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SIMPLE BIOCHEMICAL TEST FOR THE IDENTIFICATION OF BACTERIA IN LABORATORY

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Abstract *One of the most difficult task for young microbiologists is the isolation and identification of unknown bacteria form different sources in their university or collage laboratory. Most of the time they got succeeded by studying different literature but not all the time and they have to purchase ATCC stains to continue their work but ATCC are expensive for them and it is not easy for every student to buy it. Identification of bacteria is difficult but by using different simple test and proper approach, young researchers can identify bacteria in their laboratory. In this review some of the most commonly used test are mentioned for the isolation of bacteria in small scale university or collage laboratory. In this review Nine simple tests are mentioned and by using these test Young microbiologists can almost identify bacteria without any harden. Although it is just a mini review but it can be very handy for young microbiologists and young researchers of different fields to get the detail in one paper instead of reading different literature.*

Keywords *isolation, identification, Bacteria and Test*

INTRODUCTION

Researchers of bacteriology and microbiology are working day by day to understand the mechanism of different bacteria and microbes. They are isolating bacteria from different sources to use them in different field's i.e. antibiotic production,enzyme production and different dairy products. But before that they have to identify these bacteria from huge bulk of different bacterial colonies. So for this reason they need certain biochemical test to identify bacteria (Talaiekhozani et al., 2015;Gajdács et al.,2017; Muzammal et al., 2019). In this review we have mention some of the simple tests, which can be done in the laboratory to identify bacteria. Our review will be handy to the young research related to bacteriology and microbiology and besides going foe expensive ATCC strains, they can use these test to identify bacteria isolated from different sources.There are different tests to identify bacteria but in this review we will discuss some tests that are commonly used in the laboratory. These tests includes the following as mentioned below:

GRAM STAINING

Purpose

Gram staining technique was developed by the Danish physician Christian Gram in 1884.This techniques used to separate bacteria into two groups on the basis of composition of cell wall. On the basis of results we categorize bacteria into gram positive and gram negative bacteria (Sandle, 2004)

Procedure

1. Take a drop on autoclaved distill water on the clean Petri dish.
2. With the help of clean loop touch a bacteria in the petri dish and then mix it with drop of water present on the petri dish.
3. Mix it so the solution become slightly turbid and left for air dry.
4. Slightly heat the slide with the help of sprit lamp (Do not over heat) and allow to cool.
5. Put a 2 -3ml (Approx.) of crystal violet dye on the bacterial smear and leave it for 1-2 min.

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6. After that wash the slide with tap water to remove crystal violet dye.
7. Now apply 2-3ml of Gram's Iodine solution and left the slide for 1-2 min.
8. Wash the slide with tap water.
9. Now apply decolorizing solution (Alcohol) to decolorize the smear.
10. Again wash with tap water.
11. Apply 2-3ml safranin and left the slide for 1-2 min.
12. Wash the slide with tap water and allow to dry.
13. Examine the result under the microscope.

RESULTS

a) Gram Negative

Cells of gram negative bacteria will be decolorized by applying alcohol (9th step) and appears pink or red when washed with safranin (11th step)(Sandle, 2004).

b) Gram Positive

Cells of gram positive bacteria will not be decolorized (9th step) and remains violet at the end (1st step)(Sandle, 2004).

Some common bacteria and their gram staining results are in figure 1

Bacteria	Gram Stain	Shape	Size	Colony color	Media
E.coli	-	Rod	0.5 µm		Nutrient agar
Klebsiella	-	Rod	2 µm to 0.5 µm		MacConkey Agar
Acinetobacter spp	-	Rod	1.0–1.5 µm by 1.5–2.5 µm		MacConkey Agar
Proteus spp.	-	Rod	0.4-3 µm		Nutrient agar
Staphylococcus epidermidis	+	Round (Grapes)	1.5 µm		Mannitol Salt agar
Staphylococcus aureus	+	Round (Grapes)	1 µm		Mannitol Salt agar
Micrococcus luteus	+	Round (Clusters)	0.5 – 2 µm		Nutrient agar
Streptococci	+	Round Or ovoid	0.5 – 2 µm		Nutrient agar

Whitish
Pinkish
Creamy
Yellow

Figure 1:Gram staining result and colony color ofdifferent medically important bacteria

QUALITY CONTROL

For Best result and quality control, also use known bacterial specie while performing Gram staining as positive control. Not all bacteria can be separated on the basis of gram staining. There are many medically important bacteria that cannot be separated by Gram staining and their reason is in table 1.

Table 1: Important bacteria which cant be separated by gram stain adopted from Levinson and Jawetz 1996

Bacteria Name	Reason	Alternative test
Rickettsiae	Very small; Intercellular	Giemsa Stain
Chlamydiae	Very small; Intercellular	Inclusion Bodies in cytoplasm
Mycoplamsa	No cell wall	None
Tuberculosis	Dye cannot enter into the cell wall because there is too much lipid.	Acid fast stain
Legionella Pneumophilla	Poor uptake of red stain	Enhance the time of counterstain

MOBILITY TEST

Purpose

Presence of flagella on the bacteria shows its mobility. So mobility test is used to determine whether the bacteria are motile or not (Hemraj et al., 2013).

