

STUDENT STUDY PROJECT

ON

ISOLATION AND IDENTIFICATION OF MICROORGANISMS PRESENT IN DAIRY PRODUCTS AND ANTIMICROBIAL ACTIVITY OF LACTOBACILLUS AGAINST PATHOGENIC BACTERIA

Department of Microbiology

Dr.BRR Government College, Jadcherla

Mahabubnagar – 509001



Accredited by NAAC with "B⁺⁺" Grade//An ISO 9001-
2015 Institution

Mahabubnagar (DIST), Telangana state, India-509301

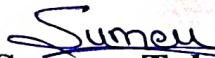
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CERTIFICATE

This is to certify that the project work entitled "Isolation And Identification of microorganisms Present in Dairy Products And Antimicrobial Activity of Lactobacillus Against Pathogenic Bacteria "Jadcherla, Mahabubnagar District, and Telangana. "is a bonafide work done by the students of III MZC (EM) Miss J.J.Lidya Rose, Miss.M.Deepika, Mr.K.Raghavendar, Mr.M.Shiva, Mr.M.Tharun under my supervision for the award of Project Work in Microbiology, Department of Microbiology, Dr. BRR Government College, Jadcherla and the work hasn't been submitted to any other College/University either in part nor in full, for the award of any degree.


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DECLARATION

We hereby declare that the project work entitled with "Isolation and Identification of Micro Organisms present in dairy products and anti microbial activity of lactobacillus against pathogenic bacteria . Jadcherla, Mahabubnagar District, and Telangana". "Is a genuine work done by us under the supervision of K.Neeraja, for the Department of Microbiology, Dr. BRR Government College, and it has not been under the submission to any other Institute/University either in part or in full, for the award of any degree.

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ABSTRACT

Isolation identification of pathogenic bacteria present of dairy products like curd butter milk Lassi. In the processes of making diary products like Curd ,Lassi,Buttermilk they use water, Water contain pathogenic bacteria such as Salmonella and E-coli it may cause disease to human beings. Lactobacillus may have ability to fight against the pathogenic bacteria .We collected the samples from different areas of Jadcherla, Mahabubnagar dist and isolated for lactobacillus. Identified lactobacillus ,Biochemical tests or done to check the lactobacillus had production of Biochemical ,finally we done antimicrobial test against salmonella and E-coli.

INTRODUCTION

INTRODUCTION

Now a days everyone using dairy products for there daily needs like [Curd,Buttermilk,Lassi] etc...In the process of making local dairy products they use more amount of water which contain pathogenic bacteria it may cause so many food bond diseases like fever illness so, our aim is to collect the samples from some places in jadcherla. Isolate the lactobacillus from samples. Antimicrobial test were done to each and every sample against salmonella and E-coli these are the major and common bacteria which may cause illness for our body if lactobacillus can resist the salmonella and E-coli the diary products are good for our human body.

OBJECTIVES

- Isolation and identification of Lactobacillus
- Identification of Lactobacillus
- Antimicrobial test
- Biochemical tests for Lactobacillus cultures

**REVIEW
OF
LITERATURE**

REVIEW OF LITERATURE

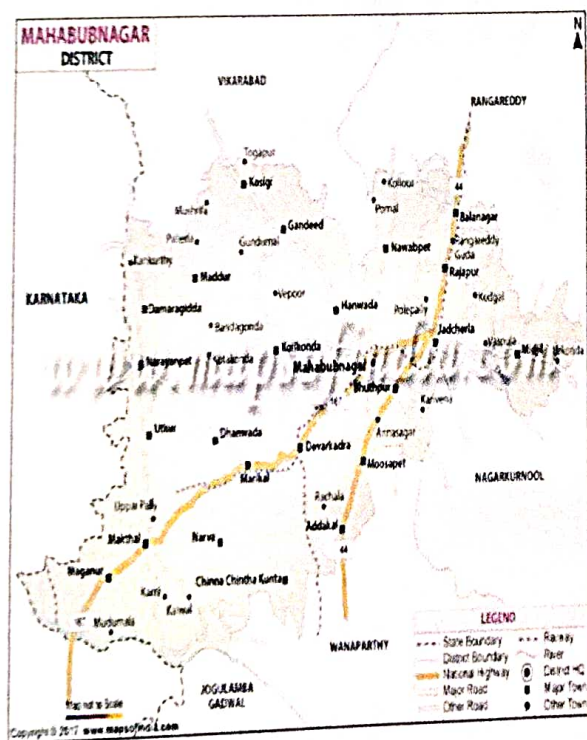
- Microbial diversity and functionality of traditional fermented milk products of India: Current scenario and future perspectives
- RH Mallappa, C Balasubramaniam, BH Nataraj... - International Dairy ..., 2021 Elsevier
- 'Lactic Acid Bacteria: Probiotic Characteristic, Selection Criteria, and its Role in Human Health (A Review)'
- R Gupta, K Jeevaratnam, A Fatima - ... Fatima. Lactic Acid Bacteria ..., 2018
- Isolation of exopolysaccharides producing lactic acid bacteria from dairy products
- P Patil, A Wadehra, K Munjal... - Asian Journal of Dairy ..., 2015
- Isolation and identification of coliform bacteria from spicy yogurt milk and plain butter milk sold in different markets of Dhaka CityA Azam - 2017

