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TOPIC: LAWN METHORD OF BACTERIAL CULTURE

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Introduction

Bacterial lawn culture is a fundamental method in microbiology that involves inoculating an agar plate with a dense bacterial suspension to achieve a confluent, uniform growth. In this technique, individual colonies are no longer distinguishable, creating a continuous layer of cells that provides an ideal platform for evaluating the effects of antimicrobial agents. This method is particularly useful in antimicrobial susceptibility testing, where clear zones of inhibition around antibiotic disks can be accurately measured (Acharya Tankeshwar, 2023; Jung & Hoilat, 2021).

The lawn culture technique is not only employed in clinical diagnostics but also plays a critical role in research settings. It enables microbiologists to assess bacterial responses under standardized conditions, thereby improving the reproducibility and reliability of results. Such consistency is vital for studying bacterial physiology, screening novel antimicrobial compounds, and monitoring trends in antibiotic resistance, which has become a growing public health concern (Lagier et al., 2015; Ramanan et al., 2018).

Selective and differential media further enhance the utility of lawn cultures. For instance, MacConkey agar is widely used for the cultivation of gram-negative bacteria because its formulation—including bile salts and crystal violet—suppresses the growth of gram-positive organisms. Moreover, MacConkey agar differentiates bacteria based on their ability to ferment lactose; lactose-fermenting bacteria produce characteristic pink to red colonies due to acid-induced color changes in the neutral red indicator (Jung & Hoilat, 2021; Nikaido, 1996). In contrast, nutrient agar serves as a general-purpose medium, supporting a wide variety of non-fastidious

microorganisms by providing essential nutrients such as peptone and beef extract, which are crucial for bacterial growth in diverse environments (Madigan et al., 2018).

By integrating these media with the lawn culture method, laboratories can not only cultivate and maintain bacterial populations effectively but also perform detailed analyses for pathogen identification and susceptibility testing. This integration is key to advancing our understanding of microbial ecology and improving clinical outcomes through timely and precise antimicrobial therapy.

In summary, the lawn method of bacterial culture is a versatile and essential tool in both clinical and research microbiology. Its ability to generate uniform bacterial growth—when combined with the use of selective and differential media—provides a robust framework for studying bacterial behavior, evaluating antimicrobial efficacy, and ultimately guiding effective treatment strategies.

