RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: *Abhijit Roy*

ROLL NO: *276*

DEPARTMENT: *MICROBIOLOGY*

YEAR: *UG-3*

PROJECT TOPIC: *Isolation and characterization of airborne microorganism*

GUIDED BY: *Arindam Roy and Chandan Rai*

Acknowledgements:

I would like to express my hearty gratitude to Dr. Arindam Roy and Asst. Prof. Chandan Rai for their guidance. I would also like to thank my all departmental faculty members and authorities of Ramakrishna Mission Vidyamandira for providing me this learning opportunity.

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INTRODUCTION:

Microbes are ubiquitously present in every living and non-living matter and can survive galore in the air, water and even in all the extremes of the environment. Isolation of microbes present in a particular site requires specific isolation culture medium. Microorganisms in air are present in spore form and can be cultured by nutrient agar plating in open air exposure. In this project, we collected air microbes by agar plating in different sites in the building with different exposure time for different agar plates. The isolated microbes are first cultured,then purified and further subcultured to study their morphological characteristics followed by biochemical assays. The experiments performed to study the morphological characteristics are gram staining, endospore staining and biochemical assays performed to study the biochemical activities are Indole production test, methyl red test, Voges-Prauskaur test, Citrase activity test, Urease production test, Gelatinase production test, TSI agar test, starch test. All the tests performed show either positive or negative results by the colour change of the respective media. If there occur any change of the colour of the respective media, the test can be considered positive and if not, it can be considered negative. Each and every experiment here follows different principle as well as procedure, even the detailed layout of the microbial activity in the respective medium. The experiments also tell about the interaction between microbial exoenzymes and there cognate or non-cognate substrates present in the medium. Not only that, they also tell surely about the activity of the microbial community to produce either the respective exoenzymes or not.

Methodology:

- **Gram Staining**:

The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolorized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple colour. Counterstain, which is usually positively-charged **safranin**, is applied last to give decolorized Gram-negative bacteria a pink or red colour.

- **Endospore staining:**

The endospore stain is a differential stain used to visualize bacterial endospores. Endospores are formed by a few genera of bacteria, such as Bacillus. By forming spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.

Aprimary stain (malachite green) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green permeate the spore wall) the endospores by heating. In this technique heating acts as a mordant. Endospores and vegetative cells are visualized as green and red colour respectively.

- **BIOCHEMICAL TESTS:**

1.IMViC tests:

a) Indole test:

Some bacteria split amino acid tryptophan into indole and pyruvic acid using the enzyme called tryptophanase. Indole can be detected with Kovac's reagent. Along with differentiation of enterics, Indole test can also be used for species differentiation. A positive result is shown by the presence of a red or red-violet color in the surface alcohol layer of the broth. A negative result appears yellow.

b) Methyl red test:

The methyl red test (MR test) is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the MR test. A negative MR test is indicated by a yellow color in the culture medium, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

c) Voges-Proskauer test:

The Voges-Proskauer test is to determine the ability of some microorganisms to produce a neutral end product 2,3-butanediol from glucose fermentation. A cherry red colour indicates a positive result, while a yellow-brown colour indicates a negative result on the addition of barritt's (reagent a and b) to the overnight cultured bacterial sample.

d)Citrate test:

Bacteria are inoculated on a medium containing sodium citrate and a pH indicator such as bromothymol blue. The medium also contains inorganic ammonium salts, which are utilized as sole source of nitrogen. Use of citrate involves the enzyme citrase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and carbon dioxide (CO_2). Production of sodium bicarbonate ($NaHCO_3$) as well as ammonia (NH_3) from the use of sodium citrate and ammonium salts results in alkaline pH. This results in a change of the medium's colour from green to blue.

2.Urease test:

Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

3. Catalase test:

The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. Catalase positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

4. Starch hydrolysis:

Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

5. Gelatin production test:

This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinases that hydrolyze gelatin.The reaction occurs in two sequential steps: in first reaction gelatinases hydrolyze gelatin into polypeptides and then polypeptides are further converted into amino acids.

Positive**:** Partial or complete liquefaction of the inoculated tube at 4°C. The control tube remains solidified even after exposure to cold temperature.

Negative: At the end of the refrigeration, the control tube and the test tube both remain completely solidified.

6. TSI test:

To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

- An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.
- An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.
- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.

- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture:

a) Colony morphology:Circular, Entire, Convex, Filamentous
b) Colony colour: White
c) Gram staining: Positive
d) Cell shape: Rod
e) Cell arrangement: Single & in chain formation
f) Endospore staining: after 4-5 days starvation,endospore will form.
g) Capsule staining: No capsule observed around cells.

Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	positive
2	Endospore		After 4-5 days starvation,endospore will form.	positive
3	TSI test	TSI medium	(a)yellow butt (b)Red slant	Glucose fermentation occurred, With no H_2S production
4	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	 (a)MR-VP (b)MR-VP (c)Trp. broth (d)citrate agar	 (a)same result with the control sample (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d) green colour turned into deep blue	 (a) negative (b)negative (c)negative (d)positive
5	Urease activity test	Urea agar	Media colour become pink	Positive

6	Catalase activity test	Nutrient agar	air bubbles forms on addition of Hydrogen Peroxide	Positive
7	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

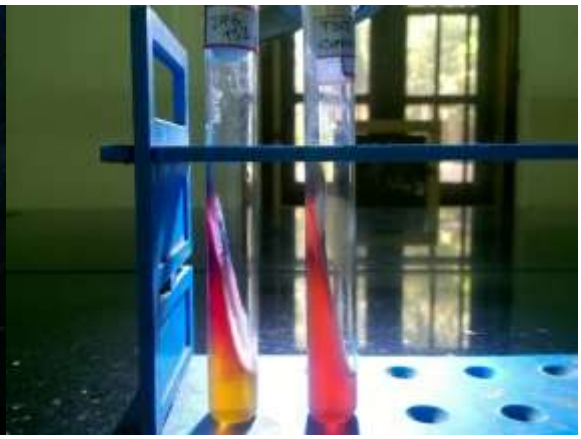
Bacterial colony on nutrient agar

IMViC Test results (Indole, MR, VP, Citrate)

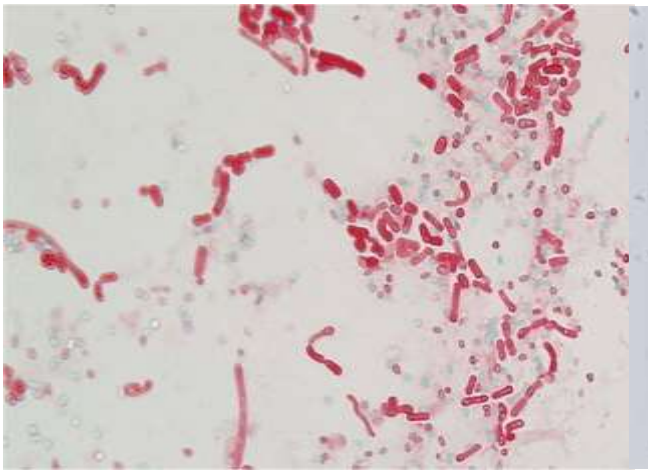
Urease test

Gelatinase test

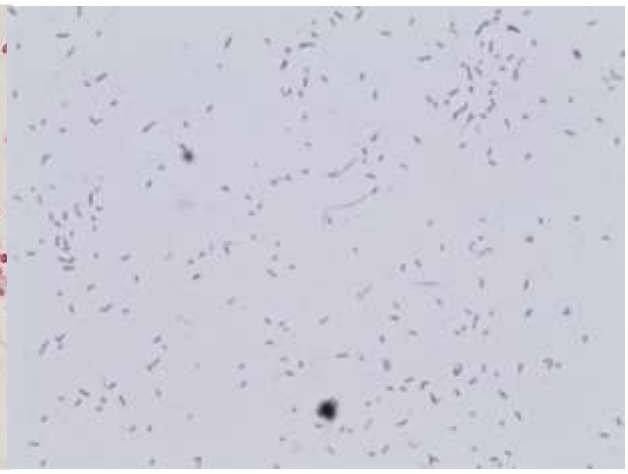
Starch test



TSI test



Endospore staining (positive)



Gram staining (positive)

<u>Conclusion:</u>

From the above result we can conclude that, the bacteria which are obtained from the air exposure method may be *<u>Bacillus</u> sp.*

RAMAKRISHNA MISSION VIDYAMANDIRA
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Citrate utilisation test:

citrate utilization test is used to detect the ability of an organism which can utilize **citrate** as a sole source of carbon for their metabolism with resulting alkalinity. The citrase enzyme hydrolyses the **citrate** to form oxaloacetic acid and acetic acid.

Growth on the medium even without colour change will be considered as positive. A colour change in the medium would be observed if the test organism produces acid or alkali during its growth. The usual colour change observed is from **green (neutral) to blue (alkaline)** as a positive result**.** No growth will be observed as result of negative result.

Other biochemical tests :

Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urea agplate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

Catalase test

The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. Catalase positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

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Blackening of the medium: Occurs in the presence of H2

Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture

Shape of arrangement : Rod
Cultural characteristics : Round,small,convex
Cell arrangement : single cell arrangement
Gram staining : positive
Endospore staining : after 4-5 days starvation, endospore will not form..
Capsule staining : No capsule observed around cells.

Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	positive
2	Endospore		After 4-5 days starvation,endospore will form.	positive
3	capsule		No result found	negetive
4	H_2S production	TSI medium	Absence of extensuve blacking in the bult of the TSI medium	negetive
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)develpoment of a deep rose colour in the culture 15 mins following the addition of Barritt's reagent. (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d)blue coloration appears on the surface on the slant.	(a)positive (b)negetive (c)negetive (d)positive
6	Urease activity test	Urea agar	Same result with the control sample	Negative
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H_2O_2	Positive
8	Gelatin liquefaction	Gelatin agar	Gelatin hydrolysis	positive
9	Starch hydrolysis	Starch agar plate	Same result with the control	negetive

Comment [WU1]: Cell

Comment [WU2]: But the picture shows that the cells are circular in shape

Comment [WU3]: Which method did you follow?
No result found is incorrect. You may write no clear zone found around cell.

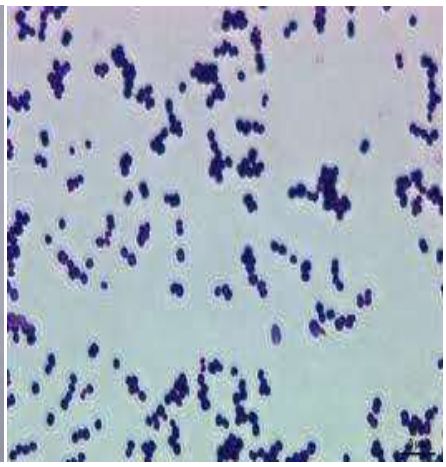
Comment [WU4]: bleckening

Comment [WU5]: You may write no change of colour after adding methyl red reagent

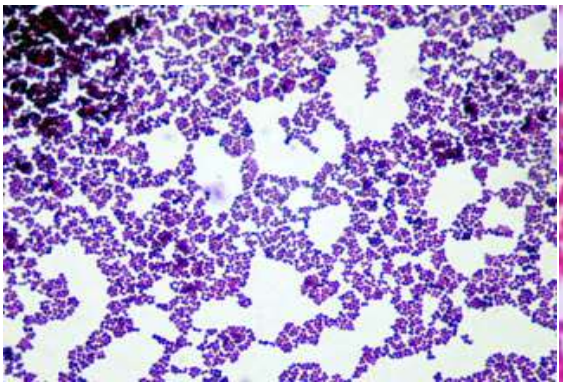
Comment [WU6]: Culture remains liquid at low temperature