STUDY AREA

STUDY AREA

Jadcherla is a census town in Mahabubnagar district of the Indian state of Telangana. It is located in Jadcherla mandal in Mahabubnagar revenue division. In 2011, it was upgraded from village to a census town, along with 11 other villages. It is a historical town and is known for its cultural heritage. Recently Jadcherla has been made a Municipality. Common crops in Jadcherla locality are cotton (*Gossypium*) chili (*capsicum Annuum*) cluster bean (*Cyamopsis tetragonoloba*). We selected cluster bean (*Cyamopsis tetragonoloba*) for our project.

Jadcherla is located at 16.7738°N 78.1367°E and at an altitude of 14 m (46 ft). The town is spread over an area of 550 km² (210 sq mi). Jadcherla is located 86 km from Hyderabad 130 km from Kurnool and 21 km from Mahabubnagar. There exist some of the historical religious structures. The Hindu temples include 12th century Chennakeshava temple, Anjaneya temple, Maisamma temple, Parushaveri temple and Ranganayaka temple. The Jain shrine also exists by the name Gollatha Gudi. Other notable landmarks of the town are Nachiketa Tapovanam, Sitammajalu waterfall, Mayuri nursery etc



**METHOD
AND
MATERIALS**

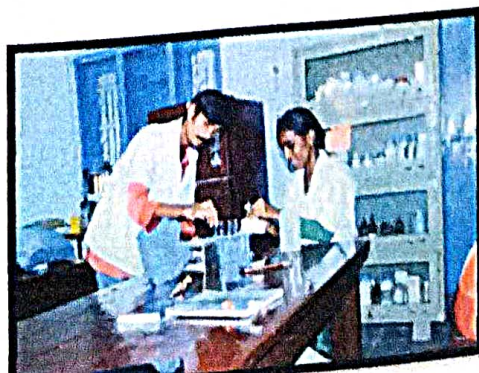
COLLECTION OF CURD, BUTTER MILK, LASSI SAMPLES:-

- Collection of samples from different areas locality of Jadcherla Mahabubnagar district
- To collect the samples we use sterile Capped Test Tubes and sterile gloves
- We collect samples from different Dairies and juice point in locality of Jadcherla
- We collected Samples from Different Areas of Jadcherla
- We took some photos from gps camera while collecting the samples from different shops
- Over all 15 samples are collected
- All the samples are isolated
- We did serial dilution for each sample
- For each serial dilution we have taken 10 test tubes
- First tube has dilution of sample it is 10
- Inoculate on the plates for 4 to 7 days at 37°C



Serial dilution :

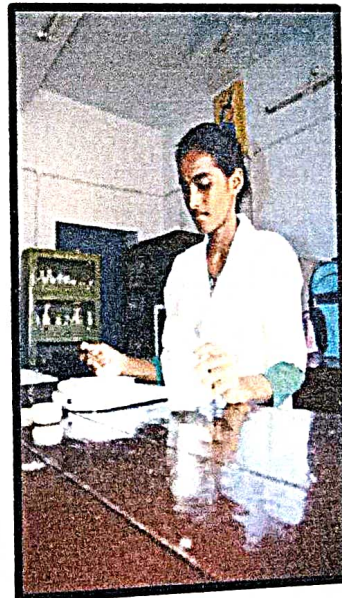
- Serial dilution method for estimating viable count of bacteria
- Prepare ten test tubes and pipette out the water in tubes .
- Pipette out the 10ml of water in 10^{-1} tube . 10^{-2} - 10^{-10} add 9ml of water .
- Sterile the tubes by autoclave and cottonplugs to cover the tubes .
- Sterilize for 15min at 121°c
- Remove the tubes using heat- resistant gloves and allow to cool . when tubes reached to room temperature.
- Add the collected 1ml sample in 10^{-1} tube
- Pipette out 1ml of solution in to 10^{-2} test tube
- Pipette out 1ml of solution in to 10^{-3} test tube
- Pipette out 1ml of solution in to 10^{-4} test tube
- Pipette out 1ml of solution in to 10^{-5} test tube
- Pipette out 1ml of solution in to 10^{-6} test tube
- Pipette out 1ml of solution in to 10^{-7} test tube
- Pipette out 1ml of solution in to 10^{-8} test tube
- Pipette out 1ml of solution in to 10^{-9} test tube
- Pipette out 1ml of solution in to 10^{-10} test tube



METHOD AND MATERIALS

Isolation of Lactobacillus from Curd, Butter milk & Lassi

- Isolation of Lactobacillus from collected samples by using De Man, Rogosa & Sharpe Agar (MRS Agar).
- Preparation of MRS Agar Medium
- Prepare 500ml of MRS Agar medium
- Autoclave the MRS Agar medium for 15 min at 121°C 15 PSI
- Autoclave the wrapped petri plates along with media.
- Remove the media and plates after sterilization using heat resistant gloves.
- Pour the media in to plates allow to solidify it.
- Streak the samples on plates incubate at 30-35°C for 24 hours.



