

# Biochemical action of bacteria

[Science](#), [Biology](#)



OBJECTIVE: 1. To distinguish the bacteria abilities to metabolize various substrates and end products formed. 2. To observe the growth of different bacteria species in term of structures and its morphology based on different chemical substance applied. 3. To observe physiological and immunological properties utilized by different species of bacteria. INTRODUCTION: Bacteria biochemical testing can determine the types and numbers in terms of colony forming units of bacteria present in a sample of different chemical. The testing could be focused on a specific type of bacteria, medical bacteria or a broad range of environmental bacteria.

Since bacteria are present in virtually any environment, it's important to be clear why the testing is being performed. The more specific the testing is the better and the easier it is to interpret the results. Numbers and types of bacteria that should be a cause for concern depends upon several factors, including the type of bacteria present and the type of samples. *Escherichia coli* are one of the main species of bacteria living in the lower intestines of mammals. *E. coli* can be found in the intestinal tract of warm-blooded animals. The presence of *E. coli* in foods is considered to be an indication of fecal contamination.

*Staphylococcus* organisms are commonly found in the environment. Several species of *Staphylococcus* are found on the skin, intestines, nasal passages, etc. of warm-blooded animals. Some species of *Staphylococcus*, particularly *Staphylococcus aureus* can be pathogenic are capable of causing illness. *Pseudomonas aeruginosa* is widely distributed in soil, water and plants. It survives in hot tubs, whirlpools, contact lens solution, sinks and

showers. It can cause a number of opportunistic infections including infections of the skin, external ear canal and of the eye.

Nitrifying bacteria recycle organic nitrogenous materials from ammonium (the endpoint for the decomposition of proteins) to nitrates. Their presence can indicate that the water may have been polluted by nitrogen-rich organics from sources such as compromised septic tanks, sewage systems, industrial and hazardous waste sites and is undergoing an aerobic form of degradation.

The presence of denitrifying bacteria can indicate that the water has been polluted by nitrogen-rich organics from sources such as compromised septic tanks, sewage systems, industrial and hazardous waste sites. MATERIALS: 1.

Nutrient broth cultures of *Escherichia coli* . Nutrient broth cultures of *Serratia marcescens* 3. Nutrient broth cultures of *Salmonella typhimurium* 4. Nutrient broth cultures of *Bacillus subtilis* 5. Nutrient broth cultures of *Klebsiella* spp.

6. Nutrient broth cultures of *Streptococcus* spp. 7. Nutrient broth cultures of *Staphylococcus aureus* 8. Nutrient broth cultures of *Proteus vulgaris* 9. Nutrient broth cultures of *Pseudomonas fluorescens* 10. Parafilm tape 11.

Inoculating loops 12. Gloves 13. Incubator 14. Nutrient agar plate 15. Nutrient agar slants 16. Starch agar plates 17. Gelatine agar plates 18. 2 tubes Clark's-Lub medium (MR-VP medium) 19. Tryptone broth 20. 3 Kigler' slant 21. 5 tubes nitrate broth ( 0. 1%  $\text{KNO}_3$ ) 22. 5 urea broth 23. Tube containing 10ml of sterile saline 24. Glucose broths with Durham tubes and phenol red indicator 25. Lactose broths with Durham tubes and phenol red indicator 26. Sucrose broths with Durham tubes and phenol red indicator 27.

Gram's iodine 28. Kovac's indol reagent 29. Mercuric chloride solution 30. KOH-creatine solution or 40% KOH 31. F&R reagent 32. Nessler's reagent

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PROCEDURE: A. CARBOHYDRATE METABOLISM 1. Fermentation of sugars

Materials: 1. Glucose broths with Durham tubes and phenol red indicator 2.

Lactose broths with Durham tubes and phenol red indicator 3. Sucrose broths with Durham tubes and phenol red indicator 4. 18 hour nutrient broth

cultures of *E. coli* and *S. typhimurium* Procedure: 1) The small bottles of different sugars were inoculated with a loopfuls of *E. coli* and *Salmonella* spp.

2) The tubes were labelled and incubate at 37°C for 24 hours 3) All observations were recorded for presence of acid or gas production. 2.

