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Full Length Original Research Paper

Qualitative, Biochemical and Nanoparticle-Antimicrobial Analysis of Lactobacillus SPS screened from the various milk and curd samples of southern Tamilnadu

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Abstract:
To study probiotic microbes like lactobacillus species were isolated from various milk and curd samples was collected from different places such as vellore , kannamangalam , gudiyatham. Which were subjected to screening and characterized and examined for the presence of probiotic properties of lactobacillus. Lactobacillus is a genus of lactic acid bacteria and It's a group of regular,non-sporing, gram positive bacteria,rod shaped,non-motile and absence of catalase enzyme. Milk and curd samples is an important culture media for lactobacillus. Analysis for quality of milk and curd samples by mbrt test. To detect various adulterants present in milk by using specific biochemical test. The main aim of this study to isolates were obtained by growing on de man rogosa and sharpe[MRS] Agar medium. Lactobacillus was isolated for the production of silver nanoparticles was monitered by UV-spectroscopic analysis.The peak was observed between 400-450nm indicating this presence. Finally the antibacterial activity of nanoparticles were checked against lactobacillus.

Keywords: Lactobacillus, Milk, Silver Nanoparticles, Antibacterial activity.

Introduction:

The Lactobacillus species plays an important role in dairy products and it's a group of lactic acid bacteria[1]. Lactobacillus are living organisms which administered in adequate amounts of a health benefit on the host[2,3]. Lactic acid bacteria is a most common types of microbes such as lactobacillus but certain yeasts and bacilli may also be used and are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements[4,5,6]. The *Lactobacillus* genus consists of a genetically on the host and its a group of rod-shaped, gram positive bacteria, non-spore forming and grows readily at low ph values[below PH 5.0],optium temperature of 37°c[7]. Conformation of the lactobacillus by biochemical testing for the absence of catalase enzyme and the presence of fermentation of glucose[8]. Lactobacilli are essential for the maintenance of the intestinal microbial ecosystem. Colonisation of the gut with Lactobacilli starts within the first week of life. Lactobacilli has been used for the production of antimicrobial compounds by the therapeutic effect[9]. The main aim to study is the protective effect of lactobacillus from fresh cow milk and their ability to reduce the toxicity by using antibacterial effect of nanoparticles[10]. Lactobacillus used nanoparticles as an antimicrobial agent and its synthesis in a laboratory. Now, silver nanoparticles are silver ions (obtained from silver nitrate), which were used for antimicrobial agent.[11-15].

In this paper *Lactobacillus* spp. were isolated from different place milk and curd like Vellore ,kannamangalam, gudiyattam which were

subjected to preliminary screening and characterized and examined for the presence of probiotic properties of lactobacillus and differentiated on the basis of biochemical testing and antibacterial activity.

Nanoparticles is wide range of publication in the field of clinical diagnosis ,molecular biology, physics, chemistry and others. The antibacterial activity of nanoparticles are used against lactobacillus can be detected. Silver nanoparticles are the most commercialized inorganic nanoparticles due to their antimicrobial potential[16]. They also used for a number of applications such as, spectrally selective coating for solar energy absorption, biolabelling and antibacterial,antiviral and antifungal activity.[17-19]. Finally the result in the formation of antibacterial activity of silver nanoparticles against lactobacillus can be identifed. The color change from white to dark brown due to the formation of silver nanoparticles.

MATERIALS AND METHODS:

Sample Collection:

The milk and curd samples were collected from different areas like vellore, gudiyattam, kannmangalam. All samples were collected in sterile sample containers and stored in deep freeze for further processing.

MBRT Test:

The given milk and curd samples are check to the quality by using MBRT test. Take 5ml of milk and curd samples with added 0.5ml of methylene blue. The decolourized time is noted. After this samples are O.D at 550 nm and the maximum microbial count present in this samples used for streaking.

Screening of Lactobacillus:

The samples like curd, raw milk or spoiled milk are taken to isolate the lactobacillus. The MRS media was prepared in petri plates. Then the loop full of milk samples are streaked on the MRS agar plates by quadrant streaking method. This plates are incubated at 37°c for 24 to 48 hours. After incubation, the colonies were restreaked on the another plates for pure colonies. And, it is stored at 4°C for further use.

Gram Staining Test:

The bacterial smear was prepared on the microscopic slide. Then the primary stain of iodine was added to allow for 1minutes and washed with water. After the decolourizing agents of 95% ethyl alcohol was added to drop by drop for 5seconds until the slide was rinse with water immediately. The final stain of saffranin is added to allow for 45seconds. Then the slide was gently rinse with water. Finally this slide was dried and observe under microscope 100x with oil immersion.

Endospore Staining Test:

The bacterial smear was prepared on microscopic slide and heat fixed. This slide was placed in water bath and malachite green[primary stain] was added to allow for 5min. Then the slide was removed from water bath and rinsed with water. After the counter stain of safranin was flooded with slide allow for 20second and rinsed with water. Finally these slide was blot dried, observe under the microscope.

