AgriCos e-Newsletter
ISSN: 2582-7049
AgriCos e-Newsletter
Open Access Multidisciplinary Monthly Online Magazine
Volume: 02 Issue: 11 November 2021 Article No: 13

Determination of Fermentation Pathway by Methyl Red and Voges Proskauer (MRVP) Test

Shanmugaraj C.[1], Archana Anokhe[2] and Vinay Kalia[3]
[1]Research Scholar, Pathology, ICAR-Indian Agricultural Research Institute, New Delhi
[2]Scientist, Entomology, ICAR-Indian Agricultural Research Institute, New Delhi
[3]Principal Scientist, Entomology, ICAR-Indian Agricultural Research Institute, New Delhi

SUMMARY

The MRVP test is applied for the invention of the fermentation pathway, which is achieved by means of microbes to use glucose. 2, 3-butanediol and mixed acid fermentation pathways are the major two sorts of pathways involved. The steady red tone on the surface on the medium shows positive due to mixed acid fermentation pathway and yellow colour resulting negative in the methyl red test. The brownish red tone on the top of the medium shows positive due to 2, 3-butanediol fermentation pathway and yellow colour resulting negative in the voges proskauer test.

INTRODUCTION

To distinguish the facultative anaerobic enteric bacteria or acid-producing and acetoin-producing bacteria, MRVP tests are carried out jointly, because the Methyl Red test and Voges Proskauer tests are two distinct tests. The MR-VP broth was developed by Clark and Lubs, which permits each assessment to be accomplished by aliquoting parts from the same inoculated medium to various tubes. MR-VP broth that contains peptone, buffers and dextrose or glucose, which is used as a section of IMViC tests. Distinct bacteria turn glucose and dextrose into pyruvate using different metabolic pathways.

Principle

MR test:

The various types of organic acids viz. succinic, acetic, lactic, and formic acid are produced from the fermentation of glucose in the mixed acid fermentation pathway. To triumph over the phosphate buffer, they produce a massive amount of organic acids, which results in a pH of beneath 4.4. At this point, when the methyl red is added to culture broth containing pH below 4.4, red colour appears to indicate positive. This reveals that the mixed acid fermentation pathway happens within the test bacteria. At this point, when the methyl red is added to culture stock having pH above 6.0, which results in yellow colour seems negative due to the bacteria metabolizing pyruvic acid to neutral end products. This shows that the mixed acid fermentation pathway isn't used (Aryal, 2018).

VP test:

VP test carried out to identify the neutral-reacting final outcome (acetoin) when cultured in determined media. The glucose was fermented and further metabolized the pyruvic acid to end result acetyl-methyl carbinol (i.e. acetoin). 2, 3 butanediol is the end product of glucose fermentation instead of forming organic acids in the butanediol fermentation pathway. In the presence of 40% KOH and atmospheric oxygen, the final product turned into diacetyl. Due to the catalytic action of alpha-naphthol, diacetyl is turned into a red complex which shows brownish red as positive and negative shows yellow (Aryal, 2018).

Materials required are test Organisms-Nutrient broth culture, MR-VP broth, pH indicator (Methyl red), Naphthol solution (V-P solution I), 40% KOH (V-P solution II). Composition of MRVP broth are prepared by mixing Glucose/Dextrose (5 g), Peptone (7 g), Potassium phosphate (5 g) in 1000 ml distilled water

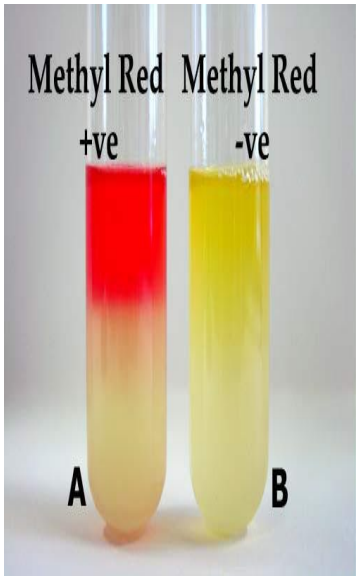
Procedure

Basic initial steps for both test:

Inoculate the test organism into MRVP broth and incubate it 48 hours at 35ºC. After growth of sufficient growth of bacteria, sample is proceeded for MR and VP test.

