A

PROJECT REPORT ON

"Isolation of Lactic Acid Bacteria from curd and preparation of probiotic food supplement".

SUBMITTED BY,

Miss Pratiksha Balasaheb Gavade.

Exam Seat No. 9308

SUBMITTED TO,

Vivekanand college, (Autonomous) Kolhapur

FOR PARTIAL FULFILMENT OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY

THE YEAR

2018-2019.

UNDER THE GUIDANCE OF,

Mr. A. L. Upadhye

Assistant Professor,

Department of Biotechnology.

"Education for Knowledge, Science and Culture"

- Shikshan Maharashi Dr Bapuji Salunkhe

Shri

Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR

(AUTONOMOUS)

DEPARTMENT OF BIOTECHNOLOGY

Certificate

This is to certify that, **Miss. Gavade Pratiksha Balasaheb.** **Exam No:** 9308 . has satisfactorily completed a Project Report **"Isolation of Lactic Acid Bacteria from curd and preparation of Probiotic food supplement"**. as a part of syllabus prescribed by SHIVAJI UNIVERSITY Kolhapur, for B.Sc. III course in Biotechnology (Entire) and this Project report represents Her bonafied work in the year 2018-2019.

Place- Kolhapur

Date- 18/03/2019

VIVEKANAND COLLEGE KOLHAPUR ESTD. JUNE 1964

Teacher in Charge

Examiner

18/3/19.

Head of Department

Department of Biotechnology (Entire/Optional)
Vivekanand College, Kolhapur.

ACKNOWEDGEMENT

I acknowledge my deep sense gratitude towards **Asst/Prof S.G.Kulkarni** Head of the Department of Biotechnology for being a great source of inspiration.

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I am also thankful to the non- teaching staff members and friends who helped us to carry out the project satisfactorily.

I record my sincere thanks to Vivekanand College, Kolhapur for allowing me to carry out my project work successfully in our college labs.

Gavade

Ms. Pratiksha Balasaheb Gavade.

DECLARATION

I hereby declare that the project work entitled "**Isolation of Lactic Acid Bacteria from curd and preparation of Probiotic food supplement**". submitted to the vivekanand college, (Autonomous) Kolhapur for the award of the degree of "Bachelor of science, Biotechnology" is the result of bonafide work carried out by me under the guidance of Asst/Prof..A.L.Upadhye.

I further declare that the results presented here have not been the basis for the reward of any other degree.

Place :- Kolhapur

Date :- 18/03/2019

PBGavade

Miss: Pratiksha Balasaheb Gavade

CONTENT

1. AIM AND OBJECTIVE

1.1 Aim: "Isolation of Lactic Acid Bacteria from curd and preparation of Probiotic food supplement".

1..2 Objectives:

- **Isolation of lactic Acid Bacteria**
- **Mass production**
- **Preparation of Food supplement (Probiotics)**
- **Determination of Total Viable count.**

4.1 REQUIRED MATERIAL

4.1.1 Reagents:

MRS medium, MRS broth, Glucose broth,Lactose broth, 2% Peptone water, Glucose phosphate broth, Kosar's citrate broth, Xylene, Kovac's reagent, Methyl red reagent, 60% α-napthal, KOH, NaCl, Sugars(lactose, Glucose, Fructose, Manitol, Sucrose, D-Xylose) H_2O_2.

4.1.2 Glassware:

Flask, Petri plates, measuring cylinder, test tubes, Conical flask, pipette, spreader, Beaker, funnel, saline tube.

4.1.3 Other Requirements:

Incubator, oven, nichrome wire loop, autoclave, tripod stand, Hot plate, weighing balance

4.2.8 STANDERD PLATE COUNT OF PROBIOTIC SAMPLE:

10 test tube containing 9 ml distilled water and 1test tube containing 10 ml distilled water are sterilized. After cooling 1 gm probiotic sample was added in the 10ml D/W containing test tube in sterile condition. Then 1 ml from this test tube is transferred in next test tube containing 9 ml d/w. This way next dilutions were prepared. With the help of sterile pipette 0.1 ml of each dilution was aseptically added on sterile MRS agar plate and spread. The plate were incubated at room temperature for 24 hours

After incubation the number of colonys per plate was counted with the help of colony count.

Observation Table:

Sr.No	No. of colonies	Dilution factor	C.F.U	Average
1.	876	10^{-1}	876×10^2	935107535570 $\times 10^2$
2.	419	10^{-2}	4190×10^2	
3.	305	10^{-3}	30500×10^2	
4.	248	10^{-4}	248000×10^2	
5.	207	10^{-5}	2070000×10^2	
6.	194	10^{-6}	19400000×10^2	
7.	149	10^{-7}	149000000×10^2	
8.	134	10^{-8}	1340000000×10^2	
9.	130	10^{-9}	13000000000×10^2	
10.	79	10^{-10}	790000000000×10^2	

RESULT :

1. ISOLATION OF ORGANISM

Curd was used for the isolation of organism by pour plate method. After 96 hours pin point white colored colonies were obtained on MRS medium. The organism was confirmed my staining, motility and biochemical test.