Comment [WU7]: You may write no clear zone around colony

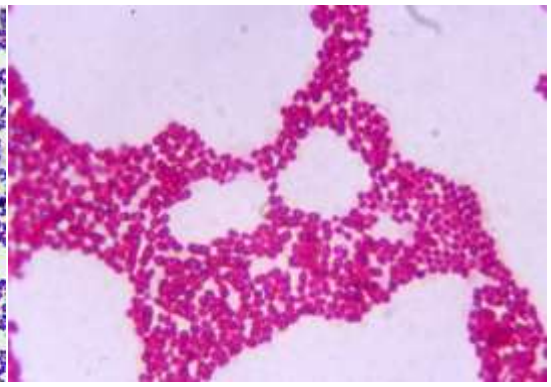
1.MAIN PLATE



2.GRAM STAINING

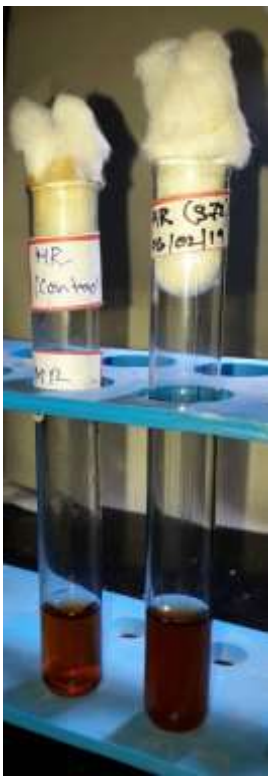


3. ENDOSPORE STAINING



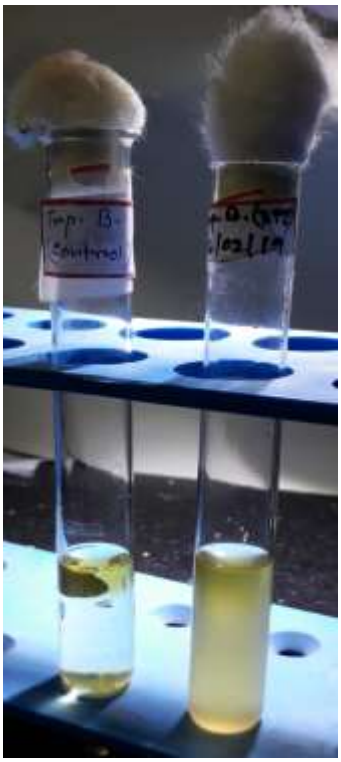
4. CAPSULE STAINING

4. H2S PRODUCTION TEST

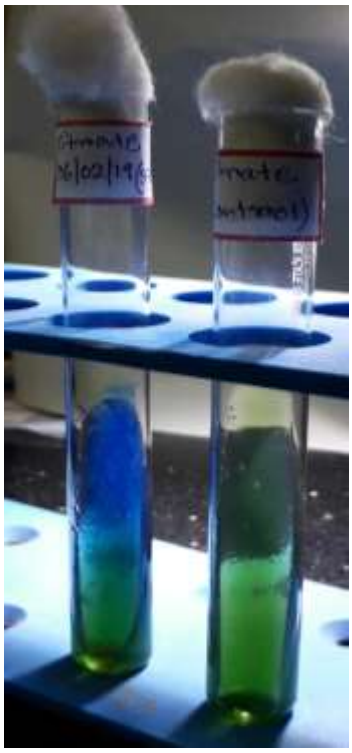


5(a).MR TEST

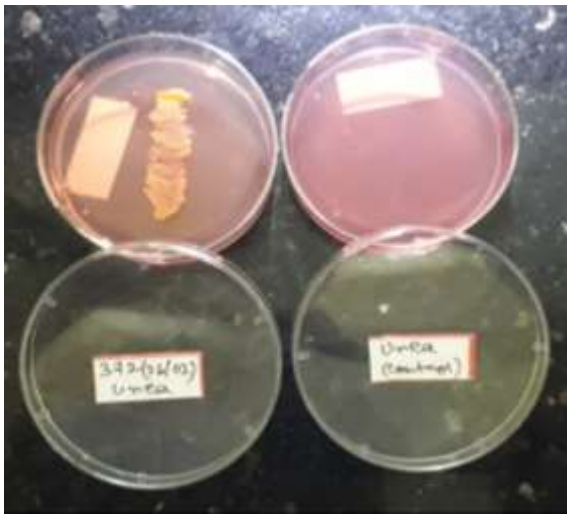
5(b).VP TEST



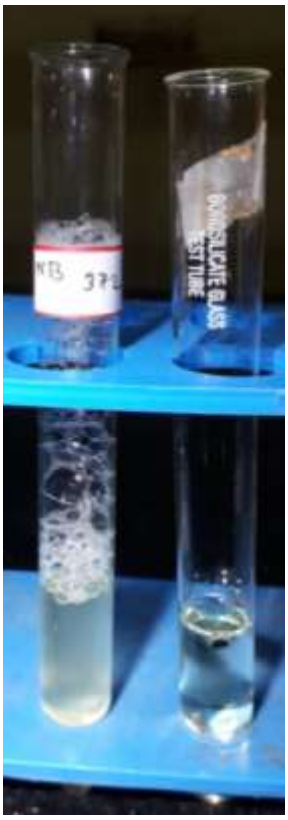
5(c).INDOLE TEST



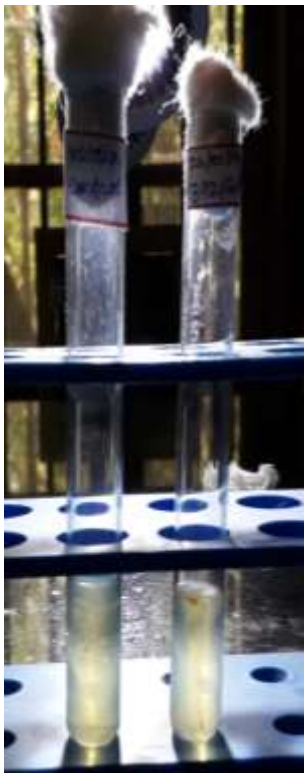
5(d).CITRATE UTILIZATION TEST



6. UREASE TEST



7. CATALASE TEST



8. GELATIN UTILIZATION TEST

9. STARCH UTILIZATION TEST

Conclusion:

From the above result we can conclude that the bacteria we obtain from air exposure method it may be *Micrococcus sp.*

Comment [WU8]: Is it a endospore forming bacteria???

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IV. Citrate test:

To determine the ability of an organism to utilize citrate as sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen.

Citrate is a sal t of citric acid. One of the metabolites in the Krebs cycle. Bacteria tha can use citrate can also extract nitrogen from the ammonium salt with the production of ammonia. Leading to alkalinisation of the medium from conversion of the NH_3^{2+} to NH_4OH.

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Negative: At the end of the refrigeration, the control tube and the test tube both remain completely solidified.

- **TSI:**

To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

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- An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.

- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.
- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

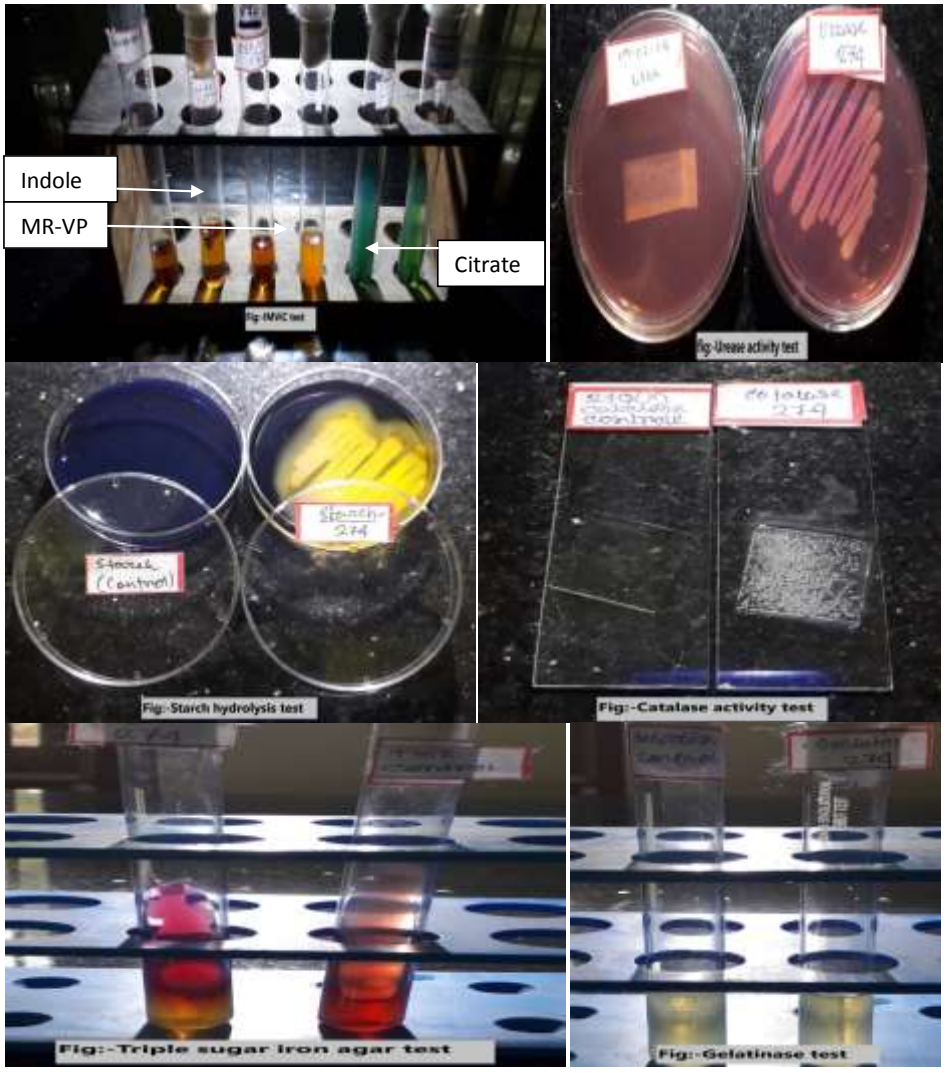
- **Description of unknown bacteria culture**

Shape of arrangement: Rod
Cultural characteristics: Round, small, convex
Cell arrangement: single cell arrangement
Gram staining: positive
Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	positive
2	TSI test	TSI medium	(a)yellow butt (b)no change in slant	Glucose fermentation has occurred
3	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)same result with the control sample (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d) no significant colour change	(a) negative (b)negative (c)negative (d)negative
4	Urease activity test	Urea agar	Colour changed to pink	Positive
5	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H_2O_2	Positive
6	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative

Comment [WU1]: Colour change does not indicate gelatin liquefaction

7	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive



Conclusion:-from the above result we can conclude that the bacteria which are obtained from the air exposure method may be *Bacillus* sp.

RAMAKRISHNA MISSION VIDYAMANDIRA

BELUR MATH

NAME- **ARKADEB BHUINYA**

ROLL-**270**

B.Sc. 3rd year(2017-20)

DEPARTMENT OF MICROBIOLOGY

PROJECT TOPIC- **ISOLATION AND CHARACTERIZATION OF AIRBORNE MICROORGANISM**

PROJECT GUIDE- **PROF. ARINDAM ROY & PROF. CHANDAN RAI**

Isolation and Characterization of Air-borne Microorganism

Acknowledgements:

I would like to express my hearty gratitude to Dr. Arindam Roy and Asst. Prof. Chandan Rai for their guidance. I would also like to thank my all departmental faculty members and authorities of Ramakrishna Mission Vidyamandira for providing me this learning opportunity.

Last but not the least,I am always thankful to my family and my lab members for motivating and supporting me in every possible way.

INTRODUCTION:

Microbes are ubiquitously present in every living and non-living matter and can survive galore in the air, water and even in all the extremes of the environment. Isolation of microbes present in a particular site requires specific isolation culture medium. Microorganisms in air are present in spore form and can be cultured by nutrient agar plating in open air exposure. In this project, we collected air microbes by agar plating in different sites in the building with different exposure time for different agar plates. The isolated microbes are first cultured, then purified and further subcultured to study their morphological characteristics followed by biochemical assays. The experiments performed to study the morphological characteristics are gram staining, endospore staining and biochemical assays performed to study the biochemical activities are Indole production test, methyl red test, Voges-Prauskaur test, Citrate activity test, Urease production test, Gelatinase production test, TSI agar test, starch test. All the tests performed show either positive or negative results by the color change of the respective media. If there occur any change of the color of the respective media, the test can be considered positive and if not, it can be considered negative. Each and every experiment here follows different principle as well as procedure, even the detailed layout of the microbial activity in the respective medium. The experiments also tell about the interaction between microbial exo-enzymes and there cognate or non-cognate substrates present in the medium. Not only that,but also they tell surely about the activity of the microbial community to produce either the respective exo-enzymes or not.

Methodology:

Gram Staining:

The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolorized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged **safranin**, is applied last to give decolorized Gram-negative bacteria a pink or red color.

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The endospore stain is a differential stain used to visualize bacterial endospores. Endospores are formed by a few genera of bacteria, such as

Bacillus. By forming spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.
A primary stain (malachite green) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green permeate the spore wall) the endospores by heating. In this technique heating acts as a mordant. Endospores and vegetative cells are visualized as green and red color respectively.

Biochemical Tests:

IMViC test:

Indole test:

Some bacteria split amino acid tryptophan into indole and pyruvic acid using the enzyme called tryptophanase. Indole can be detected with Kovac's reagent. Along with differentiation of enterics, Indole test can also be used for species differentiation. A positive result is shown by the presence of a red or red-violet color in the surface alcohol layer of the broth. A negative result appears yellow.

Methyl red test:

The methyl red test (MR test) is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the MR test. A negative MR test is indicated by a yellow color in the culture medium, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

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The Voges-Proskauer test is to determine the ability of some microorganisms to produce a neutral end product 2,3 butanediol from glucose fermentation. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result on the addition of barritt's (reagent a and b) to the overnight cultured bacterial sample.

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Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

Catalase test

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Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

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TSI:(H2S Production Test)

To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

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- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture

a) Colony morphology : Circular, Entire, Convex.
b) Colony colour : Yellowish, not bright.
c) Cell shape : Spherical (Cocci).
d) Cell arrangement: Diplococcus, some in Tetrad arrangement.
e) Gram staining: positive
f) Endospore staining: No endospores are found after 7 days incubation at 35°C in NB media
g) Capsule staining: No capsule observed around

Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram staining		Cells retain the purple colour of primary stain	Positive
2	Endospore-staining		No endospore formation	Negative
3	H2S production	TSI medium	(a)yellow butt (b)no change in slant	Glucose fermentation has occurred
4	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)same result with the control sample (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d) no significant colour change	(a)negative (b)negative (c)negative (d)negative
5	Urease activity test	Urea agar	Media ; No such color change	Negative
6	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H2O2	Positive

Comment [WU1]: Write what you observed. Like no change in colour or red ring formation

Comment [WU2]: ??