Identification of lactobacillus,

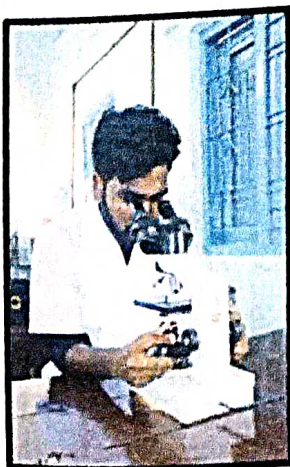
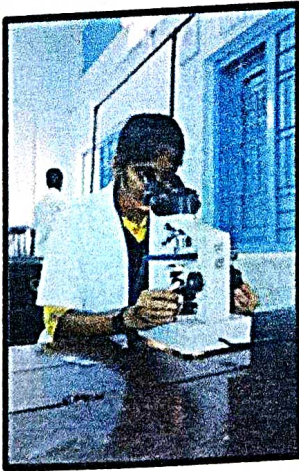
- Identification of lactobacillus by Gram staining

Gram staining

- Gram staining is the common important techniques in microbiology. This test differentiate the bacteria in to Gram positive and Gram negative bacteria, which helps in the classification and differentiation s of microorganisms.
- Procedure of Gram Staining Take a clean, grease free slide.
- Prepare the smear of suspension on the clean slide with a loop of sample.

Air dry and heat fix

- Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
- Flood the gram's iodine for 1 minute and wash with water.
- Then, wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
- Add saffanine for about 1 minute and wash with water. Air dry, Blot dry and Observe under Microscope.





Biochemical Test:

Test for Indole Formation (Indole Test)

Aim:

To identify the metabolic formation and accumulation of indole by test bacterial culture

Requirements :

Indole test medium broth tubes Kovac's reagent

Test organism Other routine lab requirements

Procedure :

- Prepare actively growing cell suspension of test organism in broth or saline
- inoculate into indole test broth tubes) and incubate at optimum temperature for 48-96 hrs .
- Add 0.5 ml of Kovac's reagent to fermented tube(s) and shake gently.
- Formation of red color in the alcohol layer indicates positive for indole test
- Observe the tubes and result.

Methyl Red Test

Requirements :

Glucose phosphate peptone water tubes Test bacterial culture

Procedure :

- Methyl red indicator solution Other routine lab requirements
- Prepare suspension culture of actively growing bacterial test organism.
- Inoculate into glucose phosphate peptone water tube) and incubate for 48 hrs at optimum growth conditions
- Add about 5 drops of methyl-red indicator solution to each tube, mix and observe immediately
- Bright red color indicates positive test for methyl red and yellow color indicates negative

Test for Catalase Production :

Requirments :

Actively growing culture in a slant

Hydorgen peroxide liquid commercially available

Other routine lab requirements

Procedure :

- Make a thick suspension of agar grown culture in broth that either or slide or in tubes
- Add a few drops of hydrogen peroxide.
- Observe evolution of bubbles indicating formation of oxygen from hydrogen peroxide as a result of catylaseaction

Test for Urease Activity

Requirments :

Urease testing medium (chrislensen's medium) slants

Test culture

Other routine lab requirements

Procedure :

- Inoculate the actively growing test culture heavily throughout the ager slant surface
- Incubate for growth at optimum conditions
- Observe for change in color of medium to purple-pink from 4hrs 4 days

Change color is due to Urease activity rating in formation of NH_2

Test for Citrate Utilization

Requirements :

Koser's liquid citrate medium (ph6.8)

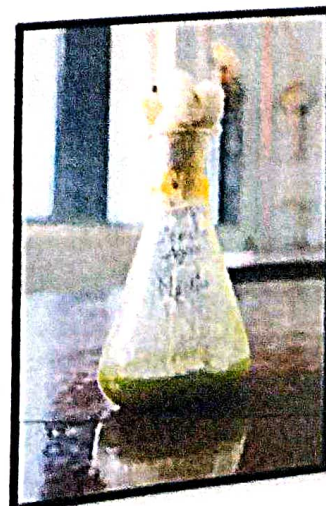
Bromothymol blue indicator solution

Simmon's citrate (ph6.8)