Methyl Red (MR) Test:

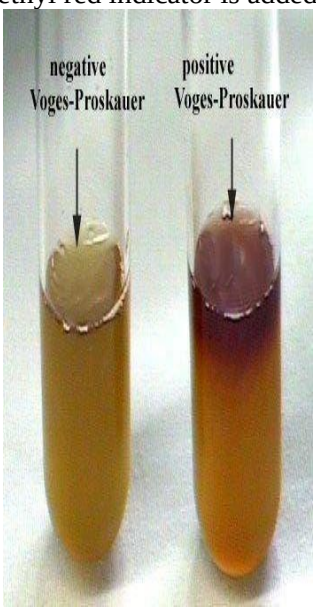
3-5 drop of methyl red is added to glass tube containing test organism and result were observed based on the colour change. If a Constant red tone appears on the upper surface of the medium, after the addition of the methyl red indicator, the result will be affirmative and If a yellow colour appears on the surface of the medium, after the addition of the methyl red indicator, the result will be negative.



(Source:https://microbiologyinfo.com/methyl-red-mr-test-principle-procedure-and-result-interpretation/)
Fig: Results of Methyl Red test, (A) Methyl Red Positive, (B) Methyl Red Negative

Voges-Proskauer Test:

1-2 drops of naphthol solution (V-P Solution I) and 2-3 drops of 40 percent KOH (V-P Solution II) were added to the test tube containing target organism and shake for 30 sec. Afterwards sample is exposed to oxygen for 10-15 minutes and colour of the medium is recorded (McDevitt, 2009) (Sourav, 2020). If a brownish-red hue appears on the surface of the medium 15 minutes to 1 hour after the methyl red indicator is added, the result will be affirmative. If a yellow tone remains on the medium 15 minutes to 1 hour after the methyl red indicator is added, the result will be negative.



(Source: https://microbeonline.com/voges-proskauer-test-principle-procedure-results/)
Fig: Results of Voges-Proskauer test

Examples

Organisms	**Results**		**Uses:**
	MR	**VP**	Initially, this Paired MR-VP tests were used to differentiate the members of the family *Enterobacteriaceae*, but now used also to characterize the *Actinobacteria*.
Escherichia coli	+ve	–ve	
Enterobacter spp.	– ve	+ve	
Serratia marcescens	– ve	+ve	

Limitations

- It is suggested that more biochemical tests need to be carried out in addition to this test for complete identification.
- The MR test is not carried out unless the medium is kept for a minimum 48 hrs. Running early results gives false positives.
- Light inoculum should be used. If heavy, bacterial growth will be inhibited and yield invalid results.
- Addition of alpha-naphthol first before KOH is important in the VP test.
- The observed results beyond one hour following the incorporation of reagents yields false-positive results in the VP test.

REFERENCES

Aryal, S. (2018). Methyl Red (MR) Test - Principle, Procedure and Result Interpretation. https://microbiologyinfo.com/methyl-red-mr-test-principle-procedure-and-result-interpretation/

Aryal, S. (2018). Voges-Proskauer (VP) Test - Principle, Reagents, Procedure and Result. https://microbiologyinfo.com/voges-proskauer-vp-test-principle-reagents-procedure-and-result/

Aryal, S. (2018). Voges Proskauer (VP) Test. https://microbenotes.com/voges-proskauer-vp-test/

McDevitt, S. (2009). Methyl Red and Voges-Proskauer test protocols. *American Society for Microbiology*, *8*.

Sourav, B. (2020). Methyl Red and Voges Proskauer Test - Principle, Result. https://microbiologynote.com/methyl-red-and-voges-proskauer-test-principle-result/