Lab Report on Bacterial Growth Cultures

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Abstract

The purpose of this experiment was to determine and study bacterial growth. The primary experiment dealt with studying the growth curve of a bacterial culture. The second experiment dealt with gram and spore staining of different bacterial cultures for observation under a microscope.

1 Experiment 1

1.1 Plotting the Exponential Growth Curve of Bacteria

Absorbance of the bacterial culture incubated with a broth solution was recorded at 15-minute intervals and summarized in the following table.

Table 1 Absorbance Values of Bacterial Cultures over Time

Time (mins)	A_{600}
0	0.172
15	0.165
30	0.190
45	0.225
60	0.276
75	0.334
90	0.407
105	0.458
120	0.550

*The absorbance was recorded at 600nm

The obtained data were plotted using a scatterplot with **Time (in minutes)** as the x-axis and **$\log_2$(Absorbance)** as the y-axis. A line of best fit was graphed over the scatterplot using only the log phase absorbance values as the bacterial culture as this phase represents the exponential growth of the bacteria.

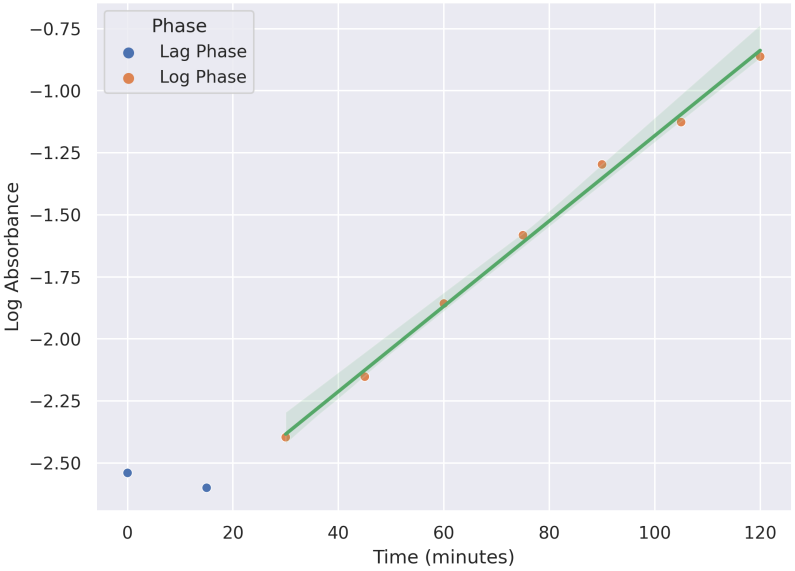

Fig. 1 Growth Curve of the Bacterial culture plotted over a 120-minute interval.

The equation of the line plotted is

$$y = 0.017x - 2.89$$

The following equation was calculated by obtaining two points in the given straight line and solving the pair of linear equations in two variables (slope and intercept). A unit increase in the y-axis relates to the doubling of absorbance. The time taken (increase in the x-axis) for the doubling gives the *doubling time* of the bacteria. Mathematically, it can be calculated by taking the inverse of the slope of the line of best fit $\Rightarrow 1/0.017 =$ **58.47 minutes**.

1.2 Calculation of Colony Forming Units

To obtain information on the concentration of colony-forming units with respect to the absorbance values, at the 60th-minute interval, the culture was taken and serially diluted to four concentrations ranging from 10^{-3} to 10^{-6}. Followingly 100μL was taken and added to the agar plate for incubation and then the colonies were visually counted. The following table summarizes the cfu/100μL at different dilutions and the calculated cfu/mL for the bacteria.

Table 2 Colonies counted over different serial dilutions. *Note: 100μL was added rather than 1mL

Time (mins)	A_{600}	Colonies on 10^{-3} dilution	Colonies on 10^{-4} dilution	Colonies on 10^{-5} dilution	Colonies on 10^{-6} dilution	Colony forming units per ml (cfu/ml)
60	0.276	TMTC TMTC TMTC TMTC	TMTC TMTC TMTC TMTC	105 ± 5 121 ± 5 127 ± 5 134 ± 5	17 ± 1 11 ± 1 12 ± 1 14 ± 1	$(121.75 \pm 25) \times 10^6$

The most optimal measurement was taken at the 10^{-5} dilution.
$\Rightarrow$ Average CFU / 100μL at 10^{-5} dilution = 121.75 ± 25.
$\Rightarrow$ CFU / 100μL at 10^0 dilution = $(121.75 \pm 25) \times 10^5$.
$\Rightarrow$ CFU / 1mL at 10^0 dilution = $(121.75 \pm 25) \times 10^6$.

2 Experiment 2

2.1 Mixed Culture Bacteria

2.1.1 Species Name

Mixed Culture Bacteria

2.1.2 Description of Bacteria under the Agar plate

Bacterial colonies were prominently visible on the agar plate. There were densely packed regions of bacteria colonies suggesting the presence of cocci packets alongside sparsely spread bacteria suggesting the presence of rod-shaped bacteria. The visible number of colonies under the agar plate was in the order of hundreds.

2.1.3 Stain Used for Microscopic Analysis

The procedure was carried out via four subsequent staining steps $\Rightarrow$ Crystal Violet (1 minute), Lugol's Solution (1 minute), Decolorization Solution (10 seconds), and Safranine Red (1 minute).

2.1.4 Description of Stained Culture

A mixture of two species of colored bacteria could be observed $\Rightarrow$

- The first species is brightly violet-colored and cocci shaped. The cells are randomly spaced and do seem to form big chains or clusters. They are however relatively close together than rod-shaped bacteria. The size of these bacteria is almost identical.