Test culture

Procedure :

- Prepare a saline suspension of actively growing test organism
- Inoculate into Koser's liquid citrate medium tubes and Simmons citrate agar slants
- Observe for turbidity [growth] in Koser's medium and change of color [from lightgreen to blue] in Simmons citrate agar slants which indicate positive for citrate utilization
- A subculture may be done in the same media to eliminate false positives
- Identify the given culture for its citrate utilization based on the test results



Antimicrobial Test:-

Requirements

- Pure cultures of lactobacillus
- E-coli and Salmonella Bacterial cultures
- Cotton swabs
- Straws
- Pipette
- Other lab requirements

Procedure


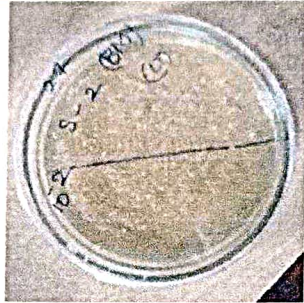
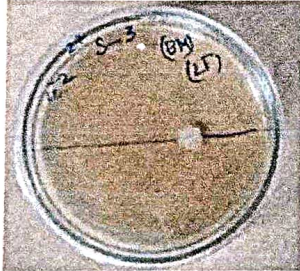


- Antimicrobial test for resistance against pathogenic bacteria by lactobacillus
- The purpose of antimicrobial test is lactobacillus have ability to resist the pathogenic bacteria (salmonella and E-coli)
- Prepare nutrient agar medium for test sterilize the agar medium
- Pour the media in to plates leave it for solidify.
- Spread the two bacterial cultures with the help of cotton swabs it helps for thin layer of growth formation on plate.
- Make holes on media with the help of straws, pipette of the lactobacillus bacterial culture in holes.
- Incubate the plates for 24hours at 30-35 c

RESULTS


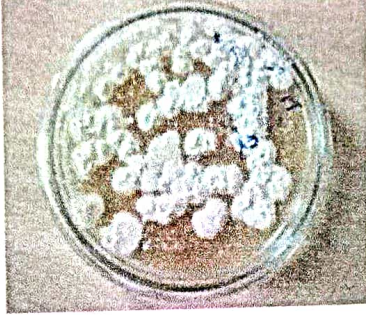
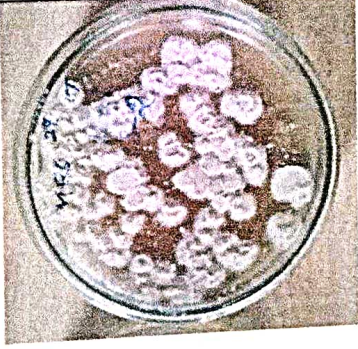


Results:

Tables:

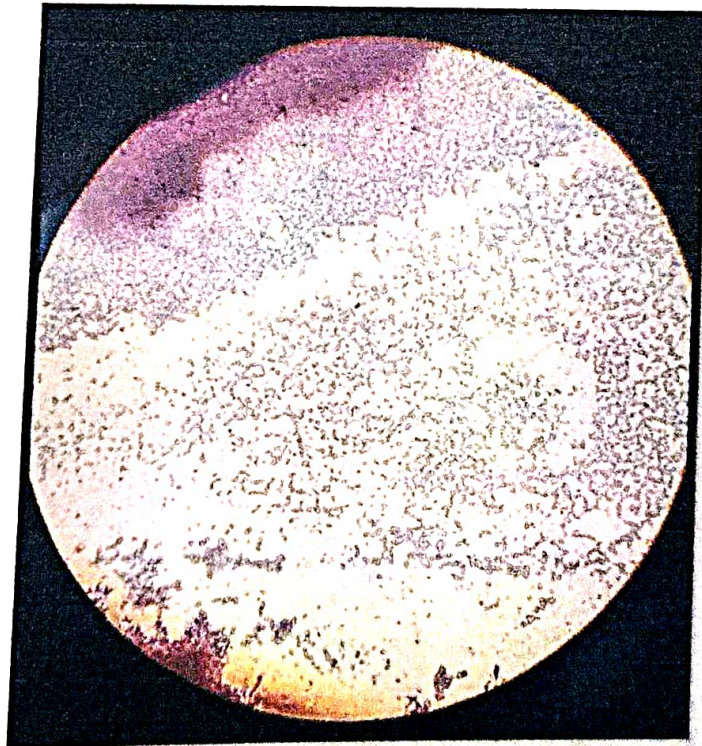
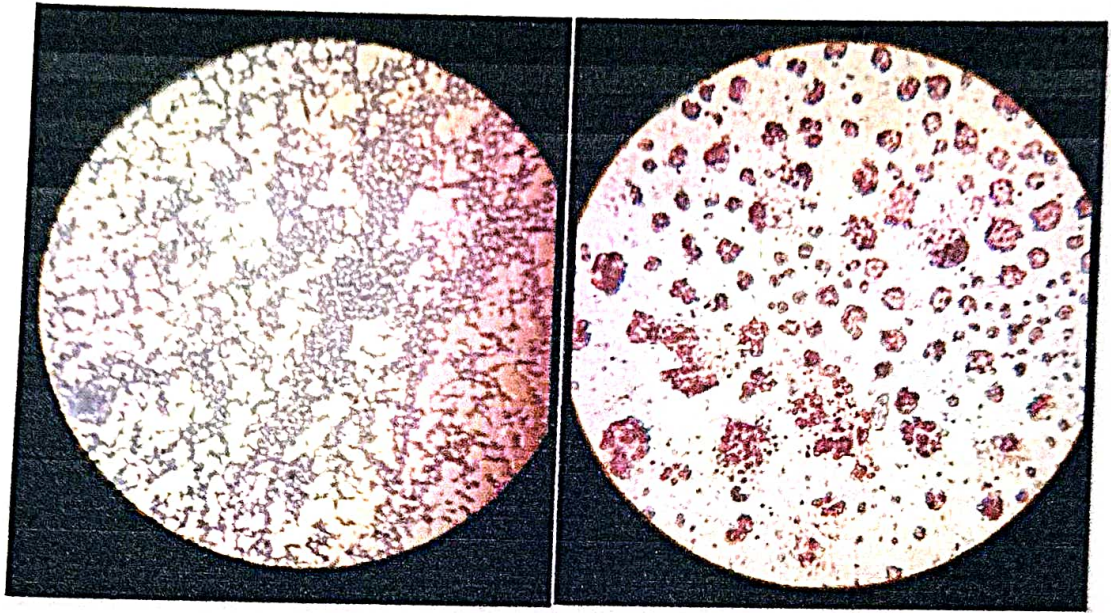
CURD	
Sample No	Isolation Plate
101	
102	
103	
104	
105	

