Differentiation of Oral Streptococcal Species by Haemolysis in Blood Agar Medium in Vitro

Ruchi Sharma, Aditi Gupta

Abstract- Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy. Blood agar medium contain a typical nutrient growth medium enriched with 5% sheep blood. It is useful for encouraging growth of fastidious organisms. The Three Types of Lytic Activities seen on the plate are- clear zone around bacterial growth -RBC hemolyzed completely (Beta-hemolysis, pathogenic); green zone around growth -RBC partially hemolyzed (Alpha-hemolysis); no change around growth -RBC is not hemolyzed (Gamma-hemolysis or no hemolysis). The discrimination of oral bacteria specifically Streptococcal species can be done by haemolysis tests using blood agar medium. Other than ultramicroscopic and morphological studies this experiment also conveys the species differentiation due to its lytic activity. Thus, blood agar medium can be a useful medium for pathogenic bacterial survey and investigation. The present review has proved the importance of blood agar utilization in diagnostics and disease analysis.

Index Terms—Bacteria,Streptococcus, Blood, Agar, Haemolysis

I. INTRODUCTION

Pathogenic bacteria

These bacteria that cause bacterial infection. One of the bacterial diseases with the highest disease burden is tuberculosis, caused by the bacterium Mycobacterium tuberculosis, which kills about 2 million people a year, mostly in sub-Saharan Africa. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria such as Streptococcus (Baltimore, 2010) and Pseudomonas, and foodborne illnesses, which can be caused by bacteria such as Shigella, Campylobacter, and Salmonella. Pathogenic bacteria also cause infections such as tetanus, typhoid fever, diphtheria, syphilis, and leprosy (Kumar et al. 2007). The ability of bacterial colonies to induce hemolysis when grown on blood agar is used to classify certain microorganisms. This is particularly useful in classifying streptococcal species. A substance that causes hemolysis is a hemolysin (Ray et al. 2004).

Utility of blood agar medium

Blood agar medium contain a typical nutrient growth medium enriched with 5% sheep blood. It is useful for encouraging growth of fastidious organisms (Barron et al., 2005).

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Ruchi Sharma, Mahatma Jyoti Rao Phoole University, Jaipur, India.
Aditi Gupta, Mahatma Jyoti Rao Phoole University, Jaipur, India

It also can show results of hemolytic enzymes produced by some organisms. Remember from the selective and differential media lab, that alpha hemolysis is the incomplete breakdown of the blood cells leading to a green coloration around the colonies, beta hemolysis is the complete breakdown of RBCs leading to a clear area around the cells, and that gamma hemolysis is growth on the media with no breakdown of the blood cells and no change to the media (Wayne, 2007).

Mouth flora as sample for bacterial isolation

The human mouth provides a warm, moist environment for many species of organisms. Within your mouth lives a tremendous amount of microorganisms, including fungi, protozoa, viruses and bacteria. Dental caries or cavities are caused by bacteria and saliva is known to contain over a million bacteria per milliliter. All cavities begin with the formation of plaque. Plaque is a gummy substance on the surface of the enamel that is a mixture of various bacteria and the end products of carbohydrate hydrolysis and fermentation. The production of acids by these bacteria causes tooth decay. At a pH of 5.5 or lower, demineralization of the tough, protective enamel begins.Streptococcus and lactobacilli are two of the biggest acid producers and it now seems that Streptococcus mutans seems to be the most important one and is usually the one that initiates tooth decay (Hahn et al., 2005).

II. METHODOLOGY

Prepare required amount of blood agar medium as per lab instructions given in the powder of blood agar which has to be mixed in water and boiled. Boiling is done for 10 minutes and then cooled to 45 C. Obtain a piece of sugarfree gum or paraffin and chew for 3 minutes without swallowing. Collect your saliva in a sterile container. Chewing removes the bacteria from your teeth. Shake your saliva to resuspend the microorganisms.With a 1ml pipette, transfer 0.2 ml of saliva to tube of test agar. Mix the contents of the tube by rotating the tube vigorously between the palms of the hands. Pour plate the blood agar medium containing salivary sample in a petridish. Let the medium solidify. Incubate the petridish at 30C. after 24 hours colonies of bacteria are seen in the petridish. Blood agar plate allows for the growth of fastidious organisms and the differentiation of three hemolytic activities.

III. OBSERVATIONS

The Three Types of Lytic Activities seen on the plate are- clear zone around bacterial growth -RBC hemolyzed completely (Beta-hemolysis, pathogenic); **green**

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zone around growth -RBC partially hemolyzed (**Alpha-hemolysis**); **no change** around growth -RBC is not hemolyzed (**Gamma-hemolysis or no hemolysis**)

When alpha hemolysis (α-hemolysis) is present, the agar under the colony is dark and greenish. Streptococcus pneumoniae and a group of oral streptococci (Streptococcus viridans or viridans streptococci) display alpha hemolysis. This is sometimes called green hemolysis because of the color change in the agar. Other synonymous terms are incomplete hemolysis and partial hemolysis. Alpha hemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methemoglobin (Gerber et al., 2012).

