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Nutritional and environmental growth requirements of select bacteria on various types of media

Results:

In this experiment, the nutritional requirements of select bacteria, *Escherichia coli*, *Haemophilus influenza*, and *Beta Streptococcus sp.* Group B, were tested to differentiate between non-fastidious and fastidious organisms. This was done by streaking each select bacteria onto various types of media and observing the amount of growth and colony size. As expected, Table 1 shows no bacterial growth on the 1.5% agar plate, as it is a basal media containing only agar and water. In Table 1, *Escherichia coli* shows a trend of heavy growth while *Beta Streptococcus sp.* Group B shows a trend of medium colony growth. The last selected bacteria, *Haemophilus influenza,* shows no growth on any media other than on the Chocolate agar plate. These observations are significant as they can be used to classify the select bacteria on their degree of fastidiousness.

Table 1. Amount growth and colony size of select bacteria, *Escherichia coli* (Ec), *Haemophilus influenza* (Hi), and *Beta Streptococcus sp.* Group B (Bsp), on various media.

Various media types	Ec	Hi	Bsp
1.5% Agar	−	−	−
Mueller Hinton	+++ M	−	+ M
Blood Agar	+++ L	−	+ M
Chocolate Agar	+++ L	++ M	++ M
MacConkey	+++ S	−	+ M

Notes: −, no growth; +, minimal growth; ++, moderate growth; +++, heavy growth; S, small colony; M, medium colony; L, large colony.

In this part of the experiment, the types of haemolysis (Alpha, Beta and Gamma) of select bacteria, *Escherichia coli*, *Haemophilus influenza,* and *Beta Streptococcus sp.* Group B, were observed on blood agar plates in order to further classify the bacteria. This was done by streaking each select bacteria onto a blood agar plate and observing the haemolytic reaction. In Table 2, *Escherichia coli* exhibits a partial beta haemolysis while *Beta Streptococcus sp.* Group B exhibits a complete beta haemolysis. *Haemophilus influenza* shows no display of haemolysis as it shows no growth on the blood agar plate. These observations are significant as they can be used to further classify the select bacteria on their ability to break down blood.

Table 2. Haemolysis type exhibited by select bacteria, *Escherichia coli* (Ec), *Haemophilus influenza* (Hi), and *Beta Streptococcus sp.* Group B (Bsp), on blood agar media.

Type of haemolysis	Ec	Hi	Bsp
Complete beta (β)	−	−	✓
Partial beta	✓	−	−
Double zone	−	−	−
Alpha (α)	−	−	−
Gamma (γ)	−	−	−

Notes: −, no display of haemolysis type; ✓, exhibits haemolysis type.

The growth and lactose fermentability of select bacteria, *Escherichia coli*, *Haemophilus influenza*, and *Beta Streptococcus sp.* Group B, were examined on MacConkey agar plates in order to further classify the bacteria. This was done by streaking each select bacteria onto a MacConkey agar plate and observing the colour reaction, growth and colony size. In Table 3, *Escherichia coli* shows a colour change from the neutral red in the media to a red/pink. This represents its lactose fermentability. *Beta Streptococcus sp.* Group B displayed lactose negative properties. *Haemophilus influenza* shows no display of colour change, or lactose fermentability as there was no growth on the MacConkey agar plate. These observations are significant as they can be used to further classify the select bacteria on their lactose fermentability.

Table 3. Growth and reaction of select bacteria, *Escherichia coli* (Ec), *Haemophilus influenza* (Hi), and *Beta Streptococcus sp.* Group B (Bsp), on MacConkey agar media.

Growth and reaction	Ec	Hi	Bsp
Growth	+++ S	−	+M
Colour of media	red/pink	−	orange
Colour of colonies	pink	−	colourless
Lactose fermentability	✓	−	−

Notes: −, no growth; +, minimal growth; ++, moderate growth; +++, heavy growth; S, small colony; M, medium colony; L, large colony, ✓, exhibits fermentability.

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The effect of oxygen and carbon dioxide on the growth and colony size of select bacteria, *Staphylococcus sp.*, *Pseudomonas aeruginosa*, and *Clostridium perfringens*, was examined on blood agar plates under select environmental conditions. This was done by streaking each select bacteria onto a blood agar plate and creating three different atmospheres: anaerobic (AnO_2), carbon dioxide ($+CO_2$) and aerobic (O_2). In Table 4, *Staphylococcus sp*. shows a trend of medium and moderate growth in an oxygen concentrated environment and less growth in an anaerobic environment. *Pseudomonas aeruginosa* shows a trend of large and moderate growth in an oxygen concentrated environment and no growth in an anaerobic environment. *Clostridium perfringens* shows no growth in the O_2 and $+CO_2$ environment, however it shows minimal growth of a small colony under AnO_2 conditions. These observations are significant as they signify what types of environments the select bacteria are able to grow in.