Materials

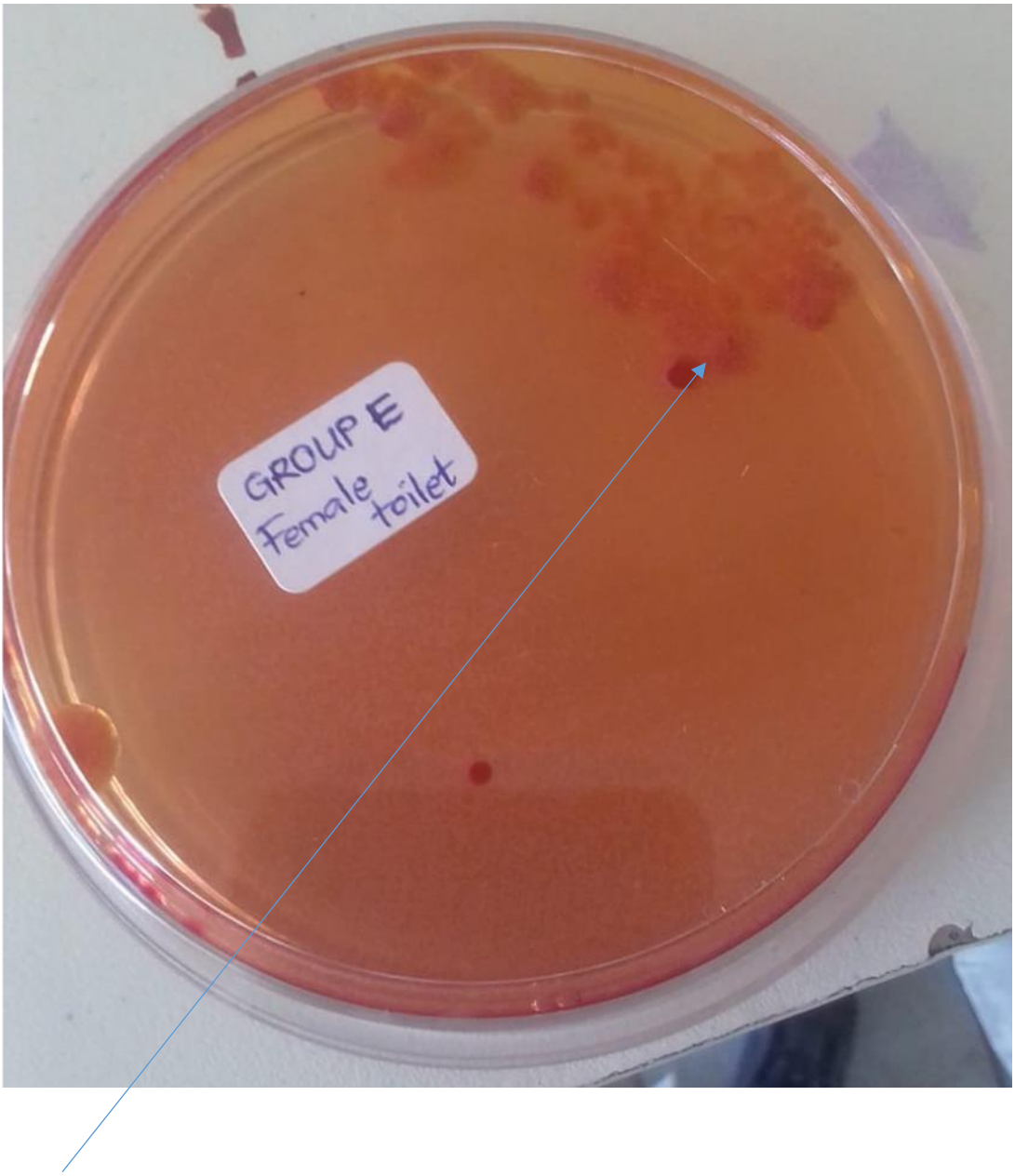
1. Bacteria
2. Heating block
3. Agar
4. Pippete
5. Vortex
6. Flask
7. Petri-dish
8. Labels

Procedure

1. Once your plate has been labelled and marked you are ready to pour your lawn. For each lawn, you will require 4 mL of top agar that has been melted by boiling and then kept at 55°C. Note that 55°C can kill many host bacteria so you must work quickly -get your working zone set up so you can work efficiently.
2. And once you have poured the top agar lawn it will start to set in a few seconds. You should be able to complete the following operation in a little under 10 seconds.
3. Attach a fresh 200 µL tip onto your P200 pipettor and aspirate 100 µL of bacterial culture.
4. Take a top agar aliquot and eject the culture into the agar. Vortex the tube for a few seconds to evenly distribute the cells while replacing the pipettor on its holder.
5. Open the lid of the Petri dish and quickly, but smoothly, pour the mixture over the solid agar surface.
6. Your main objective is not to introduce bubbles into the lawn either from the vortexing or pouring steps.
7. Replace the lid of the Petri dish, and put the empty tube back in the heat block, then gently swirl the plate for 2-3 seconds so that the molten agar forms an even layer.
8. If there are any bubbles in the top agar they can be removed with a sterile pipet tip, but working quickly before the agar is set.
9. Alternatively, once the top agar is set, the location of any bubbles can be marked on the underside of the Petri dish.
10. Set the plate to one side and allow the top agar to set. About 5 minutes, but be careful when you check - gently lift one side of the plate very slightly and if you see any movement of the top agar then place the plate back flat on the bench and wait a few more minutes.

11. Be careful because if the partially set agar moves too much the lawn will break apart and the plate will have to be discarded.