BUTTERMILK	
Sample No	Isolation Plate
201	
202	
203	
204	
205	

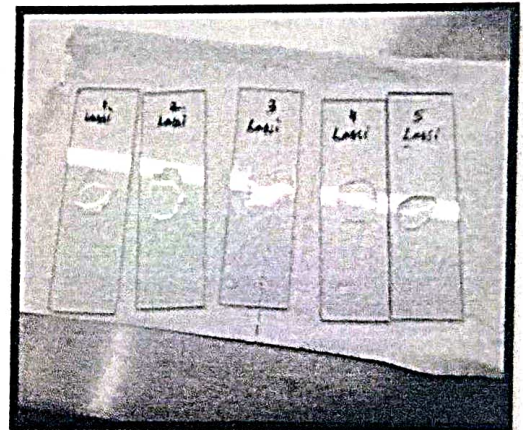
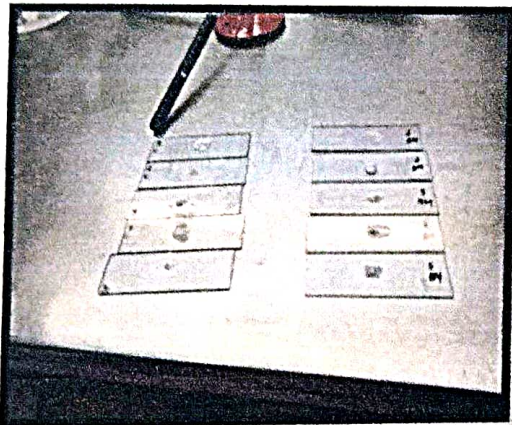
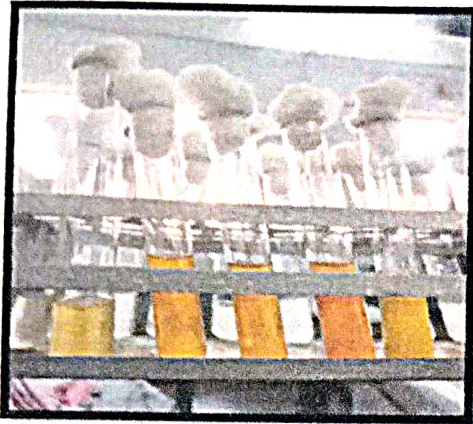
LASSI

Sample No	Isolation Plate
101	
102	
103	
104	
105	

Gram Staining:-



Biochemical Test:-



Anti Microbial Activity:-



Gram staining:-

Sample Number	Gram positive	Gram Negative
301	+	-
302	+	-
303	+	-
304	+	-
305	+	-

Sample Number	Gram positive	Gram Negative
101	+	-
102	+	-
103	+	-
104	+	-
105	+	-

Sample Number	Gram positive	Gram Negative
201	+	-
202	+	-
203	+	-
204	+	-
205	+	-