Table 4. Growth and colony size of select bacteria, *Staphylococcus sp.* (Ssp), *Pseudomonas aeruginosa* (Pa), and *Clostridium perfringens* (Cp), on blood agar media under select environmental conditions.

Environmental conditions	Ssp	Pa	Cp
Aerobic (O_2)	++ M	++ L	−
+ CO_2	++ M	++ M	−
Anaerobic (AnO_2)	+ S	−	+ S

Notes: −, no growth; +, minimal growth; ++, moderate growth; +++, heavy growth; S, small colony; M, medium colony; L, large colony.

Discussion:

In Part A of this experiment, multiple nutrional tests were performed to classify the select bacteria, *Escherichia coli*, *Haemophilus influenza*, and *Beta Streptococcus sp.* Group B. *Escherichia coli* (*E. coli*) can be identified as a non-fastidious organism, it is non-selective and will grow with the minimum nutritional requirements. However, as shown in Table 1, *E. coli* will grow better on an enriched medium, for example Blood agar and Chocolate agar media. *Haemophilus influenza* on the other hand can be classified as a fastidious organism as the bacteria only grew on the Chocolate agar medium. *Beta Streptococcus sp.* Group B (*Beta Strep*) is considered a non-fastidious organism, it displayed minimal growth and a medium colony size. None of the select bacteria grew on the 1.5% agar plate. This was expected as the media contains only agar and water, it does not provide any nutrient required for growth.

In terms of haemolysis, *E. coli* exhibits the partial beta type, shown in Table 2. According to a paper written by Everett Short and Harold Kurtz, certain strains of *E. coli* are capable of breaking down red blood cells and can be identified as either alpha or beta. However, beta hemolysin was produced on ordinary lab medium while alpha hemolysin only grew on a cooked meat medium. This coincides with the partial beta type results of the haemolysis test found in the lab. As *Haemophilus influenza* exhibited no growth on the Blood agar plate, it did not display any signs of haemolysis. In Table 2, *Beta Strep* exhibits the complete beta type of haemolysis. The bacteria completely broke down the red blood cells, creating a colourless zone. This reaction was expected, as reported by Lakshmi Rajagopal, *Beta Streptococcus sp.* Group B are beta hemolytic.

The other test performed was to examine lactose fermentability on a MacConkey agar medium. The results from Table 3 show that *E. coli* has the ability to ferment lactose. The reaction between the *E. coli* and the lactose produced acid byproducts which in turn lowered the pH and caused the visual colour change from a neutral red in the media to a red/pink colour. On the other hand, *Beta Strep* did not display the properties of a lactose fermenter as the colonies were colourless and the MacConkey medium turned orange. *Haemophilus influenza* exhibited no growth on the MacConkey agar plate, and therefore displayed no signs of lactose fermentability. However, this does not mean that it can not ferment lactose. This specific bacterium may be a slow lactose fermenter; therefore, this test can not be the sole basis for its lactose fermentability.

In Part B, three different atmospheres, on Blood agar plates, were created in order to simulate different environmental conditions in which the select bacteria, *Staphylococcus sp.*, *Pseudomonas aeruginosa*, and *Clostridium perfringens* might grow. From Table 4, *Staphylococcus sp.* was capable of growing in all three environments. It is categorized as an aerobically growing bacterium or a facultative anaerobe (Rowlinson et al. 2006). *Pseudomonas aeruginosa* is classified as a ubiquitous bacterium as it is found practically everywhere. It is capable of surviving at low-oxygen levels and of growing anaerobically. The reason for the absence of growth in the anaerobic environment in Table 4 is that *Pseudomonas aeruginosa* requires a terminal electron acceptor (Wu et al. 2005); therefore, since no growth occurred, it can be said that there were no available electron acceptors in the anaerobic atmosphere. In Table 4, there is no growth shown in either oxygen containing conditions, only the anaerobic. This is a result of *Clostridium perfringens* being an aerotolerant anaerobe (O'Brien and Melville 2000). It can tolerate oxygen but will not grow in its presence. These results are expected and coincide with the claims from Rowlinson, O'Brien, Wu and multiple other researchers.

The significance of this experiment is linked to the ever-growing methods of classifying bacteria. It is important to be able to grow and categorize bacteria in order to prevent and treat medical diseases, to develop new technologies and overall, to improve our general knowledge of bacteria. Without the study of microbiology, scientists and researchers would know nothing of how bacteria and other pathogens grow, develop and interact with other cells.

In conclusion, the nutrional and environmental requirements of select bacteria where researched in order to classify the bacteria in terms of their degree of fastidiousness, the type of haemolysis exhibited, their lactose fermentability and their ability to survive in different environmental conditions (atmospheres).

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