

# [Techniques to isolate bacterial colonies | experiment](https://assignbuster.com/techniques-to-isolate-bacterial-colonies-experiment/)

## Introduction

Bacteria are found everywhere (Campbell & Reece, 2005). They have the ability to colonize almost all habitats which means they come in wide varieties of forms. Understanding bacterial structure can therefore make it possible to understand their functions and survival. For example, just by studying the capsule layer of bacteria, one can get an idea of the pathogenicity of the bacteria. But they grow among diverse and mixed populations, where it is impossible to study a single species of bacteria. Thus it is necessary that the pure culture of bacterial colonies be obtained for studying. Pure culture is defined as a mass or group of cells arising from the same parent cell. Pure culture techniques, such as streak plate, pour plate and spread plate, isolate bacterial colonies from mixtures so that colonies comprising of the identical organisms can be studied. Isolation of pure culture is vital for characterizing a single species of bacteria­ otherwise presence of contaminants can lead to inaccurate observations (Prescott et al., 2005)

Then the isolated bacterial colonies undergo incubation at 37°C for 24 hours for all the three plates, after which, a colony can be selected and isolated again to obtain pure cultures. Another step in the experiment is to perform a differential staining method known as Gram staining, which mainly differentiates the bacteria into two categories: Gram positive and Gram negative based on their cell wall structures (Campbell and Reece, 2005).

The species of bacteria present in the given broth culture were Escherichia Coli, Staphylococcus Aureus and Bacillus Cereus. The appearances of these bacteria known in theory state that Escherichia Coli are Gram negative and rod-shaped; Bacillus Cereus are Gram positive and rod-shaped while Staphylococcus Aureus are Gram positive and cocci.

So the experiment was carried out to see if it was possible to obtain pure cultures from mixed broth by applying streak, spread and pour plate techniques.

## AIMS

The main objectives of this experiment were to understand the purpose, principles and techniques of Gram staining as well as the isolation of pure cultures. The experiment further aimed to compare the different isolation techniques, namely streak plate, spread plate and pour plate in terms of producing pure cultures, and also compare the morphological features of three types of bacteria.

## MATERIALS AND METHODS

The procedure was carried out according to the instructions stated in the pages 32-36 of the Microbiology lab manual (MIC2011, Class Notes, 2011).

## RESULTS (combined with Morning Lab, Bench 1, Group A3)

The entire experiment was carried out in three different sessions.

### Session 1

A broth culture, labelled as Culture 2, was provided and it was known to contain a mixture of Staphylococcus aureus, Escherichia coli and Bacillus cereus. Sample from the broth culture was heat-fixed and then gram stained for observation under the light microscope. Three different types of bacteria were seen and their identifiable characteristics were matched with the three kinds of bacteria known to be present in the mixture.

After the presence of all three types of bacteria was confirmed, loopfuls from the broth culture were taken in order to prepare pure cultures using three isolation techniques – streak plate, spread plate and pour plate. Once the three isolation plates were ready, they were incubated overnight at 37°C for the next session.

### Session 2

After overnight incubation at 37°C, the colonies which formed on the streak, spread and pour plate were observed under the microscope to see the distribution and the morphology of bacterial colonies. These isolation techniques helped determine which bacteria were responsible for the colony morphology.

Since distinct and well-isolated colonies were not found from the spread plate and pour plate techniques, selection of distinct and clear colonies of all three types of bacteria were made from the streak plate and observed under the microscope.

### Session 3

The isolated colonies were incubated overnight at 37°C and inoculated onto new nutrient agar (NA) plates, after which pure plates of each type of bacteria were prepared and Gram stained followed by the observation of single colonies of bacteria under the microscope.

## DISCUSSION

(1) As seen in the table-4 of results, Bacillus cereus had the largest colony with irregular shape, raised elevation, rough and dull surface, undulating edges, opaque and white in colour. They are Gram positive and rod-shaped, which are arranged in chains.

The moderate-sized colony was the Escherichia coli with a circular shape, convex elevation, smooth and glistening surface, smooth edges, transparent and yellow in colour. They are Gram negative and are seen under the microscope as short rod-shaped structures existing in pairs or single cells.

The smallest of all colonies belonged to the Staphylococcus aureus, with a circular shape, smooth and glistening surface, undulating edges, opaque in pale yellow in colour. They are Gram positive cocci arranged in grape-like clusters and they do not form spores (Mahon et al., 2007). The cellular morphology of bacteria was observed by Gram staining while the colony morphology was seen using streak plate technique. The morphology of each bacterium observed in the pure cultures matched with those in the original mixture, but the colony size in pure culture was much bigger than those in mixed culture. The probable reason for this is that multiple bacterial colonies experience competition for nutrients while colonies in pure culture had sufficient food and space. No contamination was found and individual bacterial colonies were separated on an agar surface.

