      pkgconfig_2.0.3
## [13] rlang_1.1.4        cli_3.6.3         yaml_2.3.10
## [16] xfun_0.48          fastmap_1.2.0     GenomeInfoDbData_1.2.7
## [19] withr_3.0.1        hms_1.1.3         generics_0.1.3
## [22] vctrs_0.6.5        grid_4.1.2        tidyselect_1.2.1
## [25] ade4_1.7-22        glue_1.8.0        R6_2.5.1
## [28] fansi_1.0.6        rmarkdown_2.28    farver_2.1.2
## [31] purrr_1.0.2        tzdb_0.4.0        magrittr_2.0.3
## [34] scales_1.3.0       htmltools_0.5.8.1 MASS_7.3-55
## [37] colorspace_2.1-1   labeling_0.4.3    utf8_1.2.4
## [40] munsell_0.5.1      RCurl_1.98-1.16   crayon_1.5.3
```