Simple Staining Test:

In simple staining method the bacterial smear was prepared and heat fixed and the smear was covered with methylene blue allow the dye to remain on the smear for approximately 1 minutes. After some time the exess stain was washed off the slide. Using the distilled water wash bottle ,gently washed off the exess methylene blue from

the slide by directing a gentle stream of water over the surface of the slide, excess blot was removed using bibulous paper.

BIOCHEMICAL CHARACTERISATION:

Different types of biochemistry test like Indole test, Catalase utilization test, Starch hydrolysis test, Fermentation of carbohydrate, Mannitol motility test and Methyl red test are performed.

MILK ADULTERATION TEST

Detection of Microorganism In Milk:

Take 10ml of milk into a test tube and 1ml of methylene blue indicater is added. The contents of the test tube was mixed well. A blue color is observed. This test tube is now kept in incubator at 37c for 30min. Decolourised time is noted.

Detection of Starch:

Take 3ml of milk in a test tube and incubated in boiling water bath for 5mins. After incubation the test tube is then cooled and few drops of 1% iodide solution and the content of the test tube mix well. Appearance of blue black color indicates presence starch in milk.

Detection of Acids:

Take 5ml of milk in to a test tube and added few drops of conc.sulpuric acid and gently shake the test tube. Add 0.5% ferric chloride solution drop wise in to test tube. Mix the content well development of buff colour indicates presence of benzoic acid or violet colour is observed shows the presence of salicylic acid.

Detection of Soap:

Take 10ml of milk in to test tube. Add 10ml of hot water in to test tube containing milk. Now add 1 to 2 drops of phenolphthalein indicator is added in to test tube. Gently mix the contents of the test tube. Development of pink colour on addition of phenolphthalein indicator conforms the presence of soap in milk.

Detection of Ammonium Sulphate:

Take 2ml of milk in to the test tube. Added 500μl of 2% NaOH in to the test tube. Mix the contents well and Added 500μl 2% sodium hypochloride in to the test tube and mix well. Finally 500 μl of 5% phenol in to the test tube content throughly. A light bluish colour will be observed on the test tubes. The content of the test tubes in a boiling water bath for 20seconds

PRODUCTION OF SILVER NANOPARTICLES:

In 5.5gms of mrs broth was dissolved in 100ml. Then the lactobacillus culture was innoculated in broth. This media containing lactobacillus was centrifuged at 10,000rpm for 10minutes. The pellet was removed and supernatent is taken to added 40 µl with 760 µl of tris-Hcl buffer[Ph-7.5]. After this added to 4ml of milk and leave it for 1hour. Again centrifuged at 5000rpm for 5minutes. This supernatent taken for production of silver nanoparticles. Take one test tube to added 0.1g of silver nitrate with 1ml of water and another test tube as control [without] silver nitrate. This reaction is allowed in bright condition for 72 hrs. After this solution is measured at [200-900nm]in absorbance spectrum.

RESULT AND DICUSSION:

Fig.1: Shows different milk and curd samples

Result For MBRT Test:

Table 1: The color of milk and curd samples observed was white in appearance

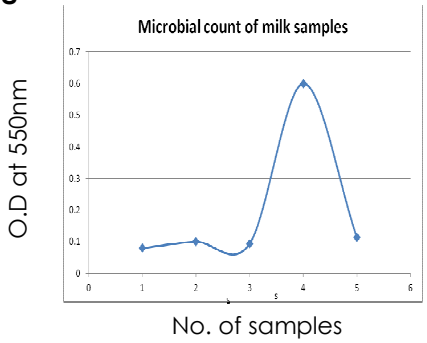
Sample Type	Sample Number	Decolourized Time	Quality
Raw Milk	1	6 hours	GOOD
	2	7 hours	GOOD
	3	6 hours	GOOD
	4	6 hours	GOOD
	5	More than 8hours	EXCELLANT
	6	More than 8hours	EXCELLENT
	7	More than 8hours	EXCELLENT
Pasturized Milk	1	More than 8hours	EXCELLENT
	2	More than 8hours	EXCELLENT
	3	More than 8hours	EXCELLENT
Curd	1	1hours	POOR
	2	2hours	POOR
	3	2and1/2hours	FAIR
	4	2and1/2hours	FAIR
	5	1and 1/2hours	POOR

Fig.2: Shows the result of MBRT test

Table 2: Shows the result of microbial count in milk

SAMPLES	O.D
SAMPLE 1	0.080
SAMPLE 2	0.10
SAMPLE 3	0.094
SAMPLE 4	0.60
SAMPLE 5	0.114

Fig.3: Shows the result of microbial count



Isolation of Bacteria

First we collect the sample from different places of like Vellore, kannamangalam gudiyattham. Bacterial colony was isolated from the different places from milk and curd sample by the streak plate technique and a pure culture was obtained by the restreaked method. First pour plate technique was done to isolate the number of colony and then the streak plate technique.