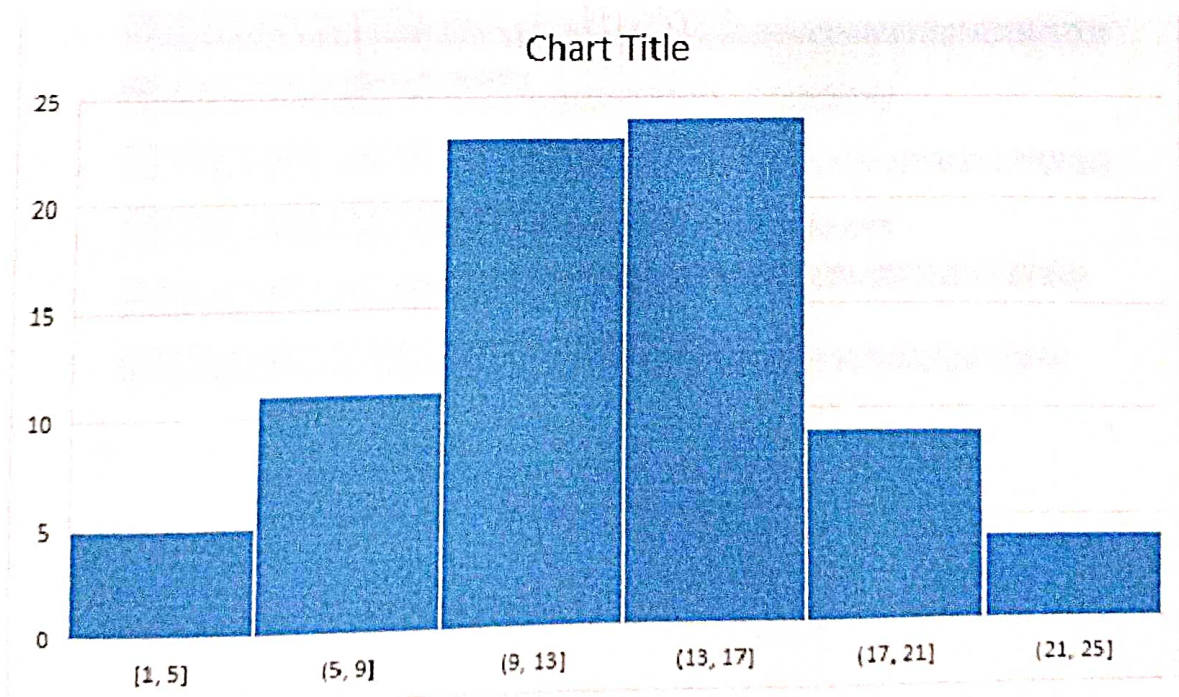
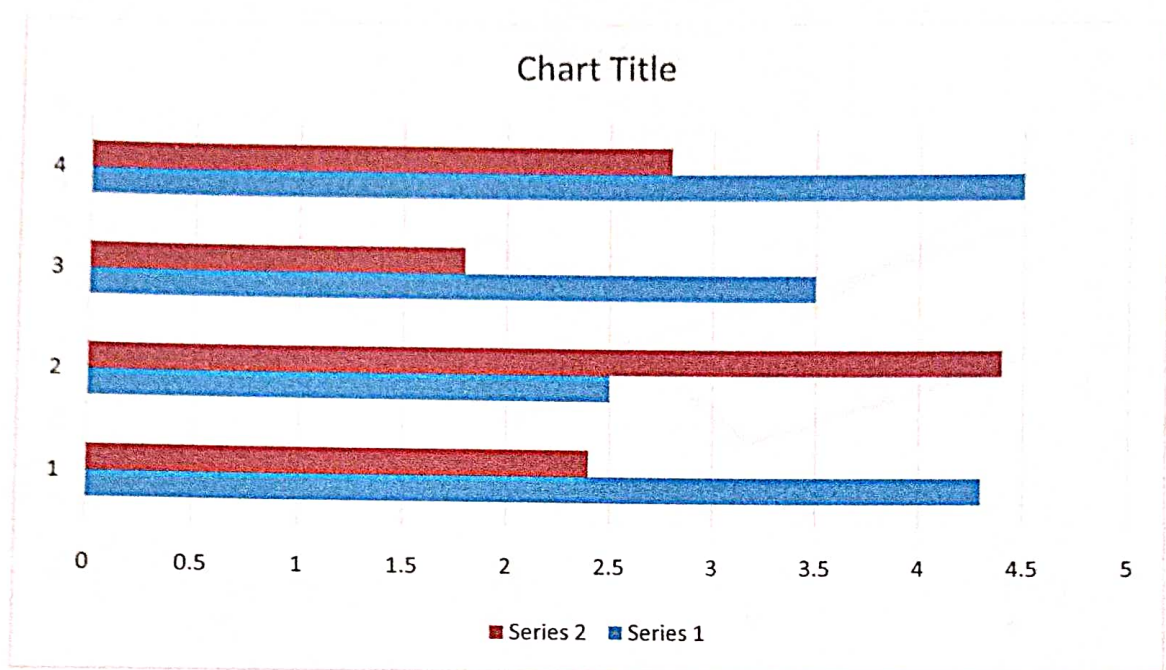
Biochemical Test :-