Comment [WU3]: ??

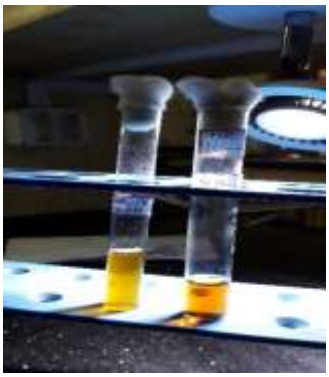
7	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

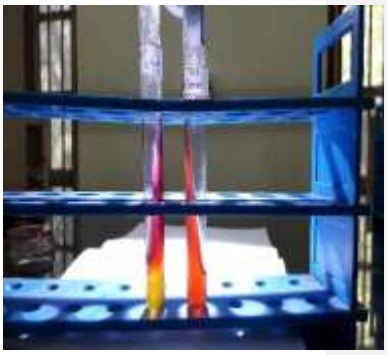
Comment [WU4]: Inoculated tubes solid after incubation

VP Test

Indol Test

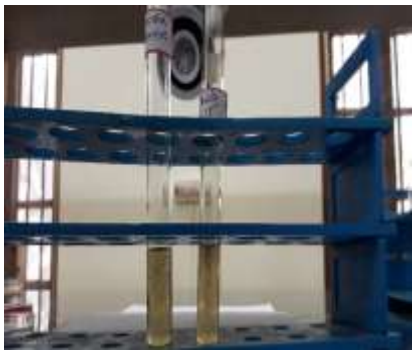
MR Test >>





Citrate Utilization Test **H2S Production Test**

Urease Test



Gelatinase Test

Catalase Test

Starch Test

Conclusion: From the above result of the project titled "Isolation and Characterization of Air-borne Microorganism" I can conclude that ,the tested bacteria is *Staphylococcus aureus.*(Staphylococcus aureus)

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: AYANAVO SAHA
ROLL NO. : 377
DEPARTMENT:MICROBIOLOGY
YEAR:UG-3
PROJECT TOPIC:Isolation and characterization of airborne microorganism
GUIDED BY: Arindam Roy and ChandanRai

ACKNOWLEDGEMENTS:

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Citrate utilisation test:

citrate utilization test is used to detect the ability of an organism which can utilize **citrate** as a sole source of carbon for their metabolism with resulting alkalinity. The citrase enzyme hydrolyses the **citrate** to form oxaloacetic acid and acetic acid.

Growth on the medium even without colour change will be considered as positive. A colour change in the medium would be observed if the test organism produces acid or alkali during its growth. The usual colour change observed is from **green (neutral) to blue (alkaline)** as a positive result**.** No growth will be observed as result of negative result.

Other biochemical tests :

Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urea agplate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

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Description of unknown bacteria culture

Shape of arrangement : Rod

Cultural characteristics : Round,small,convex

Cell arrangement : single cell arrangement

Gram staining : positive

Endospore staining : after 4-5 days starvation, endospore will not form..

Capsule staining : No capsule observed around cells.

Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	positive
2	Endospore		After 4-5 days starvation,endospore will form.	positive
3	capsule		No result found	negetive
4	H_2S production	TSI medium	Absence of extensuve blacking in the bult of the TSI medium	negetive
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)develpoment of a deep rose colour in the culture 15 mins following the addition of Barritt's reagent. (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d)blue coloration appears on the surface on the slant.	(a)positive (b)negetive (c)negetive (d)positive
6	Urease activity test	Urea agar	Same result with the control sample	Negative
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H_2O_2	Positive
8	Gelatin liquefaction	Gelatin agar	Gelatin hydrolysis	positive
9	Starch hydrolysis	Starch agar plate	Same result with the control	negetive

Comment [WU1]: cell

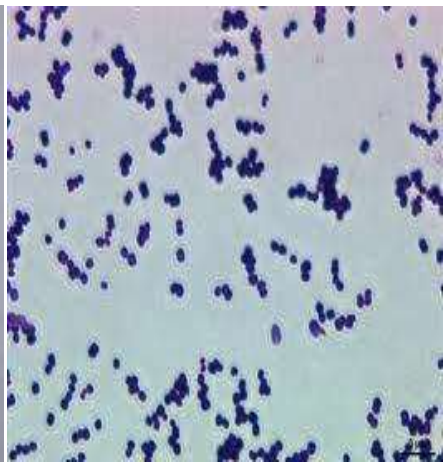
Comment [WU2]: butt

Comment [WU3]: ??. No red colouration

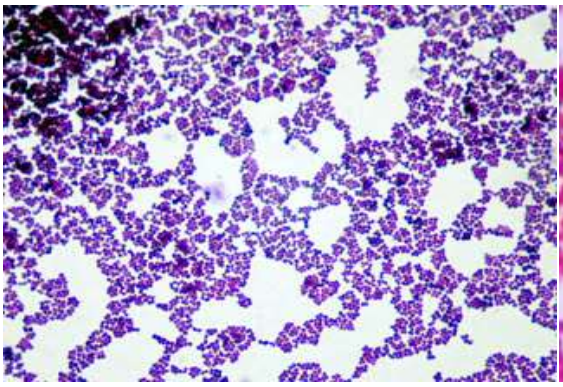
Comment [WU4]: ??

Comment [WU5]: Inoculated tube remains liquid even after freezing

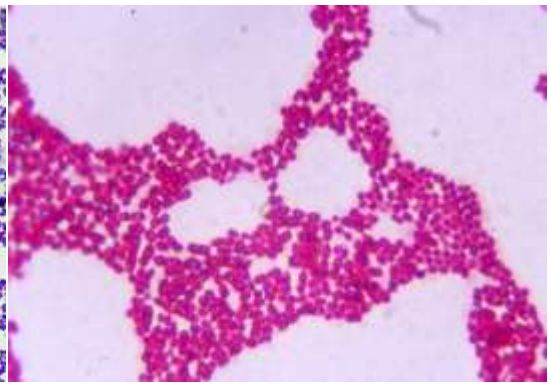
1.MAIN PLATE



2.GRAM STAINING

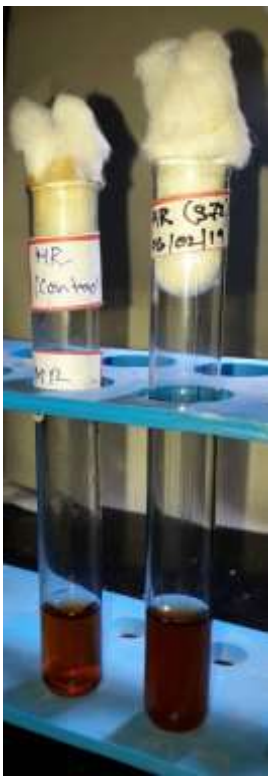


3. ENDOSPORE STAINING



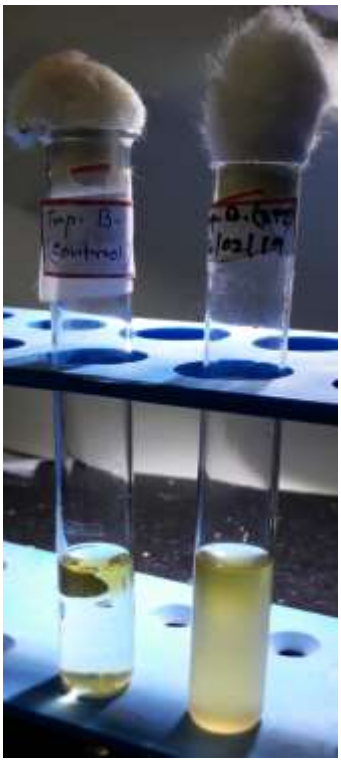
4. CAPSULE STAINING

4. H2S PRODUCTION TEST

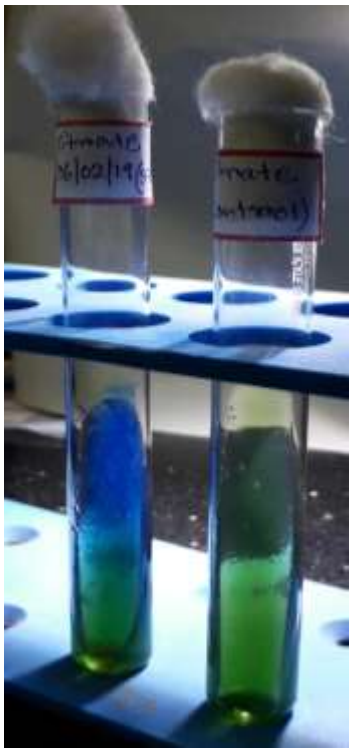


5(a).MR TEST

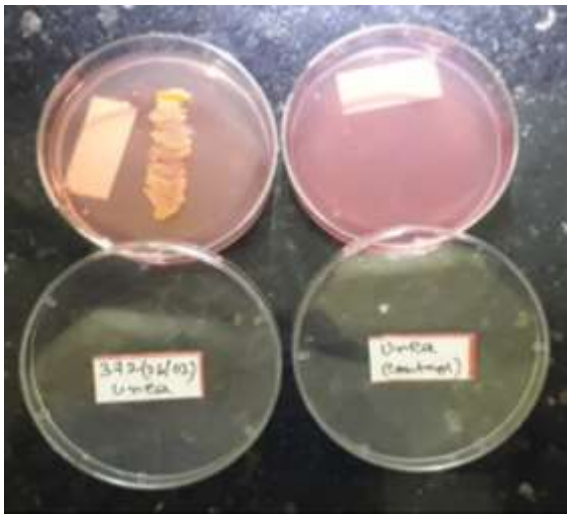
5(b).VP TEST



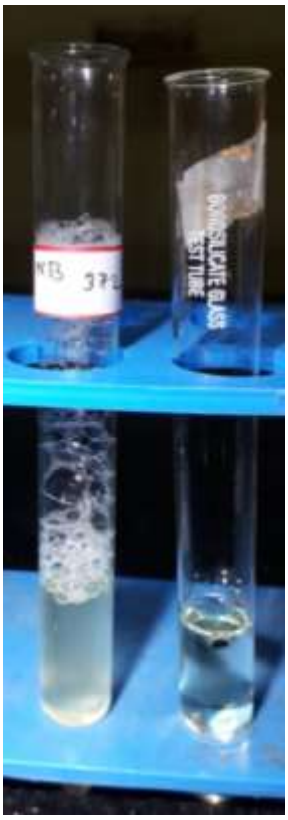
5(c).INDOLE TEST



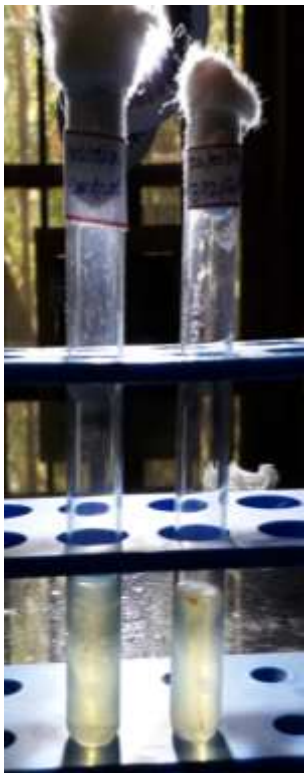
5(d).CITRATE UTILIZATION TEST



6. UREASE TEST



7. CATALASE TEST



8. GELATIN UTILIZATION TEST

9. STARCH UTILIZATION TEST

Conclusion :

From the above result we can conclude that the bacteria we obtain from air exposure method it may be *Micrococcus sp.*

Comment [WU6]: Is it endospore forming?

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME-BASANTA MALIK
YEAR-3rd year
ROLL-279
PROJECT TOPIC-ISOLATION AND CHARACTERISATION OF AIRBORNE MICROORGANISM
PROJECT GUIDE-ARINDAM ROY and CHANDAN RAI

Isolation and Characterization of Air-borne Microorganism

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Methyl red test:

The methyl red test (MR test) is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the MR test. A negative MR test is indicated by a yellow color in the culture medium, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

Voges-Proskauer test:

The Voges-Proskauer test is to determine the ability of some microorganisms to produce a neutral end product 2,3 butanediol from glucose fermentation. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result on the addition of barritt's (reagent a and b) to the overnight cultured bacterial sample.

OTHER BIOCHEMICAL TESTS

Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

Catalase test

The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. Catalase positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

Starch hydrolysis

Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

Gelatin production test:

This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinases that hydrolyze gelatine.The reaction occurs in two sequential steps: in first reaction gelatinases hydrolyze gelatine into polypeptides and then polypeptides are further converted into amino acids.

Positive: Partial or complete liquefaction of the inoculated tube at 4°C. The control tube remains solidified even after exposure to cold temperature.
Negative: At the end of the refrigeration, the control tube and the test tube both remain completely solidified.