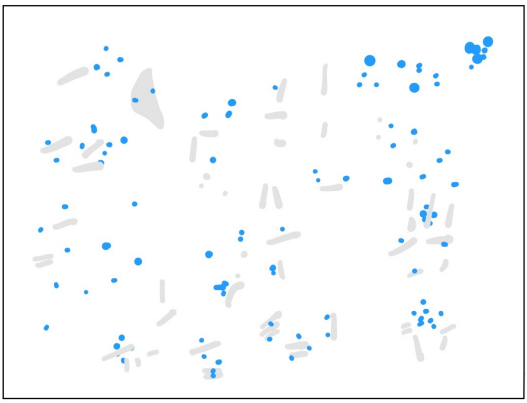

Fig. 2 The following drawing depicts the visual attributes of Mixed Stained bacteria viewed under 100x zoom.

- The second species is a dark pink colored rod-shaped bacteria. It is more sparsely distanced than the former and tends to have some angled arrangement as well as pair-wise occurrence.

2.1.5 Conclusion

According to the visual characteristics observed, the first species seems to be a gram-positive cocci bacteria that occurs in packets. The second species seems to be either a randomly sparsed or angled-arrangement gram-negative bacillus.

2.2 Bacterial Culture A

2.2.1 Species Name

Escherichia Coli.

2.2.2 Description of Bacteria under the Agar plate

Bacterial colonies were prominently visible on the agar plate. The colonies appeared to be smooth, circular, and randomly dispersed throughout the agar.

2.2.3 Stain Used for Microscopic Analysis

The procedure was carried out via four subsequent staining steps ⇒ Crystal Violet (1 minute), Lugol's Solution (1 minute), Decolorization Solution (10 seconds), and Safranine Red (1 minute).

2.2.4 Description of Stained Culture

- The bacterial colonies appear to be rod-shaped and pinkish in color with a few notable violet-stained bacteria that could be the result of contamination.
- The cells seemed to have densely occupied the entire slide.

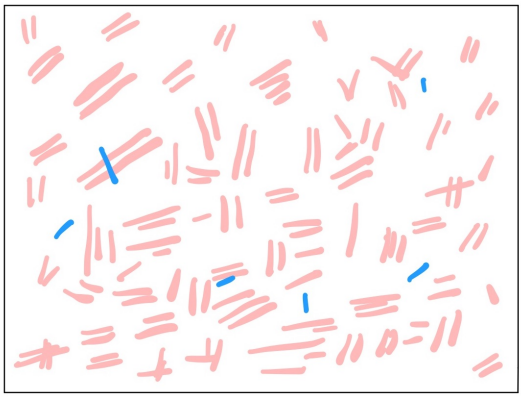

Fig. 3 The following drawing depicts the visual attributes of Bacterial Culture A viewed under 100x zoom.

- There is no chain formation or visible clustering. The arrangement is almost random.

2.2.5 Conclusion

It can clearly be verified that the absence of blue/violet stain indicates that the bacteria present is gram-negative which in fact is true with *E. Coli*.

2.3 Bacterial Culture C

2.3.1 Species Name

Bacillus subtilis.

2.3.2 Description of Bacteria under the Agar plate

Bacterial colonies were prominently visible on the agar plate. The colonies appeared to be yellowish-white colored, smooth, circular, shiny, and randomly dispersed throughout the agar.

2.3.3 Stain Used for Microscopic Analysis

Schaeffer & Fulton's method was followed for staining with Malachite Green.

2.3.4 Description of Stained Culture

- The bacterial colonies appear mostly to be bacilli-shaped and violet in color. There are cells however of different shapes that could be visible.
- These different shapes could possibly indicate the spore formation on the bacteria or possible clustering.
- Relative to the bacilli slide, these bacteria are more sparsely distanced.

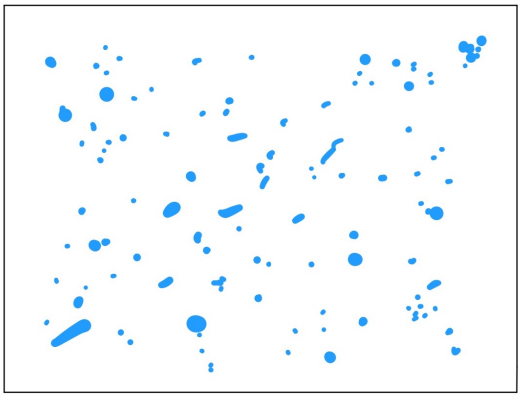

Fig. 4 The following drawing depicts the visual attributes of Bacterial Culture C viewed under 100x zoom.

2.3.5 Conclusion

It can clearly be verified that the presence of blue/violet color alongside spore-like features in some cells indicates that the bacteria present is a gram-positive sporulating culture which in fact is true with *Bacillus subtilis*.

2.4 Sources of Error

1. One of the most prominent sources of error is the contamination of the glass slides while transferring the bacteria.
2. Subsequently, as the bacteria within the colonies are densely packed making them highly sensitive to the quantity of bacteria taken from the agar plate. If an excess of bacteria is taken into the slide, the microscopic image would be heavily layered and make it difficult to distinguish visual traits.
3. The quintessential factor in viewing bacterial cultures under the microscope is the proper incubation of bacteria. If the incubation is carried out incorrectly, experimental results might be obtained incorrectly or even not at all.