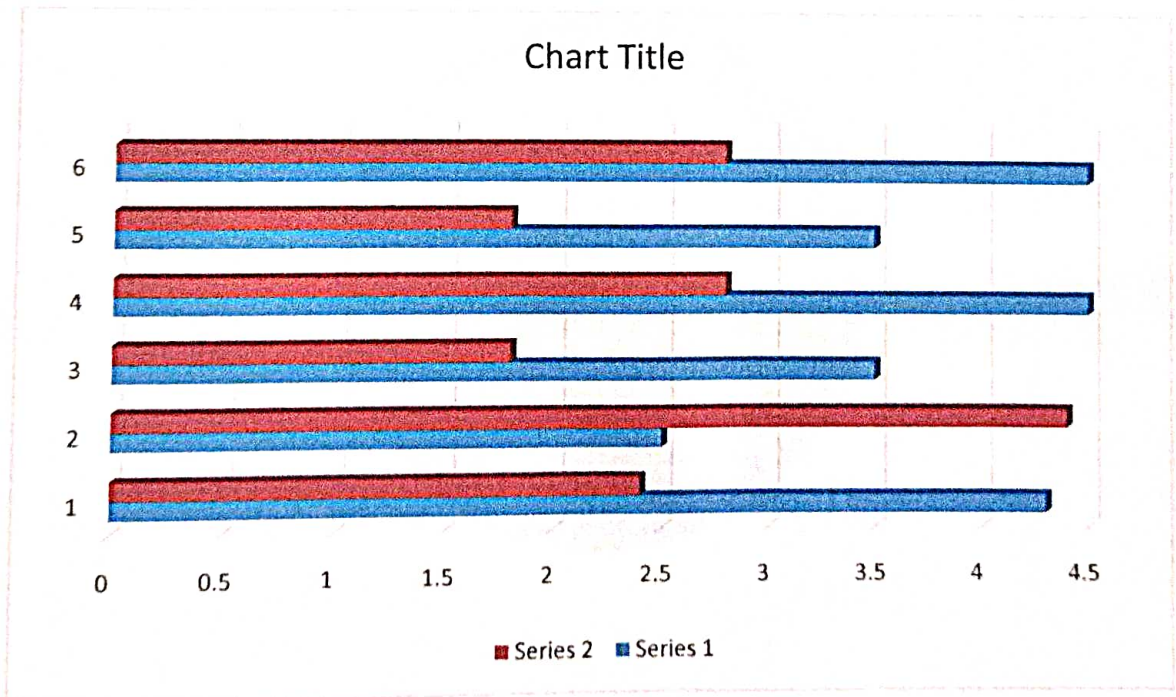
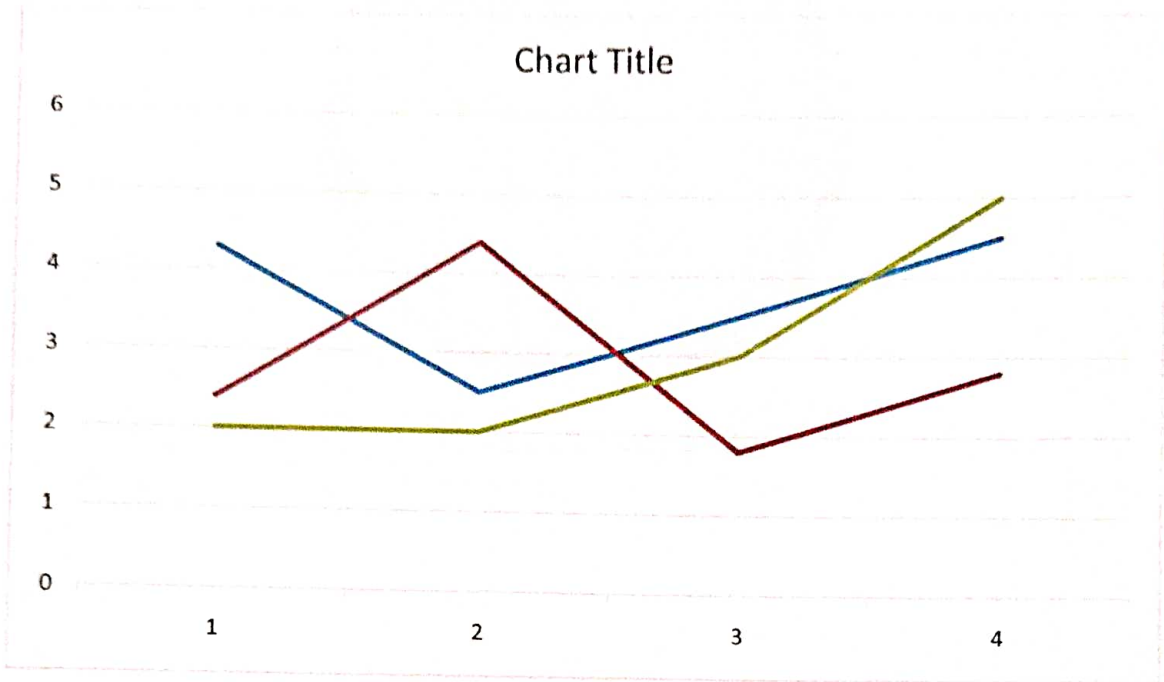
Sample No	Indole		Methyl Red		Catylase		Citrase		Urease	
	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve
201	x	√	x	√	x	√	√	x	x	√
202	x	√	x	√	√	x	√	x	x	√
203	x	√	x	√	x	√	√	x	x	√
204	x	√	x	√	x	√	√	x	x	√
205	x	√	x	√	√	x	x	√	x	√