Results



Pink-red colonies: Pink-red colonies on MAC medium indicating the presence of lactose fermenting bacteria

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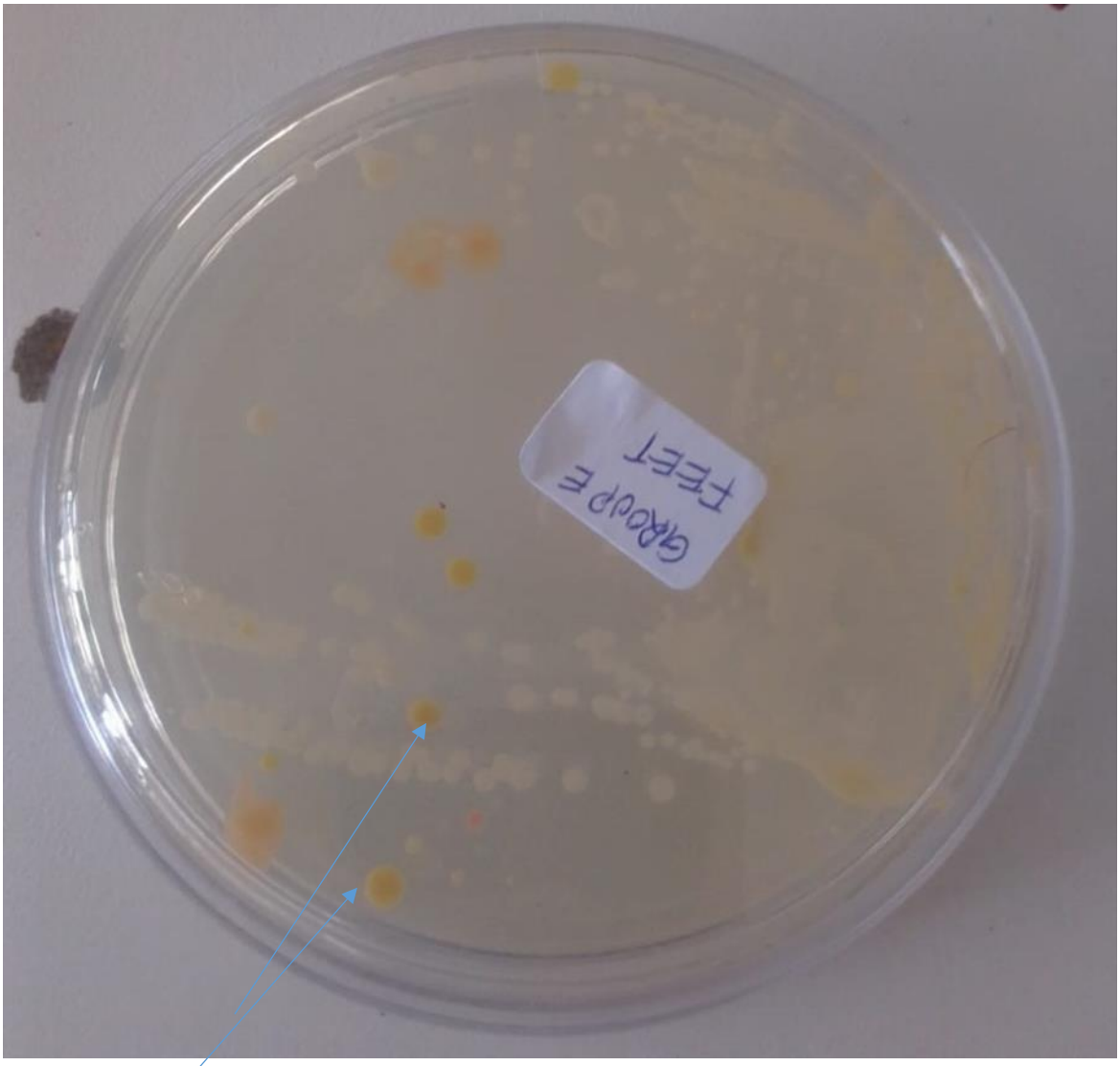

Circular, pinpoint colonies of the size 0-5 to 1 mm in diameter; light yellow colored with low convex elevation; mucoid colonies.