Hydrolysis of starch Materials: 1. Starch agar plates 2. Broth agar cultures of

*B. subtilis* and *E. coli* Procedure: 1) Starch plate was streaked with *E. coli* in

for sections and repeated for *B. ubtilis* bacteria in other starch plate. 2) The

plates were secured with parafilm, labelled and inoculated at 37°C for 24

hours. The following day 1) The plates were tested for starch hydrolysis by

flooding the pates with Gram's iodine. 2) The plates were examined and the

colonies that showed clear uncoloured zones in contrast with the blue-black

background of the starch-iodine complex were noted. 3) The extent of the

zones of hydrolysis indicated either the reddish colour zones were seen. 4)

All results and observations were recorded. B. PROTEIN AND AMINO ACID

METABOLIM 1. Indole test Materials: 1. Broth cultures of *B. ubtilis*, *E. coli*, and

*S. typhimurium* 2. 3 tubes of tryptone broth 3. Kovac's indole test reagent

Procedures: 1) The peptone water was inoculated with a loopfuls of the test

organism. 2) The tube was labelled and incubated for 24 hours. The following

day 1) The tubes were added with a few drops of Kovac's indole reagent

(dimethylaminobenzaldehyde) 2) The red or dark color indicates the

presence of indole. 4. Hydrogen sulphide Materials: 1. Broth cultures of *B.*

subtilis, E. coli, and S. typhimurium

2. 3 Kigler's slant Procedures: 1) The Kigler's slant was inoculated with a loopfuls of the test organism by the stab method. 2) The tube was labelled and incubated for 24 hours. The following day 3) The Kigler' slant was observed for production of H<sub>2</sub>S where the black precipitate along the line of growth in the Kigler's slants indicated the H<sub>2</sub>S have been produced. 4) The observations were recorded.

3. Gelatine hydrolysis test Materials: 1. Broth cultures of B. subtilis, E. coli, and S. typhimurium 2. Gelatine agar plates 3. Mercuric chloride solution Procedures: 3) The gelatine agar plates were inoculated with a loopfuls of the test organism with a single streak at the centre of the plates. 4) The plates were secured with parafilm, labelled and incubated for 24 hours. The following day 5) The plates were flooded with mercuric chloride solution. 6) The medium become opaque in regions that still contain gelatine and clear regions where gelatine has been hydrolysed.

C. VOGES-PROSKAUER TEST Materials: 1. Broth cultures of E. coli, and Klebsiella spp. 2. 2 tubes of Clark-Lub's medium (MR-VP medium) 3. KOH-creatine solution Procedures: 1) The tubes of Clark-Lub's medium (MR-VP medium) were inoculated with a loopfuls of the test organism. 2) The tubes were labelled and incubated for 24 hours.

The following day 1) The tubes were tested with Voges-Proskauer test. 2) The 0. 5ml of KOH-creatine solutuin was addd. 3) The tube was shaken vigorously for 30 seconds. 4) The red or pink color indicates the presence of acetoin.

D. CATALASE TEST Materials: 1. Broth cultures of Streptococcus spp. and Staphylococcus aureus. 2. Nutrient agar slant Procedures: 1) The nutrient agar slant was inoculated with a loopfuls of the test organism. 2) The tube was labelled and incubated for 24 hours. The following day 1) The

tubes were tested with catalase test by adding several drops of a 5% solution of hydrogen peroxide. ) The vigorous bubbling indicates the presence of oxygen. E. NITRATE REDUCTION TEST Materials: 1. Broth cultures of E. coli, Proteus vulgaris, Serratia marcescens, Pseudomonas fluorescens. 2. 5 tubes containing nitrate broth (0. 1% KNO<sub>3</sub>) 3. Nitrate test reagent Procedures: 1) The nitrate broth was inoculated with a loopfuls of the test organism. 2) The tube was labelled and incubated for 24 hours. The following day 1) The tubes were tested with 1ml of Follet and Ratcliff's (F&R reagent) 2) The orange or brown color indicates the presence of nitrate. 3) The absent of nitrate indicates that: a.

There has been no nitrate reduction b. The reduction has proceeded beyond that nitrate stage. 4) The absent of orange or brown color were further tested with small amount of cadmium to the tube. If nitrate still present, it will be catalytically change to nitrate which will then reacts with the F&R reagent in the tube. 5) In the absent of a positive nitrate result, the bubbles f H<sub>2</sub> gas was observed in the Durhams tube OR 6) The samples were tested with 1ml of Nessler's reagent. The brown or orange color indicates the presence of ammonia.