TSI:
To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

- An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.
- An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.
- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.
- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture

Shape : Rod
Cultural characteristics: Round,small,convex, white and translucent
Cell arrangement: single cell arrangement mostly
Gram staining: positive
Endospore staining: after 4-5 days starvation,endospore will not form.
Biochemical Tests ;

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	Positive
2	Endospore		After 4-5 days starvation,endospore will not form.	Negative
3	Capsule		----------	----------
4	H2S production	TSI medium	(a)yellow butt (b)no change in slant	Glucose fermentation has occurred.H2S not

				produced.
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)same result with the control sample (b)same result with the control sample. (c)presence of red colourization in the tryptophan broth after adding kovac's reagent. (d)significant colour changeobserved.	(a) negative (b)negative (c)positive (d)positive
6	Urease activity test	Urea agar	Media colour become pink	Positive
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H2O2	Positive
8	Gelatine liquefaction	Gelatine agar	No significant liquefaction	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

Comment [WU1]: Please write as I suggested

Pic: Purification by streak plate method

Pic: IMViC Test

Pic: Indole test

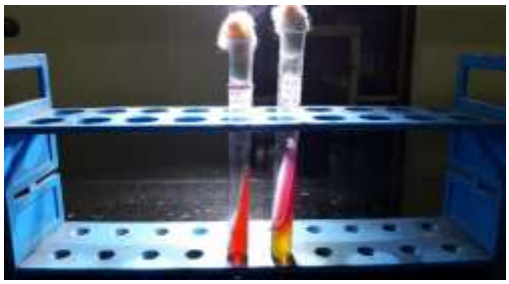
Pic: MR Test

Pic: VP Test

Pic: Citrate Test

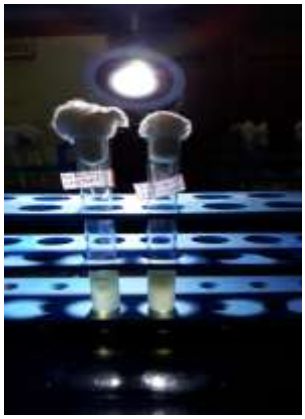
Pic: Catalase Test

Pic: Urease Test



Pic: Triple sugar ion test

Pic: starch agar Test



Pic: Gelatine agar test

Conclusion: Hence the microorganism isolated from air may be ***Bacillus*** sp.

RAMAKRISHNA MISSION VIDYAMANDRA
(Belur math : Howrah)

NAME: BASUDEB SING
ROLL NO: *273*
DEPARTMENT: *MICROBIOLOGY*
YEAR: UG-3

PROJECT TOPIC: ***Isolation and characterization of airborne microorganism***

GUIDED BY: ***Dr. Arindam Roy and Chandan Rai***

Isolation and Characterization of Air-borne Microorganism

Acknowledgements:
I would like to express my hearty gratitude to Prof. Arindam Roy and Prof. Chandan Rai for their guidance. I would also like to thank my all departmental faculty members and authorities of Ramakrishna Mission Vidyamandira for providing me this learning opportunity.
Last but not the least; I am always thankful to my family and my lab members for motivating and supporting me in every possible way.

INTRODUCTION:

Microbes are ubiquitously present in every living and non-living matter and can survive galore in the air, water and even in all the extremes of the environment. Isolation of microbes present in a particular site requires specific isolation culture medium. Microorganisms in air are present in spore form and can be cultured by nutrient agar plating in open air exposure. In this project, we collected air microbes by agar plating in different sites in the building with different exposure time for different agar plates. The isolated microbes are first cultured, then purified and further subcultured to study their morphological characteristics followed by biochemical assays. The experiments performed to study the morphological characteristics are gram staining, endospore staining and biochemical assays performed to study the biochemical activities are Indole production test, methyl red test, Voges-Prauskaur test, Citrase activity test, Urease production test, Gelatinase production test, TSI agar test, starch test. All the tests performed show either positive or negative results by the color change of the respective media. If there occur any change of the color of the respective media, the test can be considered positive and if not, it can be considered negative. Each and every experiment here follows different principle as well as procedure, even the detailed layout of the microbial activity in the respective medium. The experiments also tell about the interaction between microbial exoenzymes and there cognate or non-cognate substrates present in the medium. Not only that, they also tell surely about the activity of the microbial community to produce either the respective exoenzymes or not.

Methodology:

Gram Staining:

The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolorized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged **safranin**, is applied last to give decolorized Gram-negative bacteria a pink or red color.

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The endospore stain is a differential stain used to visualize bacterial endospores. Endospores are formed by a few genera of bacteria, such as Bacillus. By forming

spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.

A primary stain (malachite green) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green permeate the spore wall) the endospores by heating. In this technique heating acts as a mordant. Endospores and vegetative cells are visualized as green and red color respectively.

IMViC tests:

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- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture

(1)Colony morphology : Circular, Entire, Convex.
(2) Colony colour : White in colour
(3) Cell shape : Coccus
(4) Cell arrangement : arranging in line
(6) Gram staining : positive
(7) Endospore staining : No endospores are found after 7 days incubation at 35°C in NB media.
(8) Capsule staining : No capsule observed around cells.

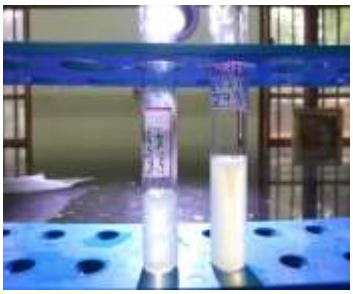
Comment [WU1]: ? Chain

Biochemical Tests (summary)

Sl no.	**Name of Experiment**	**Culture media**	**Observation**	**Result**
1	Gram stain		Cells retain the purple colour of primary stain	positive
2	Endospore		After 7days starvation, no endospore still found.	Negative
3	capsule		No result found	negative
4	H2S production	TSI medium	(a)yellow butt (b)no change in slant	Glucose fermentation has occurred with no H2S production
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)same result with the control sample (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d) no significant colour change	(a) negative (b)negative (c)negative (d)negative
6	Urease activity test	Urea agar	Media colour become pink	Positive
7	Catalase activity	Nutrient agar	Air bubbles forms on	Positive

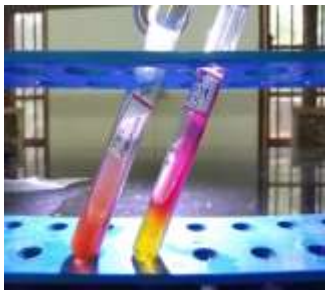
	test		addition of H2O2	
8	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

Comment [WU2]: ?? liquefaction



Gelatinase test

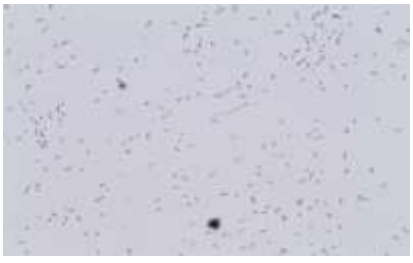
Starch test



TSI tes

Catalase test

Imvic test



Gram staning

urease test

Conclusion:

From the above result we can conclude that, the bacteria which are obtained from the air exposure method may be *Bacillus sp.*

Isolation and characterization of airborne microorganism

NAME: Biplab Ghosh
ROLL NO. : 333; YEAR: UG-3

Dept. of Microbiology
RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

GUIDED BY: Dr. Arindam Roy and Chandan Rai

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- **Methodology:**

- **Gram Staining**:

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I. Indole test:

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IV. Citrate Utilization test:

To determine the ability of an organism to utilize citrate as sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen.

Citrate is a salt of citric acid. One of the metabolites in the Krebs cycle. Bacteria that can use citrate can also extract nitrogen from the ammonium salt with the production of ammonia. Leading to alkalinisation of the medium from conversion of the NH_3^{2+} to NH_4OH.

Other Biochemical Tests:

- **Urease test:**

Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

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- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2).

Result:

- **Description of unknown bacteria culture:**

Colony morphology : Circular, Undulated, Umbonate.
Colony colour : White, not bright.
Shape of Cell: Rod Shaped
Gram staining: positive
Endospore staining: after 4-5 days starvation, endospore had not observed.

- **Biochemical Tests (summary):**

Sl no.	Name of Experiment	Culture media	Observation	Result
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4	Urease activity test	Urea agar	Colour change: light red to pink	Positive
5	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H_2O_2	Positive
6	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative
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- Figures:

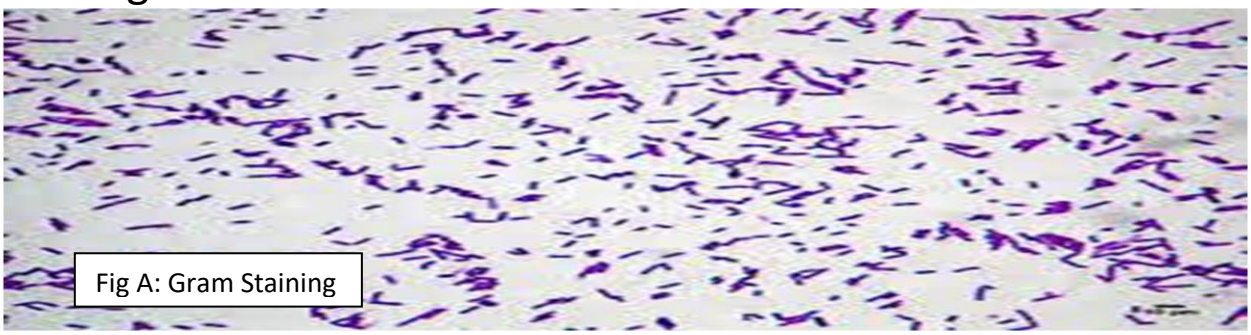


Fig A: Gram Staining

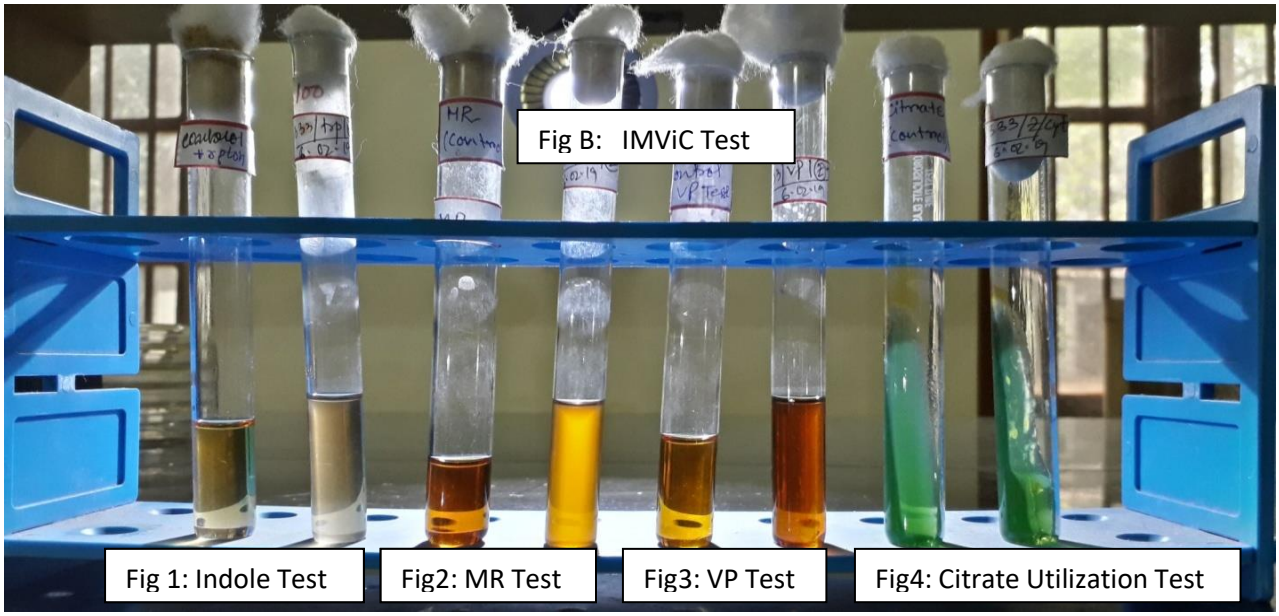
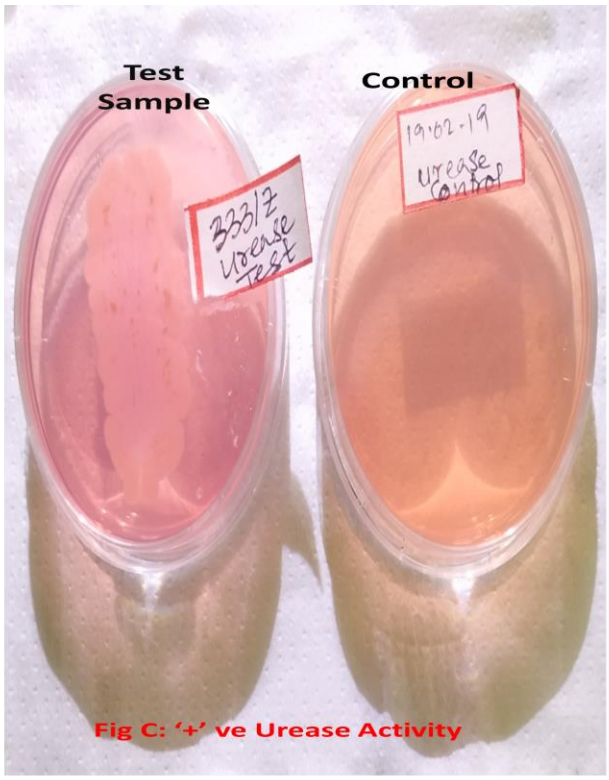
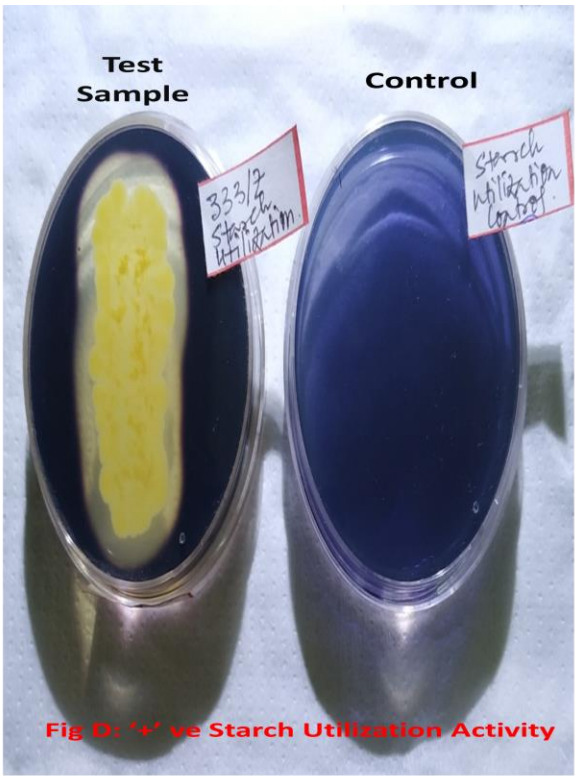
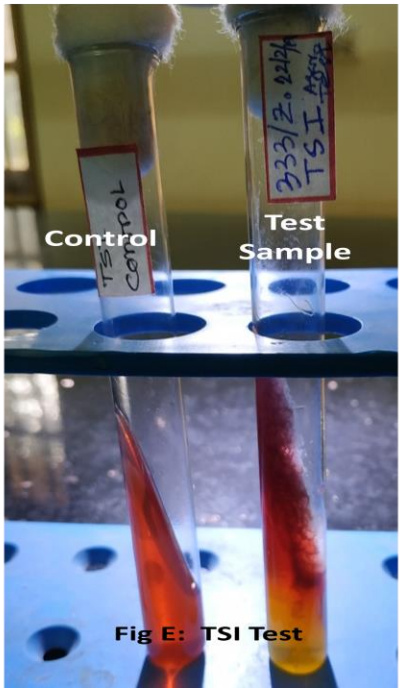