Procedure

This method is known as semi-solid medium method.

1. In this method, any unknown bacteria are inoculated in the semi-solid media in test tube/flask.
2. Incubate for 24 hours.
3. Examine the results by turbidity of media.

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RESULTS

Positive result

If bacteria are motile then the media become turbid. Clearly seen through naked eyes.

Negative Results

If bacteria are non- motile then a turbid line in the middle of the media will be form where the actual inoculum was done. Clearly seen through naked eyes(Hemraj et al.,2013)..

Quality control

For Best result and quality control, also use known bacterial specie while performing semi-solid medium methodas positive control.

CATALASE TEST

Purpose

This test is used to identify bacteria that produce catalyze enzyme. In this test hydrogen per oxide (H_2O_2) is used. Bacteria that produce catalase will decompose hydrogen per oxide (H_2O_2into H_2O and O_2(Constantinescu et al.,2001;Reiner,2010;Fung and Petrishko,1973).

Procedure

1. Make smear of the unknown bacteria on the clear slide with the help of wire loop.
2. Apply one drop on hydrogen per oxide (H_2O_2) on it.
3. Result can be seen immediately.

RESULTS

Positive Results

Bacteria are catalase positive if bubble are form (O_2) as in figure 2

Negative Results

Bacteria are catalase Negative if no bubbles are form.

Quality control

For Best result and quality control, also use known bacterial specie, while performing catalase testas positive control.

Figure 2: Catalase positive bacteria producing O_2 after applying H_2O_2

OXIDASE TEST

Purpose

In this test cytochrome oxidase as known asindophenoloxidase,is detected. Bacteria which contain this enzyme will converts colorless reagent into colored product. This test can be performed by many methods i.e. filter paper spot test, filter paper method direct plate method (TarrandandGröschel,1982;Gadeh olt, 1964).Today we will discuss filter paper test method.

Procedure-Filter Paper Test Method

1. In this test filter paper (Available in laboratory) is soaked in the 1% Kovács oxidase reagent and allow to dry.
2. Now with the help of sterilized wire loop apply a unknown bacterial specie (Present in the form of culture) on the filter paper which is soaked in in the 1% Kovács oxidase reagent.
3. Note for color changes.

RESULT

Positive result

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Bacteria are oxidase positive if color changes into dark purple.

Negative result

Bacteria are oxidase negative if no color change is detected.

Quality control

For Best result and quality control, also use known bacterial specie, while performing oxidase testas positive control.

INDOLE TEST

Purpose

The indole test is used for the ability of abacteria to reduce the amino acid tryptophan and produce indole(Miller and Wright,1982;MacWilliams, 2012)

Procedure

1. Small amount of unknown bacterial specie is applied to thetube of tryptone broth with a small amount of a pure culture.
2. Incubate for 24 hours at 37°C.
3. Add 5 drops of Kovác's reagent directly to the tube with the help of dropper.

RESULTS

Positive result

Red or pink ring will be formed on the surface of media

Negative result

No change in the media color (Remains yellow)

Quality control

For Best result and quality control, also use known bacterial specie, while performing Indole testas positive control.

GELATINASE TEST

Purpose

This is another simple test for identification of bacteria. In this test bacteria is identified by production of enzyme known as gelatin as enzyme. This enzyme liquefy gelatin (Calander et al., 2006;Nakayama et al.,2006).

Procedure

1. Unknown bacterial species are inoculated into nutrient gelatin media(stab ½ to 1 inch deep into the media) for 24-36 hours at 37°C.
2. Bacteria used in this test are of high growth in the petri dishes.

RESULTS

Positive result

Media will be liquefying. Try to pour a drop of media on your palm. Dropping of a drop will confirm positive result.

Negative result

No liquefaction occurs in media.

Quality control

For Best result and quality control, also use known bacterial specie, while performing Gelatinase test as a positive control.

DECARBOXYLASE TEST

Purpose

This test is used to determine the enzymatic activity of bacteria to decarboxylase any amino acid

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especially (Lysine) and convert into aminewith resultant alkalinity(Fay and Barry,1972;Freier et al.,1976).

Procedure

1. In this test Light inoculum will be used in lysine and decarboxylase test tubes.
2. Add1.5 to 2.5ml oil in each tube contains inoculum.
3. Incubate at 20^{0}c for 24 hours (Prolonged incubation is recommended).

RESULTS

Results of Decarboxylase test is in table 2

Table 2: Result of Decarboxylase test

Test Results	Lysine tube	Control tube
Positive	Turbid to faded purple	Yellow
Negative	Yellow	Yellow
Negative	Purple	Purple

Quality control

For Best result and quality control, also use known bacterial specie, while performing Gelatinase test as a positive control.