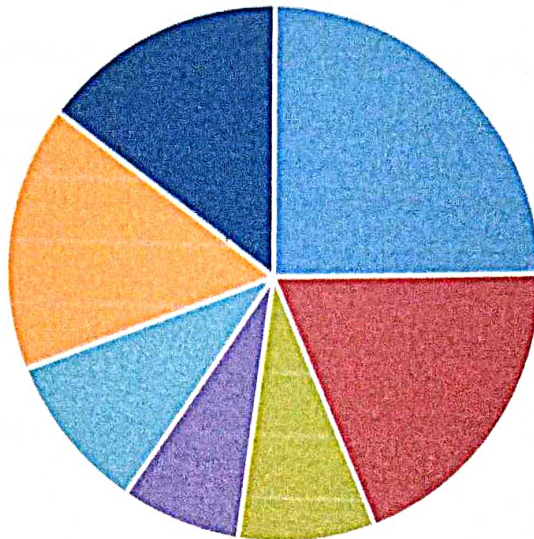
Sample No	Indole		Methyl Red		Catylase		Citrase		Urease	
	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve
101	x	√	x	√	√	x	√	x	x	√
102	x	√	x	√	x	√	x	√	x	√
103	x	√	x	√	x	√	x	√	x	√
104	x	√	x	√	√	x	x	√	x	√
105	x	√	x	√	x	√	√	xx	x	√

Sample No	Indole		Methyl Red		Catylase		Citrase		Urease	
	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve	-Ve
301	x	√	x	√	x	√	√	x	x	√
302	x	√	x	√	x	√	√	x	x	√
303	x	√	x	√	x	√	√	x	x	√
304	x	√	x	√	x	√	√	x	x	√
305	x	√	x	√	x	√	√	x	x	√

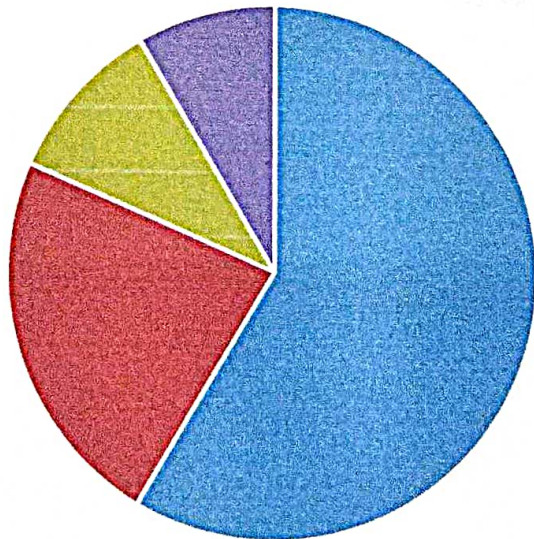
Graphs:-







■ 1 ■ 2 ■ 3 ■ 4 ■ 5 ■ 6 ■ 7



Results:

We obtained a total 15 samples from three dairy products (Curd, Buttermilk, Lassi)

Finally we observed that lactobacillus can resist in some samples but in some samples lactobacillus can't resist pathogenic bacteria like salmonella and E-coli. Local dairy products contain pathogenic bacteria. If we consume that it may cause some illness to human beings.

CONCLUSION

This is a basic study that is provided on dairy products in Jadcherla region Mahabubnagar Dist Telangana India. Lactobacillus have resistance against the Salmonella and E-coli ensure that Dairy Products in Jadcherla some are good but some are cause illness to human beings. Consume of dairy products leads to diseases. Further we will continue this project.

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