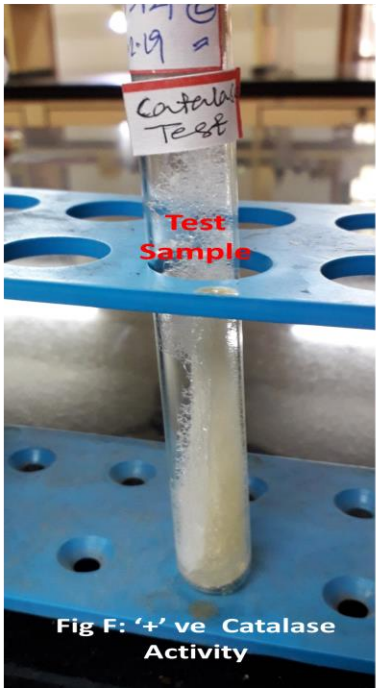
Fig B: IMViC Test

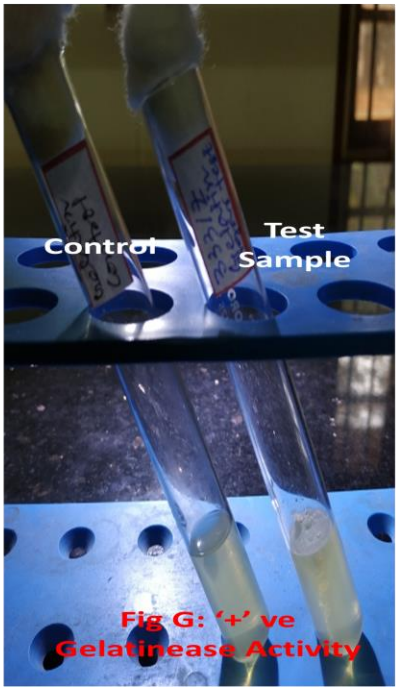
Fig 1: Indole Test Fig2: MR Test Fig3: VP Test Fig4: Citrate Utilization Test











Figures: (A). Gram Staining; (B). IMViC Test; (C). Urease Test; (D).Starch Utilization Test; (E). TSI Test; (F). Catalase Test; (G). Gelatinase Activity Test.

- **Conclusion:-**

From the above result we can conclude that the bacteria which are obtained from the air exposure method may be *Bacillus sp.*

Urease is a virulence factor found in various pathogenic bacteria. It is essential in colonization of a host organism and in maintenance of bacterial cells in tissues. Due to its enzymatic activity, urease has a toxic effect on human cells. So, the characterize organism may be a pathogenic bacteria, as it shows positive Urease Test.

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: **ONAM TAMIR**
ROLL NO. : **272**
DEPARTMENT: ***MICROBIOLOGY***
YEAR: ***UG-3***
PROJECT TOPIC: **Isolation and characterization of airborne microorganisms**
GUIDED BY: Arindam Roy and Chandan Rai

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The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolourized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple colour. Counter stain, which is usually positively-charged **safranin**, is applied last to give decolourized Gram-negative bacteria a pink or red colour.

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The endospore stain is a differential stain used to visualize bacterial endospores. Endospores are formed by a few genera of bacteria, such as Bacillus. By forming spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.

A primary stain (malachite green) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green permeate the spore wall) the endospores by heating. In this technique heating acts as a mordant. Endospores and vegetative cells are visualized as green and red colour respectively.

BIOCHEMICAL TESTS:

1. IMViC tests:

a) Indole test:

Some bacteria split amino acid tryptophan into indole and pyruvic acid using the enzyme called tryptophanase. Indole can be detected with Kovac's reagent. Along with differentiation of enteric, Indole test can also be used for species differentiation. A positive result is shown by the presence of a red or red-violet colour in the surface alcohol layer of the broth. A negative result appears yellow.

b) Methyl red test:

The methyl red test (MR test) is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the MR test. A negative MR test is indicated by a yellow colour in the culture medium, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

c) Voges-Proskauer test:

The Voges-Proskauer test is to determine the ability of some microorganisms to produce a neutral end product 2, 3 butanediol from glucose fermentation. A cherry red colour indicates a positive result, while a yellow-brown colour indicates a negative result on the addition of Barritt's (reagent A and B) to the overnight cultured bacterial sample.

d) Citrate test:

To determine the ability of an organism to utilize citrate as sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen.
Citrate is a salt of citric acid, one of the metabolites in the Krebs cycle. Bacteria can use citrate can also extract nitrogen from the ammonium salt with the production of ammonia. Leading to alkalinisation of the medium from conversion of the NH_3^{2+} to NH_4OH.

2. Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant

remains the same (light orange) if the organism being tested didn't produce urease enzyme.

3. Catalase test

The Catalase test tests for the presence of Catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. Catalase positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no Catalase enzyme to hydrolyse the hydrogen peroxide)

4. Starch hydrolysis

Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1, 6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

4. Gelatinase production test:

This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinase that hydrolyze gelatine. The reaction occurs in two sequential steps: in first reaction gelatinase hydrolyze gelatine into polypeptides and then polypeptides are further converted into amino acids.

Positive: Partial or complete liquefaction of the inoculated tube at 4°C. The control tube remains solidified even after exposure to cold temperature.
Negative: At the end of the refrigeration, the control tube and the test tube both remain completely solidified.

5. TSI:

To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulphide.

- An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.
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- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.
- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacterial culture

a) Shape of arrangement: Rod
b) Cultural characteristics: Round, small, convex
c) Cell arrangement: single cell arrangement
d) Gram staining: positive
e) Endospore staining: after 4-5 days starvation, endospore will form.
f) Capsule staining: No capsule observed around cells.

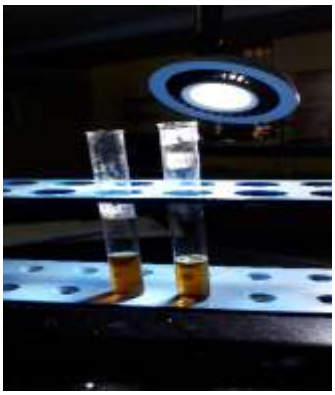
Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	Positive
2	Endospore		After 4-5 days starvation, endospore will form.	Positive
3	H2S production	TSI medium	(a) Yellow butt (b)no change in slant	Glucose fermentation has occurred
4	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a) Same result with the control sample (b) Same result with the control sample. (c) Absence of red colourization in the tryptophan broth after adding Kovac's reagent. (d) no significant colour change	(a) negative (b)negative (c)negative (d)negative
5	Urease activity test	Urea agar	Media colour become pink	Positive
6	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H2O2	Positive
7	Gelatine liquefaction	Gelatine agar	No significant colour change	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

Comment [WU1]: Colour remains unchanged

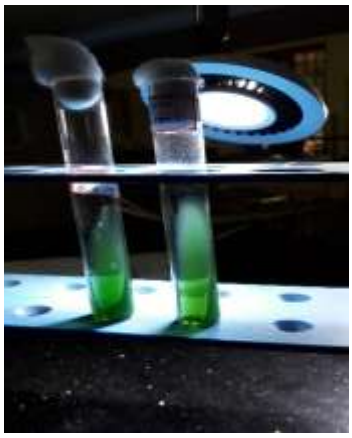
Comment [WU2]: Image shows negative result

Comment [WU3]: No liquefaction

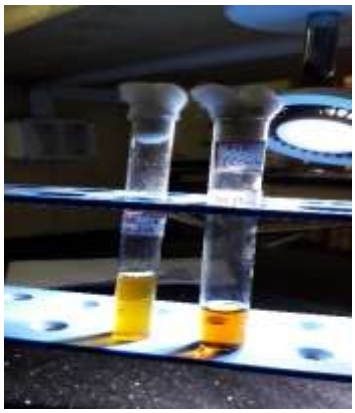


VP test

INDOLE test

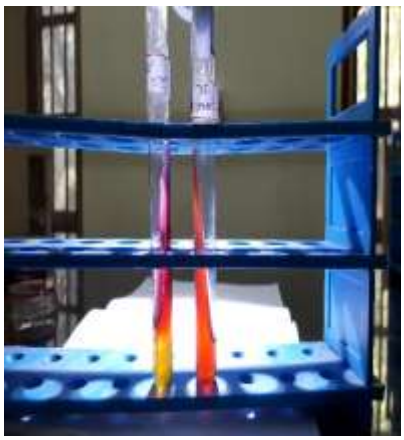


CITRATE test



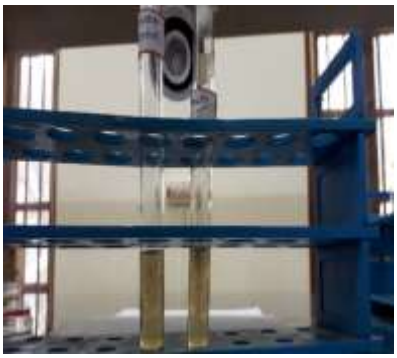
MR test

UREASE test



TSI test

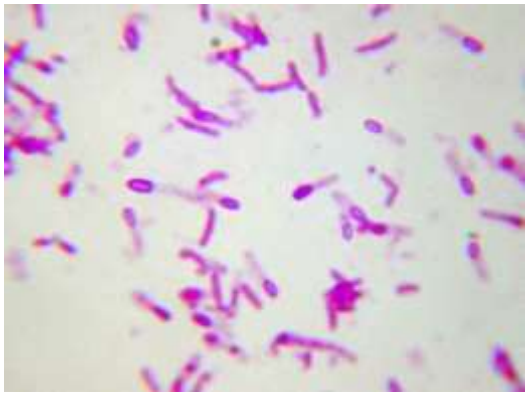
CATALASE Test



GELATINE Test

STARCH Test

GRAM STAINING (Positive)



ENDOSPORE STAINING (Positive)

Conclusion:

From the above result we can conclude that, the bacteria which are obtained from the air exposure method may be *Bacillus* sp.

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: **RISABH SAHU**
ROLL NO. : **371**
DEPARTMENT: MICROBIOLOGY
YEAR: UG-3
PROJECT TOPIC: **Isolation and characterization of airborne microorganism**
GUIDED BY: Arindam Roy and Chandan Rai

Isolation and Characterization of Air-borne Microorganism

Acknowledgements:

I would like to express my hearty gratitude to Prof. Arindam Roy and Prof. Chandan Rai for their guidance. I would also like to thank my all departmental faculty members and authorities of Ramakrishna Mission Vidyamandira for providing me this learning opportunity.