MALONATE TEST

Purpose

This test is used to identify those bacteria, which are able to utilize only sodium malonate as its only carbon source (Leifson,1933;Fleury and Magnolato,1992)

Procedure

1. Inoculate the malonate media with unknown specie of bacteriafor 24 to 48 hours at 20^{o}C.
2. Light inoculum will be used.
3. Results will be examined by color change.

RESULT

Positive Results

Malonate Media will turn deep blue from green.

Negative Results

Malonate media will remains green.

Quality control

For Best result and quality control, also use known bacterial specie, while performing Malonate test as a positive control.

ESCULIN TEST

Purpose

This test is used to identify those bacteria which has the ability to hydrolyze the glycoside esculin (aesculin) toesculetin (aesculetin) and glucose in the presence of bile (10 to 40%)(Facklam and Moody,1970;Qadri and Johnson,1981).

Procedure

1. Prepare slant of bile esculin slant.
2. Inoculate the slant with unknown bacterial specie and incubate for 24 to 36 hours at 20^{0}C.
3. Results will be examined by color change.

RESULTS

Positive result

Dark Brown or black color form. Confirm the presence of esculin positive bacteria.

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Negative result

No change in the color of the slant

Quality control

For Best result and quality control, also use known bacterial specie, while performing Esculin test as a positive control.

For overall result of all the tests see table 3.

Table 3: Overall results of all the above mention test

S NO	Test Name	Positive Result	Negative Result
1	Gram Stain	Purple Color	Pink Color
2	Motility test	Diffuse growth	Stab Line
3	CatalaseTest	Bubbles formation	Clear or No bubbles
4	Oxidase	Blue or Purple Color	No change in Color
5	GelatinaseTest	Liquid or partial liquid tube at 4^0c	Solid tube at 4^0c
6	Indole test	Red color	Yellow color
7	Decarboxylase Test	Purple Color	Yellow Color
8	Malonate Test	Blue Color	Green Color
9	Esculin Test	Dark Brown or black	No change

There are number of test available to identify bacteria depending on specie to specie but we hope the Staring from nowhere and after the end of the mentions test, researchers will be able to almost identify the bacterial specie. Step by step tests and end result is in figure 3.

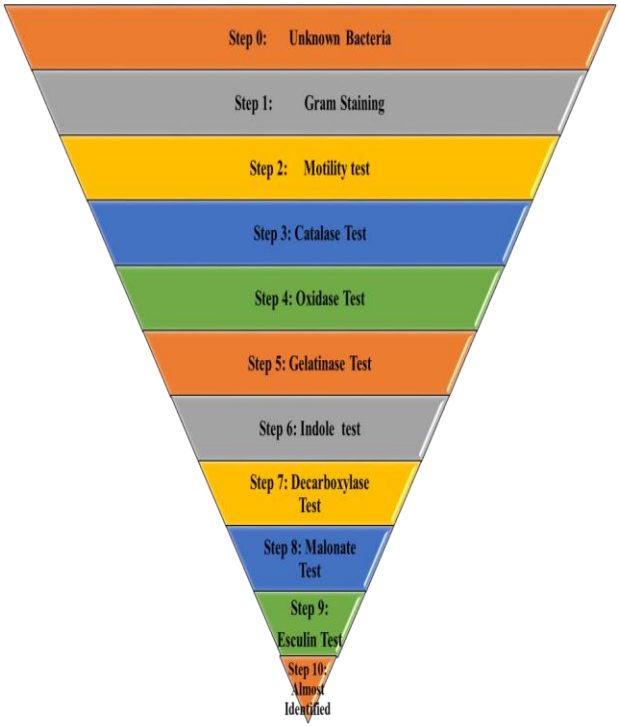

Figure3: Step by step tests and end result of tests.

Below is the detail of some of the commonly used bacteria and their test result in figure 4

CONCLUSION

There are lot of biochemical test are performed to identify bacteria. Some of those tests are easy while other is difficult. In this review we tries to highlight some of the common test available in the laboratory to identify bacteria. We hope our effort will be highly appreciated by the young researchers who find difficulty to identify bacteria in their collage research.

Figure 4: Test result of different bacteria commonly used in university and collage laboratory.

Bacteria	Test Name								
	Gram Stain	Motility test	Catalase Test	Oxidase	Gelatinase Test	Indole test	Decarboxylase Test	Malonate Test	Esculin Test
E.coli	Pink	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Negative
Klebsiella	Pink	Negative	Positive	Negative	Negative	Positive	Positive	Positive	Negative
Acinetobacter spp	Pink	Some spp are positive and other are negative	Positive	Negative	Negative	Negative	Positive	Positive	Negative
Proteus spp.	Pink	Positive	Positive	Negative	Negative	Some spp are positive and other ore negative	Positive	Positive	Negative
Staphylococcus epidermidis	Purple	Positive	Positive	Negative	Negative	Positive	Negative	Positive	Positive
Staphylococcus aureus	Purple	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Negative
Micrococcus luteus	Purple	Negative	Positive	Positive	Positive	Negative	Positive and sometime negative for some aminoacids	Negative	Positive
Streptococci	Purple	Negative	Negative	Negative	Positive	Positive	Negative	Negative	Positive

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