Pale (NLF) and pink (LF) colonies on MacConkey Agar

Discussion

MacConkey agar is a selective and differential media used for the isolation and differentiation of non-fastidious gram-negative rods, particularly members of the family *Entero bacteriaceae* and the genus *Pseudomonas*. The inclusion of crystal violet and bile salts in the media prevent the growth of gram-positive bacteria and fastidious gram negative bacteria, such as *Neisseria* and *Pasteurella*. The tolerance of gram-negative enteric bacteria to bile is partly a result of the relatively bile-resistant outer membrane, which hides the bile-sensitive cytoplasmic membrane (Nikaido, 1996). Other species specific bile-resistance have also been identified. (Provenzano, et al. 2000; Thanassi et al. 1997).

Gram-negative bacteria growing on the media are differentiated by their ability to ferment the sugar lactose. Bacteria that ferment lactose cause the pH of the media to drop and the resultant change in pH is detected by neutral red, which is red in color at pH below 6.8. As the pH drops, neutral red is absorbed by the bacteria, which appear as bright pink to red colonies on the agar. The color of the medium surrounding Gram negative bacteria may also change. Strongly lactose fermenting bacteria produce sufficient acid to cause precipitation of the bile salts, resulting in a pink halo in the medium surrounding individual colonies or areas of confluent growth. Bacteria with weaker lactose fermentation growing on MacConkey agar will still appear pink to red but will not be surrounded by a pink halo in the surrounding medium. Gram-negative bacteria that grow on MacConkey agar but do not ferment lactose appear colorless on the medium and the agar surrounding the bacteria remains relatively transparent. Lactose can be replaced in the medium by other sugars and the abilities of gram-negative bacteria to ferment these replacement sugars is detectable in the same way as is lactose fermentation (for example Farmer and Davis, 1985).

Besides differentiating on the basis of color, colonies on MacConkey medium can further be presumptively identified based on their colonial appearances (shape, size, margin, time of growth, etc.). Some of them are enlisted below:

Organism	**Type**	**Colony characteristics**
Escherichia coli	Lactose fermenter	*E. coli* gives flat, dry, pink, non-mucoid colonies with a surrounding darker pink area of precipitated bile salts.
Klebsiella spp	Lactose fermenter	Colonies typically appear large, mucoid, and pink, with pink-red pigment usually diffusing into the surrounding agar
Citrobacter spp	Late lactose fermenter	Appear as non-lactose fermenter (NLF) up to 24 hours; however, after 48 hours colonies are light pink.
Enterobacter spp	Lactose fermenter	Pink, mucoid colonies but smaller than *Klebsiella* spp.
Serratia spp	Late lactose fermenter	*S. marcescens* may be red-pigmented, especially if the plate is left at 25°C
Proteus spp	Non-Lactose Fermenter	Pale colonies with swarming, characteristic foul smell.

Shigella spp	Non-LactoseFermenter except *S.sonnei* which is a late lactose fermenter	Pale colonies,1-2 mm, flat colonies with jagged edges
Providencia spp	Non-Lactose Fermenter	Colonies are colorless, flat, 2-3 mm in diameter, and do not swarm.
Salmonella spp	Non-Lactose Fermenter	Colorless colonies, convex, 2-3 mm with a serrated margin.
Pseudomonas spp	Non-Lactose Fermenter	Colorless, flat, smooth colonies, 2-3 mm in diameter with greenish to brownish pigmentation.
Yersinia spp	Non-Lactose Fermenter	Colonies may be colorless to peach
Gram-positive bacteria		No growth

Conclusion

The results obtained on MacConkey's Media are significant for the preliminary identification and differentiation of bacteria based on their ability to ferment lactose. This selective and differential medium aids in the isolation and characterization of Gram-negative bacteria, particularly those from the *Enterobacteriaceae* family. The characteristic appearance and texture of the bacterial lawn on MacConkey's Media provide valuable information for further analysis and identification of bacterial species.

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