Last but not the least; I am always thankful to my family and my lab members for motivating and supporting me in every possible way.

INTRODUCTION:

Microbes are ubiquitously present in every living and non-living matter and can survive galore in the air, water and even in all the extremes of the environment. Isolation of microbes present in a particular site requires specific isolation culture medium. Microorganisms in air are present in spore form and can be cultured by nutrient agar plating in open air exposure. In this project, we collected air microbes by agar plating in different sites in the building with different exposure time for different agar plates. The isolated microbes are first cultured, then purified and further subcultured to study their morphological characteristics followed by biochemical assays. The experiments performed to study the morphological characteristics are gram staining, endospore staining and biochemical assays performed to study the biochemical activities are Indole production test, methyl red test, Voges-Prauskaur test, Citrase activity test, Urease production test, Gelatinase production test, TSI agar test, starch test. All the tests performed show either positive or negative results by the color change of the respective media. If there occur any change of the color of the respective media, the test can be considered positive and if not, it can be considered negative. Each and every experiment here follows different principle as well as procedure, even the detailed layout of the microbial activity in the respective medium. The experiments also tell about the interaction between microbial exoenzymes and there cognate or non-cognate substrates present in the medium. Not only that, they also tell surely about the activity of the microbial community to produce either the respective exoenzymes or not.

Methodology:

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The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolorized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged **safranin**, is applied last to give decolorized Gram-negative bacteria a pink or red color.

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spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.

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positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

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8	Gelatin	Gelatin agar	No significant colour	Negative

Comment [WU1]: No green coloured spore

Comment [WU2]: Write accordingly

	liquefaction		change	
9	Starch hydrolysis Test	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

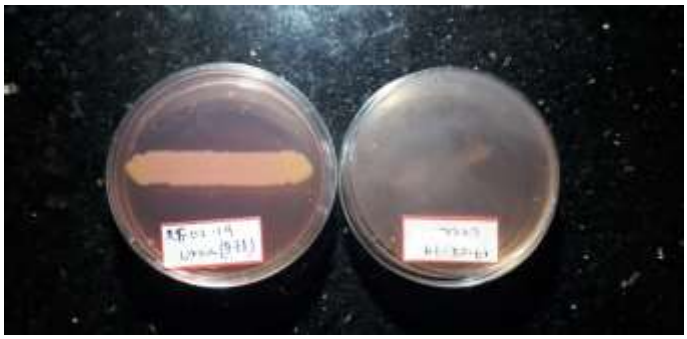

FIG: UREASE ACTIVITY TEST

FIG: STARCH HYDROLYSIS TEST

FIG: CATALASE ACTIVITY TEST

FIG:TRIPLE SUGAR IRON AGAR TEST

FIG: GELATINASE PRODUCTION TEST

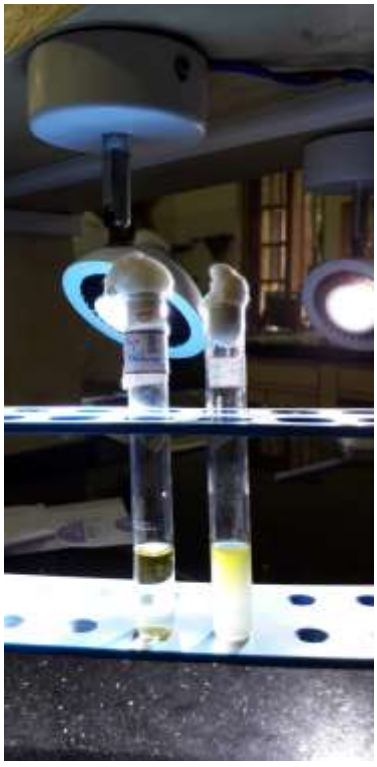

FIG: INDOLE PRODUCTION TEST

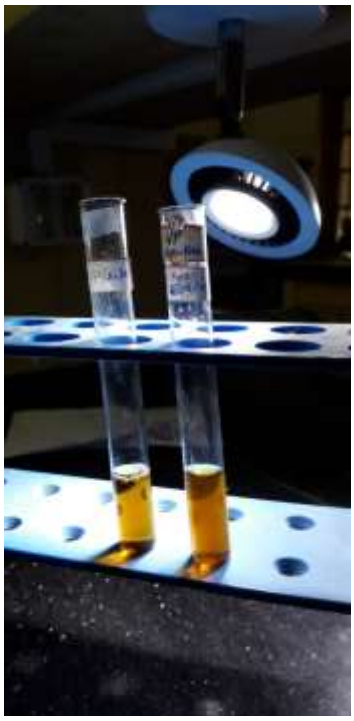

FIG:VOGES PROSKAUR TEST

FIG:CITRATE PRODUCTION TEST

FIG: METHYL RED PRODUCTION TEST

Conclusion-

Hence the microorganism isolated from the air may be *Bacillus sp.*

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME-SAYAK DAS
YEAR-3rd year
ROLL-369

PROJECT TOPIC-ISOLATION AND CHARACTERISATION OF AIRBORNE MICROORGANISM

PROJECT GUIDE-ARINDAM ROY and CHANDAN RAI

Isolation and Characterization of Air-borne Microorganism

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Comment [WU1]: ?

				produced.
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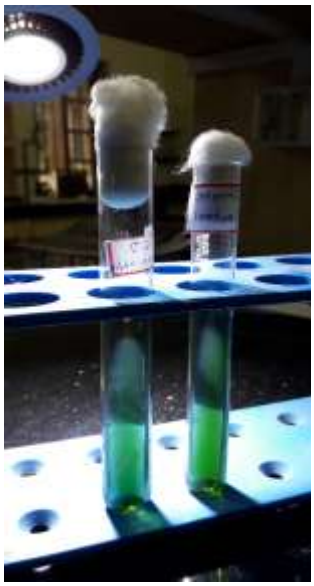
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H2O2	Positive
8	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

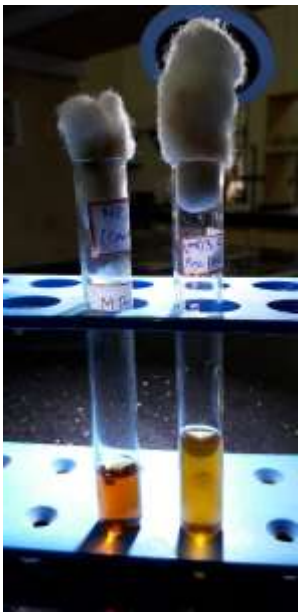
Comment [WU2]: ?

Pic:purification by streak plate method

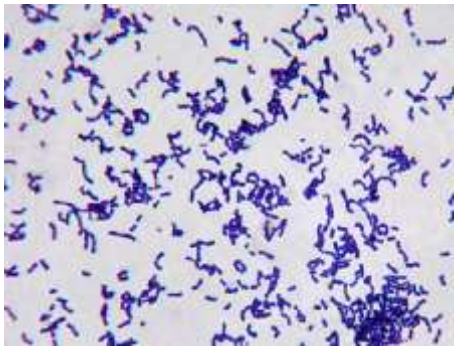
Pic:VP test



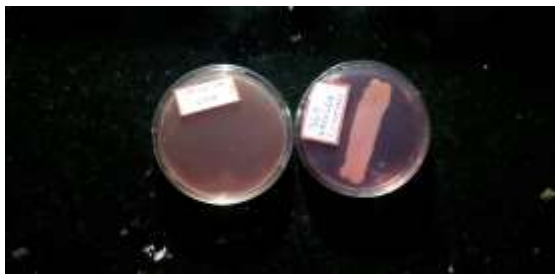
Pic:Citrate test



Pic:MR test



Pic:Gram staining technique



Pic:Urease test

Pic:Catalase test

Conclusion: Hence the microorganism isolated from air may be *Bacillus* sp.

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: SAYAN PAL

ROLL NO. : 372

DEPARTMENT: MICROBIOLOGY

YEAR: UG-3(2017-2020)

PROJECT TOPIC: Isolation and characterization of airborne microorganism.

GUIDED BY: Arindam Roy and Chandan Rai.

ACKNOWLEDGEMENTS:

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➢ Methodology:

✓ STAINING:

❖ **Gram Staining**:

The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolorized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple colour. Counter stain, which is usually positively-charged safranin, is applied last to give decolorized Gram-negative bacteria a pink or red colour.

❖ **Endospore staining:**

The endospore stain is a differential stain used to visualize bacterial endospore. Endospores are formed by a few genera of bacteria, such as Bacillus. By forming spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.

A primary stain (malachite green) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green permeate the spore wall) the endospores by heating. In this technique heating acts as a mordant. Endospores and vegetative cells are visualized as green and red colour respectively.

- ✓ **BIOCHEMICAL TESTS:**

❖ **IMViC tests:**

- **Indole test:**

Some bacteria split amino acid tryptophan into indole and pyruvic acid using the enzyme called tryptophanase. Indole can be detected with Kovac's reagent. Along with differentiation of enterics, Indole test can also be used for species differentiation. A positive result is shown by the presence of a red or red-violet colour in the surface alcohol layer of the broth. A negative result appears yellow.

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The methyl red test (MR test) is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the MR test. A negative MR test is indicated by a yellow color in the culture medium, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

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The Voges-Proskauer test is to determine the ability of some microorganisms to produce a neutral end product 2,3 butanediol from glucose fermentation. A cherry red colour indicates a positive result, while a yellow-brown colour indicates a negative result on the addition of Barrett's (reagent a and b) to the overnight cultured bacterial sample.

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hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

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The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. Catalase positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

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Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

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B. Cultural characteristics: Round, small, convex.
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E. Endospore staining: after 4-5 days starvation, endospore will not form.
F. Capsule staining: No capsule observed around cells.

Comment [WU1]: ?

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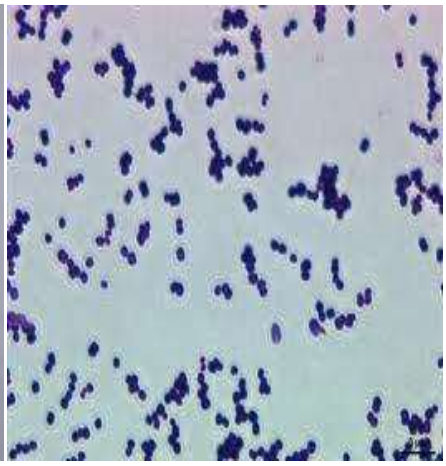
2	Endospore		After 4-5 days starvation, endospore will form.	positive
3	capsule		No result found	negative
4	H_2S production	TSI medium	Absence of extensive blacking in the butt of the TSI medium	negative
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a) Development of a deep rose colour in the culture 15 mins following the addition of Barrett's reagent. (b) Same result with the control sample. (c) Absence of red colourization in the tryptophan broth after adding kovac's reagent. (d) Blue coloration appears on the surface on the slant.	(a)positive (b)negative (c)negative (d)positive
6	Urease activity test	Urea agar	Same result with the control sample	Negative
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H_2O_2	Positive
8	Gelatin liquefaction	Gelatin agar	Gelatin hydrolysis	positive
9	Starch hydrolysis	Starch agar plate	Same result with the control	negative

Comment [WU2]: ?

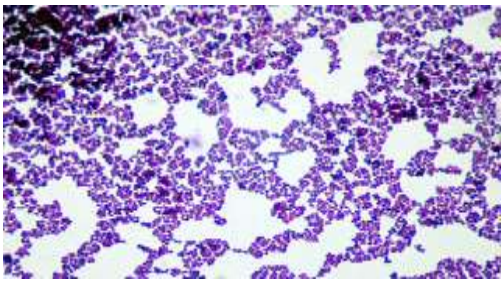
Comment [WU3]: ?

Comment [WU4]: ?