F. UREASE TEST Materials: 1. Broth cultures of E. coli, P. vulgaris, S. arcscens, P. fluorescens. 2. 5 urea broth with indicator Procedures: 1) The urea broth was inoculated with a loopfuls of the test organism. 2) The tube was labelled and incubated for 24 hours. The following day 1) The urease-positive organism produced in intense red/purple coloration of the medium after incubation. 2) All observations were recorded.

RESULTS AND OBSERVATION: Test| Observation(After 24 hours incubation)| Description| A. Carbohydrate Test 1. Fermentation of starchDurham tubes

and phenol-red indicator. 2. Hydrolysis of starch| Glucose: Lactose: Sucrose: Starch agar plates: *B. subtilis*, *E. coli* | \* Positive result for *E. coli* as tube turn yellow \* Positive result for *S. typhimurium* as tube turn yellow \* Positive result for *E. coli* as tube turn yellow \* No gas produced by *S. typhimurium* because the tube turns red. \* No gas produced by *E. coli* because the tube is slightly red. \* Positive result for *S. typhimurium* as tube turn yellow \* Positive zone of clearing. \* Negative zone of clearing. | B. Protein And Amino Acid Metabolism 1. Indole test 2. Hydrogen disulphide 3. Gelatine hydrolysis test| Tryptone broth: *B. subtilis*, *E. coli*, *S. typhimurium* Kigler's slant: *B. subtilis*, *E. coli*, *S. typhimurium* Gelatine agar plates: *B. subtilis*, *E. coli*, *S. typhimurium* | \* Negative Indole tests no color change. \* Bright fuschia at the interface is positive test for Indole. \* Negative Indole tests no color change. \* Black precipitate form shows positive sulphur reduction. \* Negative reaction. \* Positive reaction forming the black precipitate. \* Positive hydrolysis of gelatine into amino acid to be used as nutrients/gelatinase. \* Negative hydrolysis of gelatine. \* Negative hydrolysis of gelatine| C. Voges-Proskauer's Test| MR-VP medium: *E. coli*, *Klebsiella* spp. | \* Negative results of *E. coli* \* Positive results *Klebsiella* spp. | D. Catalase Test| Nutrient agar slant: *S. aureus*, *Streptococcus* spp. | *S. aureus* \* Positive catalase reaction because present of bubbles *Streptococcus* spp. \* Negative catalase reaction no bubbles present. | E. Nitrate Reduction Test| Nitrate broth: *E. coli*, *P. vulgaris*, *S. marcescens*, *P. fluorescens* | \* No color change after denitrification of ammonia. \* No color change after denitrification of ammonia. \* Turns red. Positive nitrate test shows nitrate reductase present. \* Turns red but negative catalase test. | F. Urease Test| Urea broth: *E. coli*, *P. vulgaris*, *S.*

marcescens| \* P. luorensens| \* Negative urease test because the tube remain purple. \* P. vulgaris show positive urease test from yellow to pinkish. \* S. marcescens show negative urease test because the color remain purple. \* P. fluorensens show negative urease test because the color remain purple. |

DISCUSSION: Biochemical tests of bacteria objectively to test the metabolism of carbohydrate and related products of different bacteria species, test specific breakdown of products through color changes and gas produced. Besides that, the ability of bacteria utilizes a specific substance and the metabolism of protein and amino acid by bacteria.

A. CARBOHYDRATE TEST Carbohydrate is an organic compound that consists of only carbon, hydrogen and oxygen which is basically the major carbon source of most organisms. Specific carbohydrate can be fermented by organism that incorporated in a medium producing red or acid with gas. Pinkish red color shows positive results where acidic content formed in the tube because carbon dioxide realised if fermentation occur. Negative catabolism of carbohydrate shows by yellow to colourless of Durham's tube as the solution remain alkaline in the absent of carbon dioxide gas.

Gas production can be seen as bubbles in Durham's tube. Central carbohydrate metabolism or the breakdown of sugars into smaller compounds accompanied by the production of ATP and reduction of coenzymes, follows one of several pathway. Carbohydrate utilization and fermentation will be assessed by growing cells without shaking (aeration) in defined media containing a single carbohydrate. Acid products of sugar fermentation will cause a noticeable color change in the pH indicator included in the medium.



Sugar fermentation does not produce alkaline product, however non-fermentative hydrolysis of amino acids in the peptone, present in most fermentation media, may give an alkaline reaction, which will also cause a color change in the pH indicator. Gas production, H<sub>2</sub> in particular, can be determined by placing a small, inverted Durham tube in the test medium. If gas is produced, it is trapped in the Durham tube and can be seen as a bubble. Hydrogen sulfide (H<sub>2</sub>S) is produced by bacterial anaerobic degradation of the two sulfur-containing amino acids, cysteine and methionine.