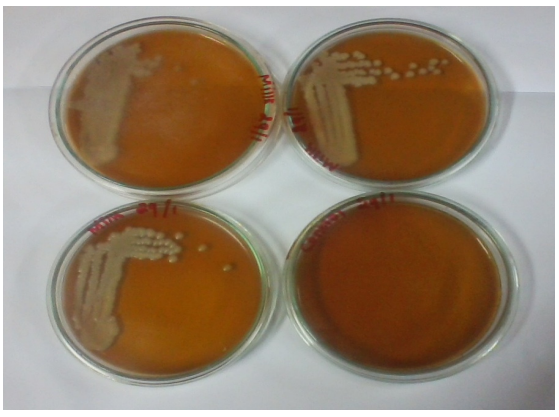


Fig.4: shows the result of colonies of lactobacillus

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STAINING OF BACTERIA:

Gram Staining:

Lactobacillus is a gram positive bacteria, rod shaped.

Endospore Staining:

This staining procedure uses Malachite Green as the primary stain for the spores.In this staining only red color vegetative cell appeared so this bacterial culture is non endospore forming cell.

Simple Staining:

This test is useful in classifying two distinct types of bacteria based on the structural difference of their cell walls. By this method we got the characteristic of bacteria is rod shaped bacteria which is the characteristic of *bacillus* species.

Table 3: Result for Biochemical Test of Lactobacillus

Biochemical Test	VIT MS1	VIT MS2
1. Indole test	- ve	- ve
2. Catalase test	- ve	- ve
3. Citrate utilization test	+ve	+ve
4. Starch hydrolysis test	+ve	+ve
5. Mannitol test	- ve	- ve
6. Methyl red test	+ve	+ve
7. Fermentation of carbohydrate test;		
Glucose	+ve	+ve
Sucrose	+ve	+ve
Lactose	+ve	+ve

Table 4: Result for Milk Adulteration

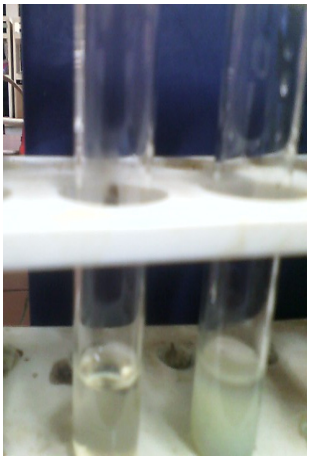
S. No.	Test of Raw Milk	Result
1.	Detection of soap	Absence
2.	Detection of microorganism	Absence
3.	Detection of formalin	Absence
4.	Detection of starch	Absence
5.	Detection of benzoic and salicylic acid	Absence
6.	Detection of ammonium sulphate	Absence
S. No.	**Test of Pasteurized Milk**	**Result**
1.	Detection of soap	Absence
2.	Detection of microorganism	Absence
3.	Detection of formalin	Absence
4.	Detection of starch	Absence
5.	Detection of benzoic acid and salicylic acid	Presence of benzoic acid
6.	Detection of Ammonium sulphate	Absence

Raw milk Pasteurized milk

Fig.5: shows the result of MBRT Test

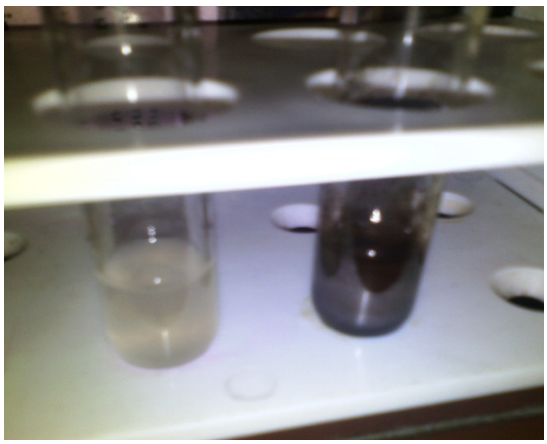
PRODUCTION OF SILVER NANOPARTICLES:

The antibacterial activity of nanoparticles against lactibacillus was identifed.This reaction is allowed in bright condition within 72 hrs. The color of test tubes changes fron white to dark brown as a result in the formation of silver nanoparticles.



Control Test

Before 72 hours



Control Test

After 72 hours

Fig.6: Shows the result of production of nanoparticles

Discussion:

Characterization and determination of lactic acid bacteria like lactobacillus isolated from the different places (Vellore, kannamangalam, gudiyattham) milk and curd sample. The collected samples were check the quality by MBRT test result was poor quality [3 samples] ,fair quality[3samples] ,good quality[4 samples] and excellent quality [6 samples]. The result for adulterant test in which there is no adulterant in milk samples. Isolated *lactobacillus* was found to be rod shaped, Gram positive and non endospore forming bacteria in nature. To determine the probiotic characteristic of these isolated strains different biochemical tests were performed such as the negative result for indole, catalase, mannitol and positive result for citrate, starch, carbohydrate methyl red test. Finally the antibacterial activity of nanoparticles[silver nitrate] used to act against lactobacillus can be identified.

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