(2) Among the three techniques used, streak plate is the most efficient way to produce well-isolated colonies, or in other words, pure colonies. In streak plate technique, the microorganisms containing many viable cells, were directly plated. However, in order for the technique to work well, there must be large populations of the organism in the original mixture (Sumbali and Mehrotra, 2009). Otherwise, the final streaks result in less or no colonies because in the streak plate method, the bacterial suspension is diluted more and more as it proceeds from the primary inoculums towards the latter streaks. Streak plate is a cost-effective and rapid-active method for separating bacteria in mixed cultures of high density and can also be repeated for achieving the desired purity. For these reasons, streak plate technique is widely used in laboratories (Pommerville, 2010). One disadvantage of streak plate is that the risk of contamination is higher since the plate is exposed to air for several times (Sumbali and Mehrotra, 2009). For more diluted populations, spread plate and pour plate are considered since they are easy to locate colonies among low density of evenly distributed populations (Willey et al., 2011). Serial dilutions reduce the microbial population (Willey et al., 2011). For spread plate, 0. 1 ml of the bacterial suspension was taken from 10-2 dilution and spread over the agar whereas for pour plate, 1 ml of the suspension was taken from the 10-2 dilution. Although spread plate can separate a bacterial colony and it is more aseptic than streak plate, it is not feasible for isolating colonies from a mixture because the method is time-consuming and the colonies are not easily differentiated. However streak plate can be used to count microbial populations (Prescott et al., 2005). Similarly, pour plates are also used for counting microbial populations. Although it has the least risk of contamination, the process is time-consuming and the colonies are hard to distinguish or count since the colonies also grow inside the agar, for which this technique is not used to isolated colonies from a mixture. Pour plate is used for isolating and counting anaerobic bacteria since these microbes cannot survive in atmospheric levels of oxygen and are therefore only found within the agar and not on the surface of the agar (Hogg, 2005).

(3) Gelatin is colourless, brittle and translucent medium (Willey et al., 2011). In this experiment, agar was used as the culture medium. Agar is preferred over gelatin for making solid media because of several reasons. First of all, unlike gelatine, agar contains complex polysaccharides that cannot be nutritionally digested or degraded by most microorganisms to form precipitates, since the incubation temperature of agar is about 20°C, which is below the optimum temperature of most organisms and this enables the agar to remain solid when microorganisms are growing on it (Hogg, 2005). Secondly, agar sets firmly and strongly, providing a stable culture medium (Willey et al., 2011). Moreover, agar has a wide range of pH from 5 to 8 and also solidifies below 40°C, for which liquid agar can be poured over the temperature sensitive nutrients without damaging them. Furthermore, the melting point of solid agar is above 100°C and therefore can be used to culture thermophiles (Bauman, 2006). Finally, agar, being porous, can also show motility of the bacteria (Prescott et al., 2005).

(4) Unlike bacterial colonies with heavy growth, the well-separated colonies do not have shortage of food or space, which prevents over-crowding and competition for resources and thus the bacterial growth rate is higher, for which they appear larger. Growth rate of bacterial cells in well-separated areas is further enhanced by the higher diffusion rate of the oxygen uptake by the cells and releasing of toxic metabolites out of the cell (Willey et al., 2011). On the other hand, bacteria in areas of high growth face competition for food and space for which their growth rate as well as survival rate is lower. This is probably the reason why Staphylococcus aureus, grew initially, but then stopped.

In this experiment, once the bacteria were isolated, they were Gram stained and checked to ensure that no contamination had taken place and that each colony had single species of bacteria. (5a) An individual colony might contain more than one type of bacterium due to contamination. Common reasons for contamination are improper sterilization of inoculating loop or absence of aseptic techniques or sometimes even under sterilized environments. Contaminants are also present in the extracellular slime layer of bacteria and also in those bacteria which are joined in a network of chains. Another reason for not having the same type of bacterium in a colony, although very rare, is mutation and this results in the presence of multiple mutant strains of bacteria being present in a single colony (Pommerville, 2010). (5b) Bacteria reproduce asexually by a process called binary fission where a parent cell divides into and subsequent divisions take place. In this way, a colony can arise from multiple cells of same or different species of bacteria which are deposited together on a solid medium and this is how a colony may not always have the same parent cell. Even when bacteria exist in clusters, not necessarily are they from a single parent cell (Hogg, 2005).