Hydrogen sulfide is released as a by-product when carbon and nitrogen atoms in the amino acids are consumed as nutrients by the cells. Under anaerobic conditions the sulfhydryl (-SH) group on cysteine is reduced by cysteine desulfurase. Ferrous ammonium sulfate-indicator. H<sub>2</sub>S reacts with ferrous sulfate forming the black precipitate Sodium thiosulfate is reduced to sulphite/thiosulfate The Kligler's Iron test is used to detect liberation of H<sub>2</sub>S gas by bacteria growing on an excess of these sulfur-containing amino acids. The agar contains high levels of peptones or sources of cysteine and methionine and ferrous sulfate as an indicator.

When H<sub>2</sub>S is produced, the ferrous ion reacts with it to give ferrous sulfide, an insoluble black precipitate. In starch hydrolysis test Iodine must be on the plate to visualize the zone of clearing surrounding the bacteria. This zone indicates starch was broken down to dextrans, maltose, and glucose. B. PROTEIN AND AMINO ACID METABOLISM Indole test measures the ability of bacteria to split indole from tryptophan molecule but in term of biochemistry,

Indole test is one of the metabolic degradation products of the amino acid tryptophan.

Bacteria that possess the enzyme tryptophanase are capable of hydrolysing and deaminating tryptophan with the production of Indole, pyruvic acid and ammonia. Positive reaction showed by *E. coli*, *P. vulgaris* and negative results observed in *Klebsiella* and *Salmonella* from observation in the Indole test. Development of fuchsia red color at the interface of the reagent and the broth within seconds after adding the reagent is indicative of the presence of Indole and is a positive test. Kovac's reagent detects if tryptophan has been hydrolyzed to indol or tryptophanase.

Gelatin is the protein derived from the animal protein collagen, has been used as a solidifying agent in food for a long time besides nutrient gelatine as an early type of solid growth medium. One problem is that many bacteria have the ability to hydrolyze or liquefy the gelatin. This gelatin liquefaction ability forms the basis for this test. C. VOGES-PROSKAUER TEST The production of acetoin by bacteria is performed through Voges Proskauer Test to determine the ability of the organisms to produce neutral end product acetyl methyl carbinol (acetoin) from glucose fermentation.

Negative results gained from *E. coli* meanwhile positive reaction gives by. Changing of color to red pinkish color at the surface of the medium indicated positive results and yellow color at the surface of the medium show negative reaction. The KOH reagent should not be excessively added to the sample because excess KOH may mask weak VP positive reactions. The MR test will be positive for organisms that have complete pathways for mixed acid fermentation. The Voges-Proskauer (VP) test determines whether a specific

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neutral metabolic intermediate, acetoin, has been produced instead of acid from glucose.

Acetoin is the last intermediate in the butanediol pathway, which is a common fermentation pathway in *B. subtilis*. The tests are complementary in the sense that often a bacterium will give a positive reaction for one test and a negative reaction for the other. The three possible patterns of results where the acetoin fermentation pathway, detected by the VP test, two molecules of pyruvate condense and two molecules of CO<sub>2</sub> are released. The 4 carbon intermediate that is formed, acetoin, contains a carbonyl group. The acetoin acts as a terminal electron acceptor with the carbonyl group being reduced to a hydroxyl group.

The reduced product, butanediol, is excreted by the bacteria and acetoin is oxidized to diacetyl by alkaline -naphthol, which forms a red complex with creatinine. D. CATALASE TEST Catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria except *Streptococcus* spp. Hydrogen peroxide forms as one of the oxidative end product of aerobic carbohydrate metabolism. If hydrogen peroxide allowed accumulating in the bacterial cells it becomes lethal to the bacteria. Catalases help in converting H<sub>2</sub>O<sub>2</sub> to water and oxygen.

In the catalase test performed, *Streptococcus* spp gives negative reaction as for *S. aureus*, the positive reaction occurred. One of the by-products of oxidation-reduction in the presence of O<sub>2</sub> during aerobic respiration is hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). This compound is highly reactive and must be degraded in the cytoplasm of the cell producing it. It can be especially damaging to molecules of DNA. Most aerobes synthesize the enzyme

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catalase, which breaks down  $H_2O_2$  into water and oxygen. The  $O_2$  gas is identified by the production of bubbles from a concentrated cell suspension.