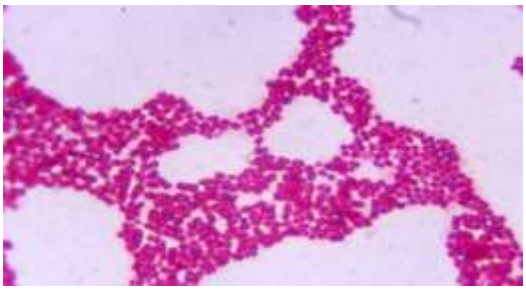
1.MAIN PLATE



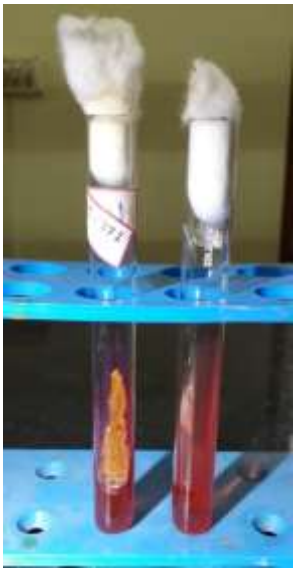
2.GRAM STAINING



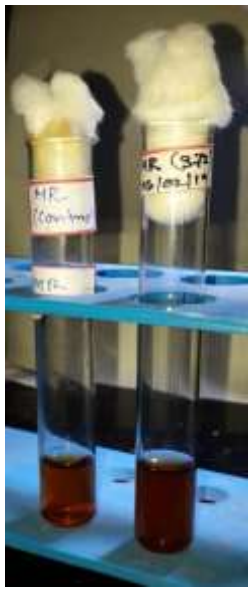
3. ENDOSPORE STAINING



4. CAPSULE STAINING

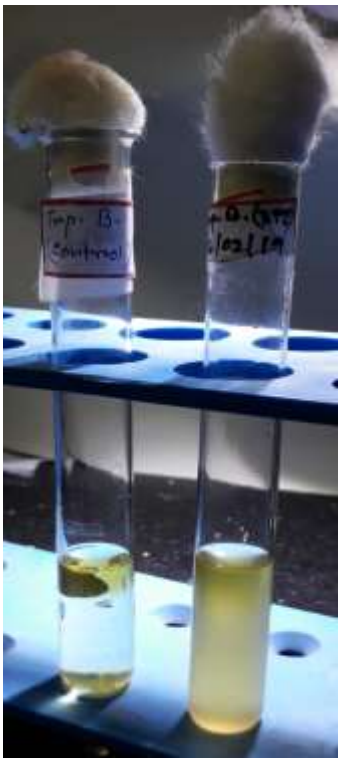


4. H2S PRODUCTION TEST

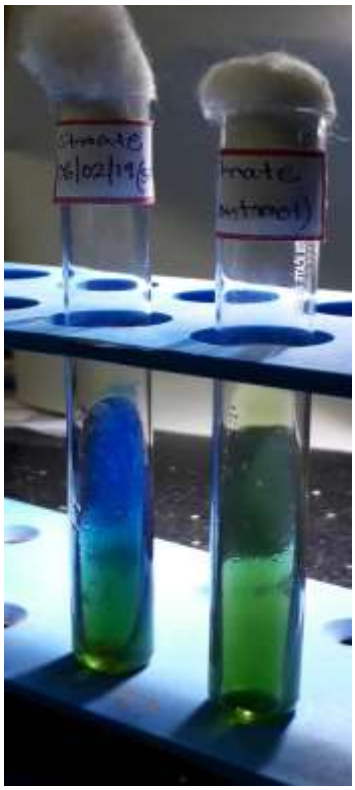


5(a).MR TEST

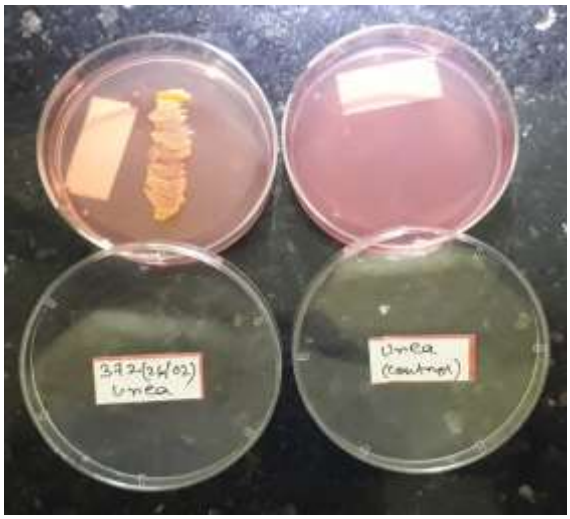
5(b).VP TEST



5(c).INDOLE TEST

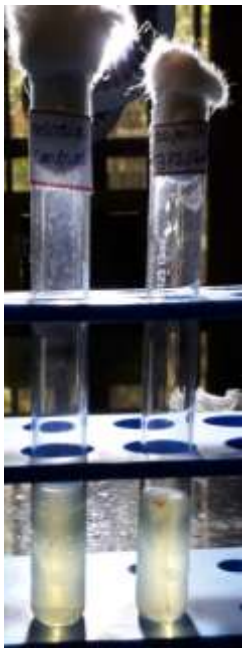


5(d).CITRATE UTILIZATION TEST



6. UREASE TEST

7. CATALASE TEST



8. GELATIN UTILIZATION TEST

9. STARCH UTILIZATION TEST

➢ Conclusion:

From the above result we can conclude that the bacteria we obtain from air exposure method it may be *Micrococcus sp.*

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: **SAYANDIP SANTRA**
ROLL NO. : **275**
DEPARTMENT: MICROBIOLOGY
YEAR: UG-3

PROJECT TOPIC: **Isolation and characterization of airborne microorganism**

GUIDED BY: Asst.Prof. Dr. Arindam Roy and Asst.Prof. Chandan Rai

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d) Citrate test:

To determine the ability of an organism to utilize citrate as sole carbon and energy source for growth and an ammonium salt as the sole source of nitrogen.
Citrate is a salt of citric acid, one of the metabolites in the Krebs cycle. Bacteria can use citrate can also extract nitrogen from the ammonium salt with the production of ammonia. Leading to alkalinisation of the medium from conversion of the NH_3^{2+} to NH_4OH.

2. Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease

plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

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Biochemical Tests (summary)

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2	Endospore		After 4-5 days starvation, endospore will form.	positive
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4	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)same result with the control sample (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d) no significant colour change	(a) negative (b)negative (c)negative (d)negative
5	Urease activity test	Urea agar	Media colour become pink	Positive
6	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H2O2	Positive
7	Gelatin liquefaction	Gelatin agar	No significant colour change	Negative

Comment [WU1]: Green coloured oval spore inside red coloured cell

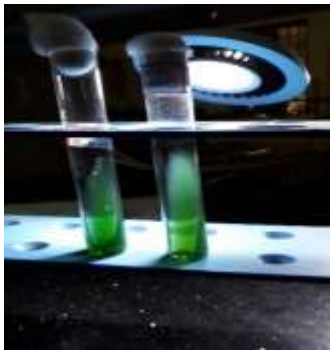
Comment [WU2]: Change as mentioned

Comment [WU3]: No liquefaction

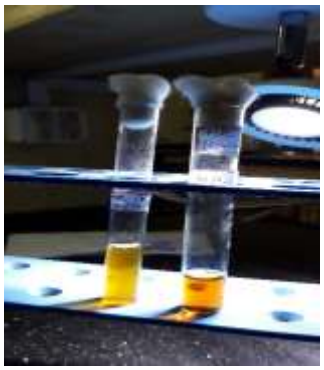
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

VP test

Indol test

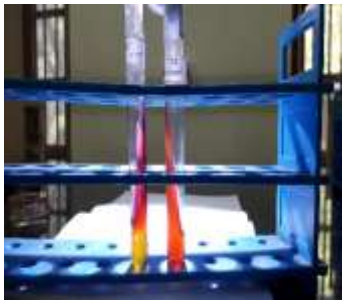


Citrate test

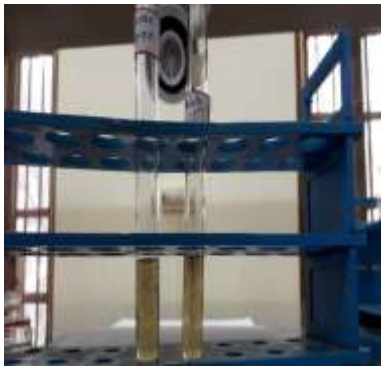


MR test

Urease test



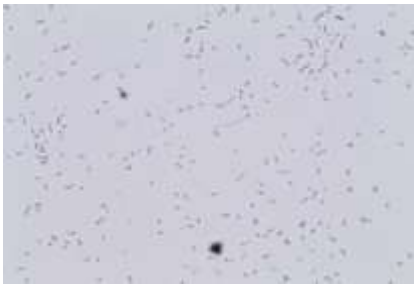
TSI test



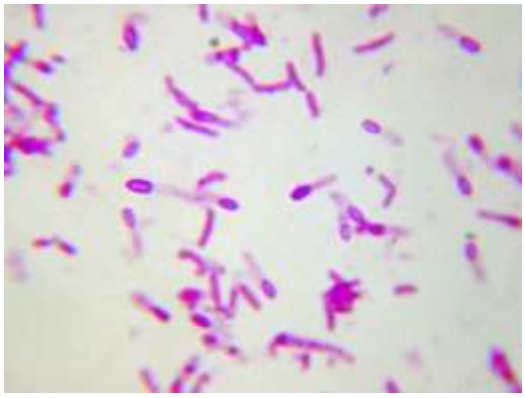
Gelatinase test

Catalase test

Starch test



Gram staining (gram positive, rod)



Endospore staining (positive)

Conclusion:

From the above result we can conclude that, the bacteria which are obtained from the air exposure method may be *Bacillus sp.*

RAMAKRISHNA MISSION VIDYAMANDIRA
BELUR MATH, HOWRAH

NAME: SAYANTAN GHOSH
ROLL NO. : 363
DEPARTMENT:MICROBIOLOGY
YEAR:UG-3
PROJECT TOPIC:Isolation and characterization of airborne microorganism
GUIDED BY: Arindam Roy and ChandanRai

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Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

Gelatin production test:

This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinases that hydrolyze gelatin.The reaction occurs in two sequential steps: in first reaction gelatinases hydrolyze gelatin into polypeptides and then polypeptides are further converted into amino acids .

Positive: Partial or complete liquefaction of the inoculated tube at 4°C. The control tube remain solidified even after exposure to cold temperature.

Negative: At the end of the refrigeration, the control tube and the test tube both remain completely solidified.

TSI:

To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.

An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.

An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.

Blackening of the medium: Occurs in the presence of H2

Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture

Shape of arrangement : Rod
Cultural characteristics : Round,small,convex
Cell arrangement : single cell arrangement
Gram staining : positive
Endospore staining : after 4-5 days starvation, endospore will not form..
Capsule staining : No capsule observed around cells.

Biochemical Tests (summary)

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	positive
2	Endospore		After 4-5 days starvation,endospore will form.	positive
3	capsule		No result found	negetive
4	H_2S production	TSI medium	Absence of extensuve blacking in the bult of the TSI medium	negetive
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)develpoment of a deep rose colour in the culture 15 mins following the addition of Barritt's reagent. (b)same result with the control sample. (c)absence of red colourization in the tryptophan broth after adding kovac's reagent. (d)blue coloration appears on the surface on the slant.	(a)positive (b)negetive (c)negetive (d)positive
6	Urease activity test	Urea agar	Same result with the control sample	Negative
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H_2O_2	Positive
8	Gelatin liquefaction	Gelatin agar	Gelatin hydrolysis	positive
9	Starch hydrolysis	Starch agar plate	Same result with the control	negetive

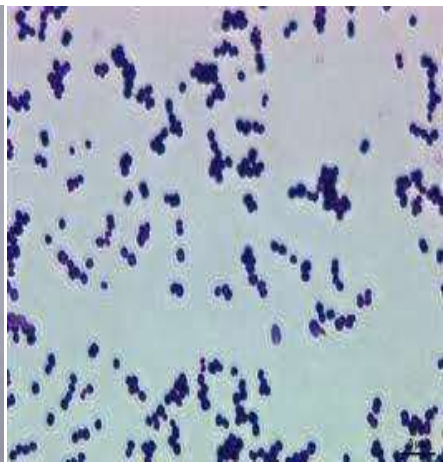
Comment [WU1]: ?

Comment [WU2]: ?

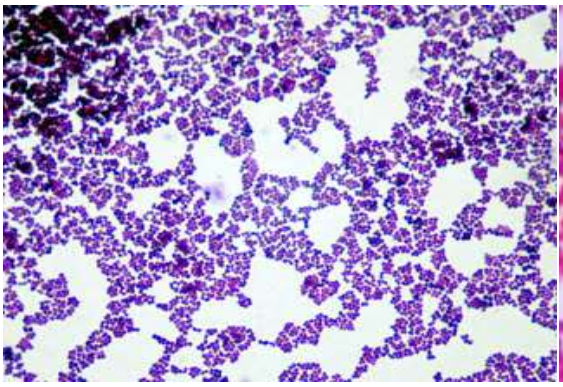
Comment [WU3]: ?

Comment [WU4]: ?