(6) Passing the dried smear through the Bunsen flame is known as heat fixing. By doing so, the microorganisms are killed and attached firmly to the slide due to the coagulation of the proteins; otherwise they would wash off with the stains. Heat fixing also alters the structure of the microorganisms, preserving the general morphology, so that they become permeable to stains (Sumbali and Mehrotra, 2009).

(7) A whole colony would result in a thick smear, which not only appear overlapping and crowded under the microscope, but also prevent the diffusion of the dyes across the cell, for which the alcohol would not be able to effectively decolorize the cells, causing majority of the cells to be stained purple. Also different types of bacterial cells have different staining methods which may get confusing. Therefore, it would get very difficult to identify individual cells.

(8) There is no association between bacterial cell shape and reaction to Gram stain. Gram staining depends on the thickness of the bacterial cell wall which is composed of peptidoglycan. Peptidoglycan consists of abundant teichoic acid, a thin layer of periplasmic space and a peptide interbridge (Prescott et al., 2005). Both Gram positive and Gram negative bacteria come in various shapes. For instance, Staphylococcus aureus (coccus-shaped) and Bacillus cereus (rod-shaped) are both Gram positive.

(9) The bacterial cell wall structure determines its Gram stain reaction. Bacterial cell walls are composed of peptidoglycan which maintain rigidity and shape of the bacterial cell and also give protection from osmotic lyses (Prescott et al., 2005). However, in Gram positive bacteria, 90% of the cell wall is made up of peptidoglycan, for which the cell walls in Gram positive bacteria is thicker than in Gram negative bacteria where the peptidoglycan content is only 5 to 20%. The decolourisation depends on the peptidoglycan content in the cell wall and therefore determines whether the cell will be Gram stained or not. Upon addition of alcohol, the pores of the thick peptidoglycan layers shrink in the cell wall of Gram positive bacteria, which results in dehydration of the layer and retaining of the stain, for which they appear purple under the microscope. As for Gram negative bacteria, the alcohol wash opens the pores of the peptidoglycan layer and the stain is not retained in the peptidoglycan layer (Prescott et al., 2005).

(10) The step which is very crucial in determining the outcome of Gram staining is the decolourisation with alcohol. The cell wall structure of Gram positive bacteria allows the crystal-violet iodine dye complex (CV-I complex) to be retained within the thick peptidoglycan layer when alcohol is added. On the contrary, the cell wall structure of Gram negative bacteria allows the CV-I complex to be removed from the peptidoglycan layer upon addition of alcohol. It is for the decolourization step that the Gram negative bacteria appear pink when counterstained with Safranin and Gram positive ones appear purple, otherwise CV-I complex would be present in both types of bacteria and both would be seen purple under the microscope.

(11) Nonetheless every step in Gram staining is necessary to obtain the correct result. If any of the steps is eliminated, the results can change and are misleading. The table below shows the various outcomes when the steps in Gram staining are changed.

When crystal violet wash is eliminated in step A, none of the cells will get the purple colour and thus all cells are stained pink when washed with Fuchsin. In step B, when the iodine wash is eliminated, the CV-I complex does not form and the crystal violet molecules are not large enough to be retained in the peptidoglycan. Thus they are washed off with the water and alcohol, for which even the Gram positive cells appear pink. In step C, with the elimination of alcohol wash, decolourization is not done, for which all cells, including the Gram negative one where the CV-I complex masks the pink colour, are stained purple (Willey et al., 2011). When Fuchsin wash is eliminated in step D, no change is brought on the Gram positive cells, but the Gram negative cells appear colourless, the reason being that after washing off the crystal violet with alcohol, no counterstain is added to the Gram negative cells (Willey et al., 2011).

A limitation in this experiment is not performing the Gram stain when the species of bacteria were identified on the streak plate and again streaked on the nutrient agar plates. The bacterial species were of taken based on their colony morphology. However a Gram stain could have confirmed if the colonies comprised of the expected bacteria or not.

## CONCLUSION

It was mandatory that aseptic techniques be followed when experimenting with microorganisms. Compared to spread plate and pour plate techniques, streak plate was found to be the most efficient and also the easiest way to isolate bacterial colonies. Pure cultures of three types of bacteria that were obtained included Staphylococcus aureus (rod-shaped and Gram positive), Bacillus cereus (cocci-shaped and Gram positive) and Escherichia coli (rod-shaped and Gram negative). Other than that, importance of heat fixing and Gram staining was understood. Gram staining proved to be a very good method for observing the bacterial cellular features and hence, differences between Gram positive and Gram negative bacteria were clarified. It was also understood that reaction of a bacterial cell to the Gram stain is determined by the decolourization step.