The test for catalase is simple and usually very reliable. It is a major method of distinguishing between *Staphylococcus* (catalase positive), *Streptococcus* (catalase negative), and *Enterococcus* (catalase negative), although some strains of *Enterococcus faecalis* may be positive. Catalase production is generally associated with aerobic organisms, since  $H_2O_2$  is a toxic by-product of aerobic growth, but not always.

**E. NITRATE REDUCTION TEST**  
Nitrate reduction test basically test the ability of organism to reduce the nitrate to nitrites or free nitrogen gas.

In order to determine either the bacteria can reduce nitrate, the test organism is inoculated into nitrate reduction broth, undefined medium that contains large amounts of nitrate ( $KNO_3$ ). After incubation, reagent added simultaneously reacts with nitrite and turn to red color, indicating a positive nitrate reduction. If there is no color change at this step, nitrite is absent. If the nitrate is unreduced and still in its original form, this would be a negative nitrate reduction result. However it is possible that the nitrate was reduced to nitrite but has been further reduced to ammonia or nitrogen gas.

This would be recorded as positive nitrate reduction result. Under anaerobic conditions, some bacteria are able to use nitrate ( $NO_3^-$ ) as an external terminal electron acceptor. This kind of metabolism is analogous to the use of oxygen as a terminal electron acceptor by aerobic organisms and is called anaerobic respiration. Nitrate is an oxidized compound and there are several steps possible in its reduction. The initial step is the reduction of nitrate ( $NO_3^-$ ) to nitrite ( $NO_2^-$ ). Several possible products can be made from further

reduction of nitrite. Possible reduced end products include the following  $N_2$ ,  $NH_3$  (ammonia),  $N_2O$  (nitrous oxide).

Bacteria vary in their ability to perform these reactions, a useful characteristic for identification. A medium that will support growth must be used and the cells must be grown anaerobically. Growth in the presence of oxygen will decrease or eliminate nitrate reduction. There are many possible end products of nitrate reduction such as nitrite, nitrogen gas ( $N_2$ ), nitrous oxides, ammonia, and hydroxylamine. The disappearance of nitrate or the appearance of the end products. The test relies on the production of nitrous acid from the nitrite. This, in turn, reacts with the iodide in the reagent to produce iodine.

The iodine then reacts with the starch in the reagent to produce a blue color. Since some of the possible products of  $NO_3^-$  reduction are gaseous, a Durham tube is sometimes inverted in the culture tube to trap gases. This being the case, it is important to pre-test the medium to ensure no detectable nitrite is present at the beginning, and, in the case of a negative test, to reduce any nitrate to nitrite to determine whether the nitrite was also reduced. If nitrite is produced, it reacts with hemoglobin to give a bright red color, instead of the dark red color of hemoglobin.

It is this reaction that is responsible for the color of meats, such as hot dogs, which are preserved with sodium nitrite. The blood agar test has the advantage of no color change occurring if the nitrite is further reduced. F. UREASE TEST Urease test mainly highlighted to determine the ability of the organism to split urea forming 2 molecules of ammonia by the action of the enzyme Urease with resulting alkalinity. Negative reaction shown by E. coli

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meanwhile *Klebsiella* spp. shows positive result. Extra precaution needed because both the urease test medium depend upon the demonstration of alkalinity that not specific for urease.

Moreover the protein hydrolysis may result I alkalinity hence false positive may be seen in *Pseudomonas*. The false positivity can be eliminated by control test using the same medium without urea as recommendation. Urea is a nitrogenous waste product of animals. Some bacteria can cleaved it to produce carbon dioxide and ammonia. The ammonia is a nitrogen source for amino acid biosynthesis as well as for synthesis of other nitrogen-containing molecules in the cell. The urease test was devised to distinguish *Proteus* species from other enterics.

The medium described here is buffered enough so that weak urease producers appear negative. The production of ammonia raises the pH of the medium. The indicator phenol red is present in the broth. Phenol red is orange-yellow at pH below than 6. 8, and turns bright pinkish-red at pH higher than 8. 1. Hence, a positive urea test is denoted by the change of medium color from yellow to pinkish red. **CONCLUSION:** Based on the laboratory, different bacteria species have different abilities to metabolize various substrates and end products formed were able to be observed and distinguished.