Beta hemolysis (β-hemolysis), sometimes called complete hemolysis, is a complete lysis of red cells in the media around and under the colonies: the area appears lightened (yellow) and transparent. Streptolysin, an exotoxin, is the enzyme produced by the bacteria which causes the complete lysis of red blood cells. There are two types of streptolysin: Streptolysin O (SLO) and streptolysin S (SLS). Streptolysin O is an oxygen-sensitive cytotoxin, secreted by most Group A streptococcus (GAS), and interacts with cholesterol in the membrane of eukaryotic cells (mainly red and white blood cells, macrophages, and platelets), and usually results in β-hemolysis under the surface of blood agar. Streptolysin S is an oxygen-stable cytotoxin also produced by most GAS strains which results in clearing on the surface of blood agar. SLS affects immune cells, including polymorphonuclear leukocytes and lymphocytes, and is thought to prevent the host immune system from clearing infection. Streptococcus pyogenes, or Group A beta-hemolytic Strep (GAS), displays beta hemolysis (Shaikh et al. 2010).

If an organism does not induce hemolysis, the agar under and around the colony is unchanged, and the organism is called non-hemolytic or said to display gamma hemolysis (γ-hemolysis). Enterococcus faecalis (formerly called "Group D Strep") displays gamma hemolysis.

IV. RESULTS AND DISCUSSION

The history of blood agar, as we know it today, is uncertain. The inclusion of blood as a nutritive supplement in culture media may pre-date the use of agar. In their 1903 Manual of Bacteriology, Muir and Ritchie list its inclusion before they discuss “agar-agar” as a replacement for gelatin as a solidifying agent.

In the same discussion, however, they note that Robert Koch preferred plates poured by mixing bacterial inocula with melted gelatin rather than streaking material on the surface. Koch recommended media that were “firm, and where possible, …transparent…” It appears that pour plates were the standard procedure for many years due largely to problems with surface contamination upon incubation. (It should be noted that, initially, agar “plates” were, indeed, sterilized flat glass plates, not Petrie dishes as we know today.) (Rickhard, 2008)

Blood Agar is a general purpose enriched medium often used to grow fastidious organisms and to differentiate bacteria based on their hemolytic properties (Rogers, 2008).

V.CONCLUSION

The discrimination of oral bacteria specifically Streptococcal species can be done by haemolysis tests using blood agar medium. Other than ultramicroscopic and morphological studies this experiment also conveys the species differentiation due to its lytic activity. Thus, blood agar medium can be a useful medium for pathogenic bacterial survey and investigation. The present review has proved the importance of blood agar utilization in diagnostics and disease analysis.

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REFERENCES

1. Kumar, Vinay; Abbas, Abul K.; Fausto, Nelson; & Mitchell, Richard N. (2007). Robbins Basic Pathology (8th ed.). Saunders Elsevier. pp. 843 ISBN 978-1-4160-2973-1
2. Ray, C. George; Ryan, Kenneth J.; Kenneth, Ryan (July 2004). Sherris Medical Microbiology: An Introduction to Infectious Diseases (4th ed.). McGraw Hill. p. 237. ISBN 978-0-8385-8529-0. LCCN 2003054180. OCLC 5235 8530.
3. Rickard A H (2008). "Cell-cell Communication in Oral Microbial Communities". Molecular Oral Microbiology. Caister Academic Press. ISBN 978-1-904455-24-0.
4. Rogers A H (editor). (2008). Molecular Oral Microbiology. Caister Academic Press. ISBN 978-1-904455-24-0.
5. Shaikh N, Leonard E, Martin JM (September 2010). "Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis". Pediatrics 126 (3): e557–64. doi:10.1542/peds.2009-2648. PMID 20696723
6. HW; Gerber, MA; Kaplan, EL; Lee, G; Martin, JM; Van Beneden, C (Sep 9, 2012). "Clinical Practice Guideline for the Diagnosis and Management of Group A Streptococcal Pharyngitis: 2012 Update by the Infectious Diseases Society of America.". Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 55 (10): e86–102. doi:10.1093/cid/cis629. PMID 22965026.
7. Hahn RG, Knox LM, Forman TA (May 2005). "Evaluation of poststreptococcal illness". Am Fam Physician 71 (10): 1949–54. PMID 15926411.
8. Baltimore RS (February 2010). "Re-evaluation of antibiotic treatment of streptococcal pharyngitis". Curr. Opin. Pediatr. 22 (1): 77–82.
9. A. Wayne, 2007, Clinical Laboratory Standards Institute. Principles and procedures for blood cultures; Approved Guideline. CLSI document M47-PA: Clinical and Laboratory Standards Institute, 2007
10. Baron EJ, Weinstein MP, Dunne WMJ, Yagupsky P, Welch DF, Wilson DM, eds. Cumitech 1C, blood cultures IV. Washington, DC:ASM Press, 2005

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