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ISOLATION & IDENTIFICATION OF *LACTOBACILLUS* FROM CURD & ITS APPLICATION IN PROBIOTIC CHOCOLATE

M. Manonmani[1*], Jadhav Ashwini Ashok[2] and M. Kavitha[3]

[1]Assistant Professor, Department of Microbiology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi – 626 123.
[2,3]BSc Microbiology, Department of Microbiology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi – 626 123.

***Corresponding Author: M. Manonmani**
Assistant Professor, Department of Microbiology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi - 626 123.

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ABSTRACT
Probiotics are microorganisms that offer some form of health benefit to the host they can be found in various different foods. In the presence study the Sample were collected from homemade curd, sivakasi. The *Lactobacillus* were isolated and identified by using gram staining, biochemical test (Indole, Methyl red, Voges proskauer, Citrate and catalase test, oxidase test, carbohydrate fermantation test). Antibiotic sensitivity test were performed by using antibiotics and test were performed by using Muller Hinton Agar plates. The results were observed based on the zone formation such as (Streptomycin 23 mm, Gentamycin 25 mm). Then their Antimicrobial production test was performed by using bacterial pathogen (*Pseudomonas* 22 mm, *Shigella* 20, *Vibrio* 19 mm). Acid sensitivity test were performed by various pH (such as 7, 6, 5, 4, 3 & 2). The result was observed pH 2 at OD value in 0.048. Probiotic chocolate was prepared. It is better than to the normal chocolate because using to the *Lactobacillus* organisms.

KEYWORDS: *Lactobacillus*, MRS broth, Probiotic Chocolate.

INTRODUCTION
The word 'probiotic' comes from Greek language 'pro bio' which means 'for life' opposed to 'antibiotics' which means 'against life'. The history of probiotics began with the history of man by consuming fermented foods that is well known Greek and Romans consume very much. Probiotics mean live microorganisms that have beneficial effects on their host's health. The medical world has long been interested in nutrient properties of curd. Curd is commonly used fermented milk product in India since time immemorial. In this study lactic acid bacteria were isolated from curd and their probiotic potential was investigated (Shaikh *et al*., 2013).

Probiotics are also challenging for the industrial applications. The probiotic concept is open to lots of different applications in a large variety of fields relevant for human and animal health. Probiotic products consist of different enzymes, vitamins, capsules or tablets and some fermented foods contain microorganisms which have beneficial effects on the health of host. They can contain one or several species of probiotic bacteria. Most of products which destine human consumption are produced in fermented milk or given in powders or tablets. These capsules and tablets do not used for medicinal applications (Chakraborty A, *et al* 2015).

Chocolate is the noblest confectionery product of the unique sensory and textural properties. Chocolate mass, which is the suspension of particles derived from cocoa beans, sweetener and in some cases from milk in cocoa butter or its mixture with another fat is the semi-product used for chocolate manufacturing (Nebesny *et al*., 2007). Enrichment of chocolate with viable cells of lactic acid bacteria and development of modified technology of chocolate manufacturing to provide survival of these bacteria would contribute to enhanced beneficial impact of this product on human health. This approach is of importance because chocolate is one of favorite foodstuffs for children (Rad *et al*., 2014).

Among all lactic acid bacteria, the genus Lactobacillus has some beneficial characteristics which make it useful for the industrial applications.

METERIALS AND METHODS
The samples were collected as Homemade Curd in sivakasi. Isolation of Probiotic Lactobacillus by using Serial dilution Techniques, Identification of Probiotic *Lactobacillus* by using Gram staining and Biochemical

test. Characterization studies of Probiotic *Lactobacillus* were performed by using Antibiotic susceptibility test, Antimicrobial activity test and Acid sensitivity test.

Preparation of Probiotic chocolate

List of ingerdians used for the preparation of Probiotic chocolate

- *Lactobacillus* culture (1 ml)
- Cocoa powder (20 gm)
- Skim milk powder (10 gm)
- Milk cream(10 gm)
- Sugar

Table: 1 Antibiotic Sensitivity Test.

S.No	Antibiotic	Result					
		25 µg	50 µg	100 µg	200µg	250µg	300µg
1.	Streptomycin	-	-	-	19 mm	21 mm	23 mm
2.	Gentamycin	20 mm	19 mm	22 mm	21 mm	20 mm	25 mm

Collection of Lactobacillus Culture

24 hrs *lactobacillus* cultures were prepared. The culture was centrifuged at 45 min, 1200 rpm. After that, the culture supernatant was used for the preparation of Probiotic chocolate.

Preparation of Probiotic chocolate

Cocoa powder was used in preparing for probiotic chocolate. 20 gm Cocoa powder, 10 gm skim powder and 10 gm milk cream added with little amount of water and ingredient were heated under the low flame, and then the sugar was added to it and mixed well. (Mishra *et al.*, 2014).

RESULT AND DISCUSSION

In the presence study also the probiotic *Lactobacillus* microorganism was isolated from homemade curd sample.

In this study the isolation of Probiotic *Lactobacillus* by using Serial dilution Techniques. The *Lactobacillus* were identified by using gram staining, biochemical test (Indole, Methyl red, Voges prosakuer, Citrate and catalase test, oxidase test, carbohydrate fermentation test). (Holt *et al.*, 1994).

Similarly, In our study also the isolation of Probiotic *Lactobacillus* were performed by using serial dilution technique. *Lactobacillus* isolated and identified by using gram staining, biochemical test (Indole, Methyl red, Voges prosakuer, Citrate and catalase test, oxidase test, carbohydrate fermentation test).

Antibiotic sensitivity test were performed by using antibiotics (Streptomycin, Penicillin, Tetracycline, Gentamycin) and test were performed by using Muller Hinton Agar. (Mishra *et al.*, 2014). Similarly, In the presence study were performed by using antibiotic, resistance of *Lactobacillus* showed the better resistance capacity in streptomycin 23 mm and Gentamycin 25 mm antibiotics.

Antimicrobial activity test were performed by using bacterial pathogen *e.coli*. (Bilkova *et al.*, 2011). Antimicrobial activity test were performed by using bacterial pathogen. (*Pseudomonas* 22 mm, *Shigella* 20 mm, *Vibrio*19 mm). The result was observed in zone formation.

Table: 2 Antimicrobial Activity Test.

S.no	Bacteial Pathogen	Rrsult in mm
1.	*Vibrio*	19 mm
2.	*Pseudomonas*	22 mm
3.	*Shigella*	20 mm

Acid sensitivity test were performed by determined in various pH (such as 7, 6, 5, 4, 3 & 2), (Mishra *et al.*, 2014). In the experiment were performed by determined in various P^H (such as 7, 6, 5, 4, 3 & 2). The best result were observed in pH 2 at 0.048 nm.

Table: 3 Acid Sensitivity Test.

S.No	Ph	OD Value at 600 nm
1.	7	1.569
2.	6	1.548
3.	5	0.098
4.	4	0.075
5.	3	0.064
6.	2	0.048

Application in prepared the probiotic chocolate. (Prathamesh S, 2014). Probiotic chocolate is better than to the normal chocolate because using to the *lactobacillus* organisms.

CONCLUSION

Probiotic chocolate is better than to the normal chocolate because using to the *Lactobacillus* organisms. The result of this study relevant to Probiotic bacteria, were support a healthy digestive tract.

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