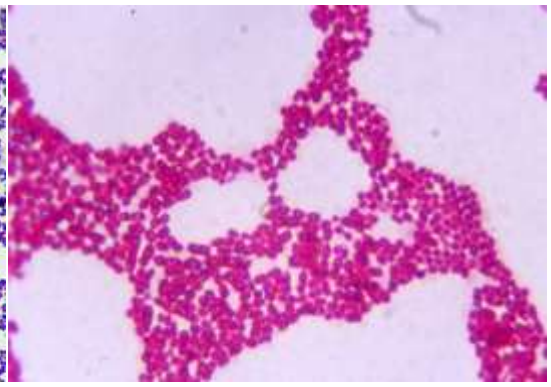
1.MAIN PLATE



2.GRAM STAINING

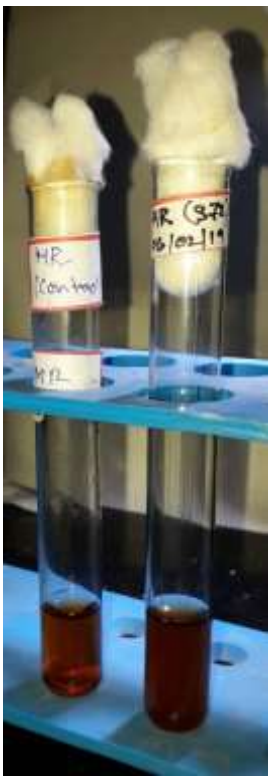


3. ENDOSPORE STAINING



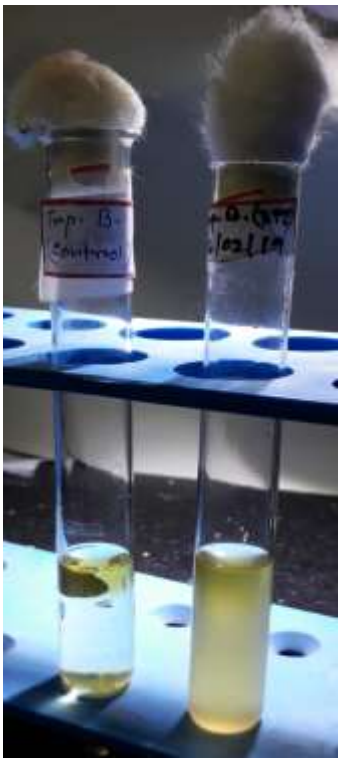
4. CAPSULE STAINING

4. H2S PRODUCTION TEST

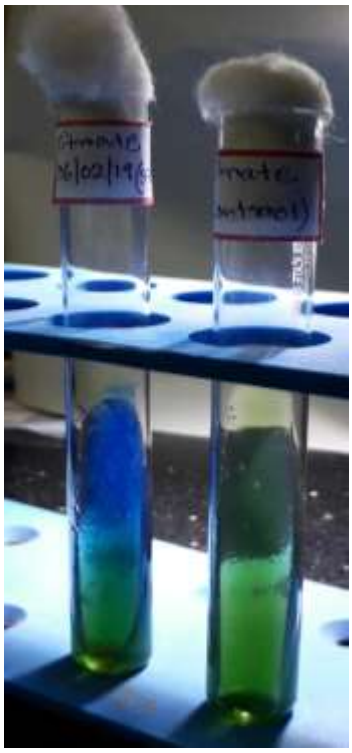


5(a).MR TEST

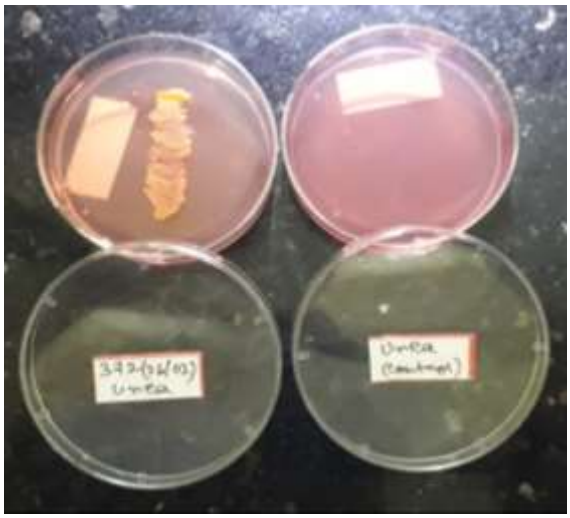
5(b).VP TEST



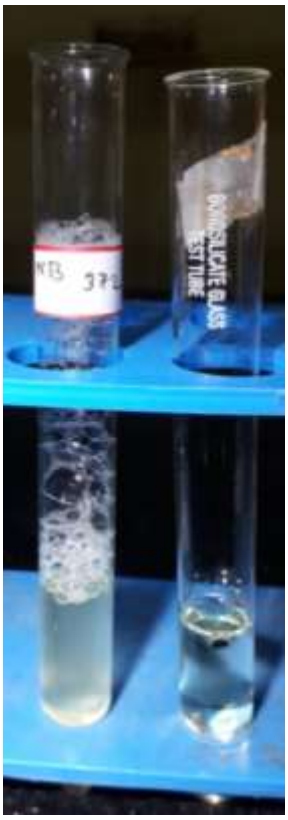
5(c).INDOLE TEST



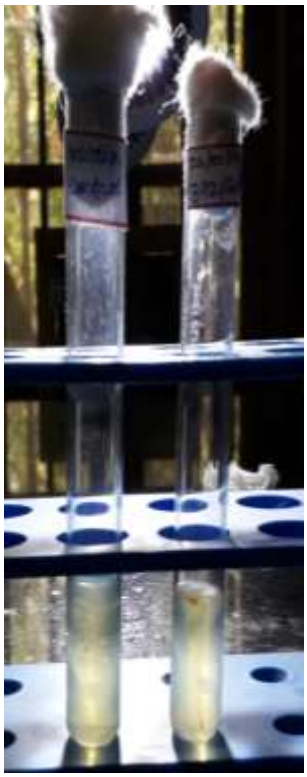
5(d).CITRATE UTILIZATION TEST



6. UREASE TEST



7. CATALASE TEST



8. GELATIN UTILIZATION TEST

9. STARCH UTILIZATION TEST

Conclusion :

From the above result we can conclude that the bacteria we obtain from air exposure method it may be *Micrococcus sp.*

Comment [WU5]: Endospore forming?

RAMAKRISHNA MISSION VIDYAMANDIRA

BELUR MATH

NAME- **SRIJON MUNSHI**

ROLL-**370**

YEAR- **B.Sc. 3rd year(2017-20)**

PROJECT TOPIC- **ISOLATION AND CHARACTERIZATION OF AIRBORNE MICROORGANISM**

PROJECT GUIDE- **PROF. ARINDAM ROY and PROF. CHANDAN RAI**

DEPARTMENT OF MICROBIOLOGY

Isolation and Characterization of Airborne Microorganism

Acknowledgements:

I would like to express my hearty gratitude to Prof. Arindam Roy and Prof. Chandan Rai for their guidance. I would also like to thank my all departmental faculty members and authorities of Ramakrishna Mission Vidyamandira for providing me this learning opportunity.

Last but not the least; I am always thankful to my family and my lab members for motivating and supporting me in every possible way.

INTRODUCTION:

Microbes are ubiquitously present in every living and non-living matter and can survive galore in the air, water and even in all the extremes of the environment. Isolation of microbes present in a particular site requires specific isolation culture medium. Microorganisms in air are present in spore form and can be cultured by nutrient agar plating in open air exposure. In this project, we collected air microbes by agar plating in different sites in the building with different exposure time for different agar plates. The isolated microbes are first cultured,then purified and further subcultured to study their morphological characteristics followed by biochemical assays. The experiments performed to study the morphological characteristics are gram staining, endospore staining and biochemical assays performed to study the biochemical activities are Indole production test, methyl red test, Voges-Prauskaur test, Citrase activity test, Urease production test, Gelatinase production test, TSI agar test, starch test. All the tests performed show either positive or negative results by the color change of the respective media. If there occur any change of the color of the respective media, the test can be considered positive and if not, it can be considered negative. Each and every experiment here follows different principle as well as procedure, even the detailed layout of the microbial activity in the respective medium. The experiments also tell about the interaction between microbial exoenzymes and there cognate or non-cognate substrates present in the medium. Not only that, they also tell surely about the activity of the microbial community to produce either the respective exoenzymes or not.

Methodology:

Gram Staining:

The structure of the organism's cell wall determines whether the organism is gram positive or negative. When stained with a primary stain and fixed by a mordant, some bacteria are able to retain the primary stain by resisting decolourization while others get decolorized by a decolourizer. After decolourization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively-charged **safranin**, is applied last to give decolorized Gram-negative bacteria a pink or red color.

Endospore staining:

The endospore stain is a differential stain used to visualize bacterial endospores. Endospores are formed by a few genera of bacteria, such as Bacillus. By forming spores, bacteria can survive in hostile conditions. Spores are resistant to heat, desiccation, chemicals, and radiation.

A primary stain (malachite green) is used to stain the endospores. Because endospores resist staining, the malachite green will be forced into (i.e., malachite green permeate the spore wall) the endospores by heating. In this technique heating acts as a mordant. Endospores and vegetative cells are visualized as green and red color respectively.

IMViC tests:

Indole test:

Some bacteria split amino acid tryptophan into indole and pyruvic acid using the enzyme called tryptophanase. Indole can be detected with Kovac's reagent. Along with differentiation of enterics, Indole test can also be used for species differentiation. A positive result is shown by the presence of a red or red-violet color in the surface alcohol layer of the broth. A negative result appears yellow.

Methyl red test:

The methyl red test (MR test) is used to identify bacteria producing stable acids by mechanisms of mixed acid fermentation of glucose. When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the MR test. A negative MR test is indicated by a yellow color in the culture medium, which occurs when less acid is produced (pH is higher) from the fermentation of glucose.

Voges-Proskauer test:

The Voges-Proskauer test is to determine the ability of some microorganisms to produce a neutral end product 2,3 butanediol from glucose fermentation. A cherry red color indicates a positive result, while a yellow-brown color indicates a negative result on the addition of barritt's (reagent a and b) to the overnight cultured bacterial sample.

OTHER BIOCHEMICAL TESTS

Urease test: Urease test is a procedure used to find out the organism's ability to split urea by producing an enzyme urease. The test is performed using urease plate. The colour of the plate changes from light orange to pink if the organism being tested is positive for urease. On the other hand, the colour of the slant remains the same (light orange) if the organism being tested didn't produce urease enzyme.

Catalase test

The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. Catalase positive reaction: Evident by immediate effervescence (bubble formation) Catalase negative reaction: No bubble formation (no catalase enzyme to hydrolyse the hydrogen peroxide)

Starch hydrolysis

Starch agar is a differential medium that tests the ability of an organism to produce certain exoenzymes, including a-amylase and oligo-1,6-glucosidase, that hydrolyse starch. Starch agar is a simple nutritive medium with starch added. Since no colour change occurs in the medium when organisms hydrolyse starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolysed starch

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This test is used to determine the ability of an organism to produce extracellular proteolytic enzymes, gelatinases that hydrolyze gelatin.The reaction occurs in two sequential steps: in first reaction gelatinases hydrolyze gelatin into polypeptides and then polypeptides are further converted into amino acids.

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To determine the ability of an organism to ferment glucose, lactose, and sucrose, and their ability to produce hydrogen sulfide.

- An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.
- An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.
- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.
- Blackening of the medium: Occurs in the presence of H2
- Gas production: Bubbles or cracks in the agar indicate the production of gas (formation of CO2and H2)

Description of unknown bacteria culture

Shape : Rod
Cultural characteristics: Round,small,convex, white and translucent
Cell arrangement: single cell arrangement mostly
Gram staining: positive
Endospore staining: after 4-5 days starvation,endospore will not form.
Biochemical Tests ;

Sl no.	Name of Experiment	Culture media	Observation	Result
1	Gram stain		Cells retain the purple colour of primary stain	Positive
2	Endospore		After 4-5 days starvation, endospore will not form.	Negative
3	Capsule		----------	----------
4	H2S production	TSI medium	(a)yellow butt (b)no change in slant	Glucose fermentation has occurred.H2S not

Comment [WU1]: ?

				produced.
5	IMViC test (a)MR test (b)VP test (c)Indole production (d)Citrate utilization	(a)MR-VP (b)MR-VP (c)Trp broth (d)citrate agar	(a)same result with the control sample (b)same result with the control sample. (c)presence of red colourization in the tryptophan broth after adding kovac's reagent. (d)significant colour changeobserved.	(a) negative (b)negative (c)positive (d)positive
6	Urease activity test	Urea agar	Medium colour becomes pink	Positive
7	Catalase activity test	Nutrient agar	Air bubbles forms on addition of H2O2	Positive
8	Gelatine liquefaction	Gelatine agar	No hydrolysis of gelatine	Negative
9	Starch hydrolysis	Starch agar plate	Clear zone forms surrounding streak line on addition of iodine solution	Positive

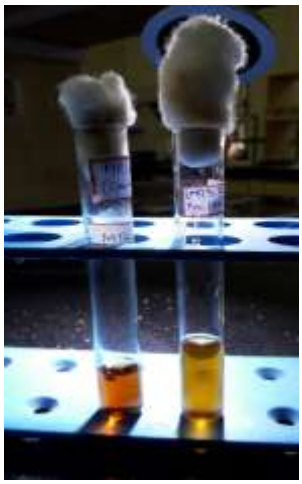
Comment [WU2]: ?

Comment [WU3]: ?

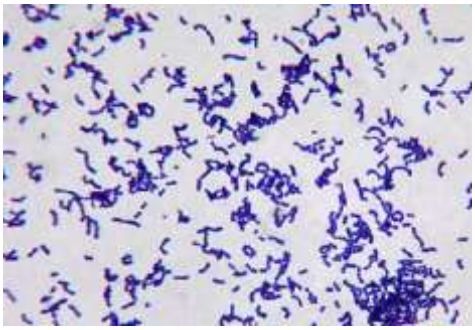
Pic: purification by streak plate method

Pic: VP test

Pic: TSI test



Pic: MR test



Pic : Gram staining

Pic: Catalase test

Pic: Urease test

Pic: Gelatinase test

Conclusion: Hence the microorganism isolated from air may be ***Bacillus*** sp.