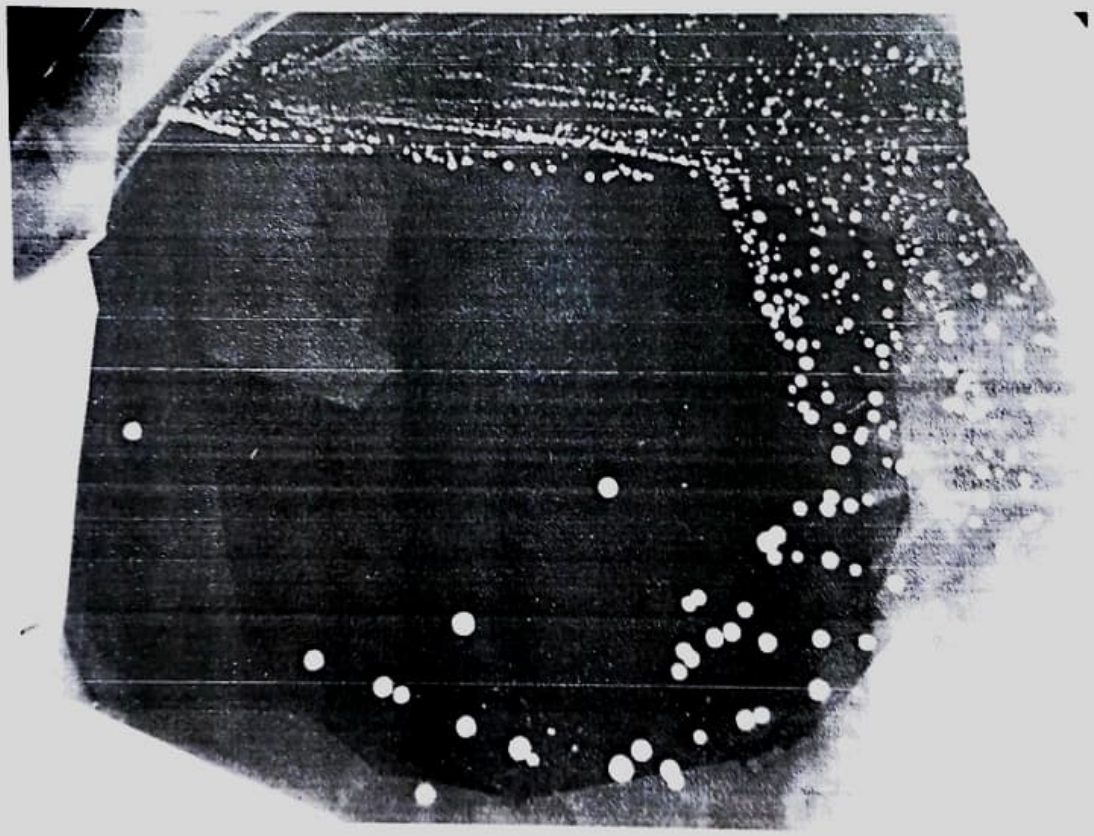

Fig. 1 : MRS medium

After 96 hour pin point white colored colonies were obtained

2. IDENTIFICATION OF ORGANISM

Colony characters of *Lactobacillus spp* grown on MRS medium for 96 hours under anaerobic conditions.

SIZE	SHAPE	COLOUR	MARGIN
0.3 mm	Circular	White	Entire
SURFACE	**ELEVATION**	**CONSISTENCY**	**OPACITY**
Smooth	Convex	Moist	Opaque

Gram staining	Gram Positive
Motility	Motile

Table.No.1. Morphological characters of isolated colony

Catalase test Negative

Sugar fermentation test	Gas production	Acid production
• **Lactose**	Positive	Positive
• **Glucose**	Positive	Positive
• **Sucrose**	Positive	Positive
• **Manitol**	Positive	Positive
• **Fructose**	Positive	Positive
• **D-Xylose**	Positive	Positive

Indole test	**Negative**
Methyl-Red	Negative
Voges proskauer test	Negative
Citrate Utilization test	Negative

Table.No.2. Morphological & Biochemical Characteristics.

➢ **Gram Staining**

Fig.2 Microscopical view

Slide shows violet color and rod shaped organism which indicates that the organism were Gram positive.

CONCLUSION

- ✓ Lactic acid from lactic acid producing bacteria of the genus *Lactobacillus* was successfully produced which was then separated from the broth.
- ✓ Alternative sources of probiotics, such as non-dairy fermented food products, present an advantage in the search for new probiotic strains.
- ✓ Increasingly, these probiotic sources are being selected for use in people who are lactose intolerant.
- ✓ The selection of probiotics from different sources involves screening for non-pathogenic microbes followed by an evaluation of basic properties, including acid and bile tolerance, an ability to adhere to gut epithelial cells, an ability to combat against pathogens in the GI tract, and the safety-enhancing property of an inability to transfer any antibiotic resistance genes to other bacteria.
- ✓ Probiotic food helps in restoring the disturb microflora of the intestine.
- ✓ It in digestion of Lactose in lactose intolerance people.

COMPOSITION OF MRS MEDIUM

(De Man, Rogosa & Sharpe medium)

Dextrose - 20g

Peptic digestion of animal tissue - 10g

Beef extract - 10 g

Yeast extract - 5 g

Sodium acetate - 5g

Dipotassium phosphate - 2g

Ammonium citrate - 2g

Tween®80 - 1g

Magnesium sulphate - 0.1g

Manganese sulphate - 0.05g

Cycloheximide - 3g

Agar- Agar - 15 g

Distilled Water - 1000 ml

pH - 6.5