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Substantiation of Position of Bacteriocin on Plasmid

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Abstract: *Lactobacillus rhamnosus L43 strain isolated from marine water. Lactobacillus isolate was sequenced which shown high similarity with reference strain Lactobacillus rhamnosus ZY Accession number KC012630.1. These results indicated the potent strain produce bacteriocin. Plasmid curing has been carried out for mapping bacteriocin producing gene on plasmid DNA of Lactobacillus rhamnosus L43. Cells growing on nutrient medium above 10 mg/ml concentration of ethidium bromide were selected to observe the effect of curing.*

Keywords: Antibacterial Activity, Bacteriocin, *Lactobacillus Rhamnosus* L43, Plasmid Bearing Bacteria

I. INTRODUCTION

1.1 Plasmid bearing LAB

The plasmids of both Gram-negative and Grampositive bacteria are involved in metabolic activity, membrane protein production, the establishment of progressive infection and resistance. Many successful attempts have been made to isolate and sequence the genes of plasmids from many different species, including Escherichia coli, Staphylococcus, Streptococcus, Salmonella, Listeria and lactic acid bacteria (LAB) spp. The first plasmid isolation was carried out from LAB (1)

Jamuna and Jeevaratnam in 2004 reported that the *Lactobacilli* isolates from some traditional fermented foods, LABB (appam batter) and LABP (vegetable pickle) to produce bacteriocins. He reported that both the strains harboured multiple plasmids ranging in size from 1.5 kb to more than 10 kb. He has shown that combination of acriflavin, ethidium bromide and novobiocin could induce loss of plasmid DNA in these strains. He analysed that non cured strain of *Lactobacilli* was resistant to ciprofloxacin, colistin, gentamycin, nalidixic acid and streptomycin but after curing strain altered its response by showing sensitivity to ciprofloxacin, gentamycin and streptomycin.

II. MATERIALS AND METHODS

- **Selected Isolate:** *Lactobacillus* strain was isolated from marine water, Thirumullavaram beach, Kerala using MRS broth (Hi-Media, India) at 37^0C for 48 hrs. The isolate was identified by 16S rRNA sequencing and phylogenetic analysis. *Lactobacillus* L43 isolate was sequenced which shown high similarity with reference strain *Lactobacillus rhamnosus* ZY Accession number KC012630.1.
- **Antibiotic sensitivity Test:** Antibiogram of selected potential isolate *Lactobacillus rhamnosus (L43)* carried out using Kirby-Bauer method as proposed by Kedzia, and Koniar,

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(1980) (2). The isolate *Lactobacillus rhamnosus* was inoculated into MRS broth individually and incubated for 24 h. About 20 ml of MRS agar was seeded with the cultures of selected isolate B25 (10^7cfu/ml) mixed well, poured into sterile petri plates and allowed to solidify. Antibiotic discs (HiMedia, India) were placed upside and pressed on the top of the agar plates. The plates were incubated at 37^0C overnight. Resistance was determined in terms of zone of growth around the discs.

2.1 Confirmation of Location of Bacteriocin Expressing Marker on Plasmid

A. Plasmid Curing

Confirmation of plasmid encoded nature of gene producing bacteriocin was determined by plasmid curing. The curing of plasmid has been carried out by treating the active intact cells of *Lactobacillus rhamnosus L43* using ethidium bromide (3)**.**

2.2 Confirmation of Curing by Antibiotic Sensitivity Test and Antimicrobial Activity

Antibiotic sensitivity test was carried out as described previously (2). Both cured and uncured cultures were used to determine the pattern of antibiotic sensitivity. The bacteriocin was produced from both cured and uncured culture and antibacterial activity of this bacteriocin was determined described previously.

In both tests the response of both cultures was correlated with each other. The presence of plasmid encoded marker was confirmed by observing loss of respective activity from cured culture when compared to response of uncured culture.

III. RESULT AND DISCUSSION

3.1 Confirmation of Location of Bacteriocin Expressing Marker on Plasmid

A. Plasmid Curing

Plasmid curing has been carried out for mapping bacteriocin producing gene on plasmid DNA of *Lactobacillus rhamnosus* L43. As per survival curve it was observed that Ethidium bromide was found to be detrimental for growth of *Lactobacillus rhamnosus* L43. The LD_{50} was found to be 10 mg/ml. Cells growing on nutrient medium above 10 mg/ml concentration of ethidium bromide were selected to observe the effect of curing. Table 1 and figure 1 represent the survival curve obtained by Ethidium bromide concentrations.

B. Antibiotic Sensitivity Test After Plasmid Curing

Cured cells of *Lactobacillus rhamnosus* L43 has shown loss of resistance to antibiotics viz., streptomycin, Gentamycin, Erythromycin, Rifamycin, Vancomycin, Cefuroxime, Nalidixic acid and Co-timoxazale as compared to uncured cells. Table 2 represents comparative antibiogram of *Lactobacillus rhamnosus L43*after plasmid curing.

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C. Antibacterial Activity After Plasmid Curing

Table 3 and Photo plate 1 represents the antibacterial activity of strain *Lactobacillus rhamnosus L43* before and after plasmid curing. In both tests the response of both cultures was correlated with each other. The presence of plasmid encoded marker was confirmed by observing loss of respective activity from cured culture when compared to response of uncured culture.

Presently, we have demonstrated the plasmid mediated bacteriocin production, antibiotics resistance and immunity could be lost as the curing of plasmid. Moreover, it has been recently reported previously (6) that *Lactobacillus plantarum* strain produces bacteriocin of about 2.5 kDa which is plasmid encoded. Scolri in 1999 has reported that both the antimicrobial compound production and immunity determinants were encoded by an 8.8 kb plasmid in a *L. casei*strain of vegetable origin, while there was a plasmid associated production of bacteriocin in a *Lactobacillus* strain without affecting the immunity, indicating the possibility of the immunity genes on the chromosomes (3). Similarly, a study reports plasmid curing which was performed (7) at Institute of Molecular genetics and genetic engineering, University of Belgrade, Serbia. Similarly plasmid profile of *Lactobacillus sp*. as reported earlier (8,9). Photoplate 2 has shown the presence of plasmid of *Lactobacillus rhamnosus* L43

Table 1: Survival curve of ethidium bromide treated *Lactobacillus pentosus* B25

Sr. No.	Ethidium Bromide concentration (mg/ml)	No. of colonies Survived	No. of colonies Killed	% Mortality
1	Control	136		
2	10	87	69	50
3	12	53	83	61.02
4	14	27	109	80.14
5	16	9	127	93.38
6	18	-	-	-
7	20	-	-	-

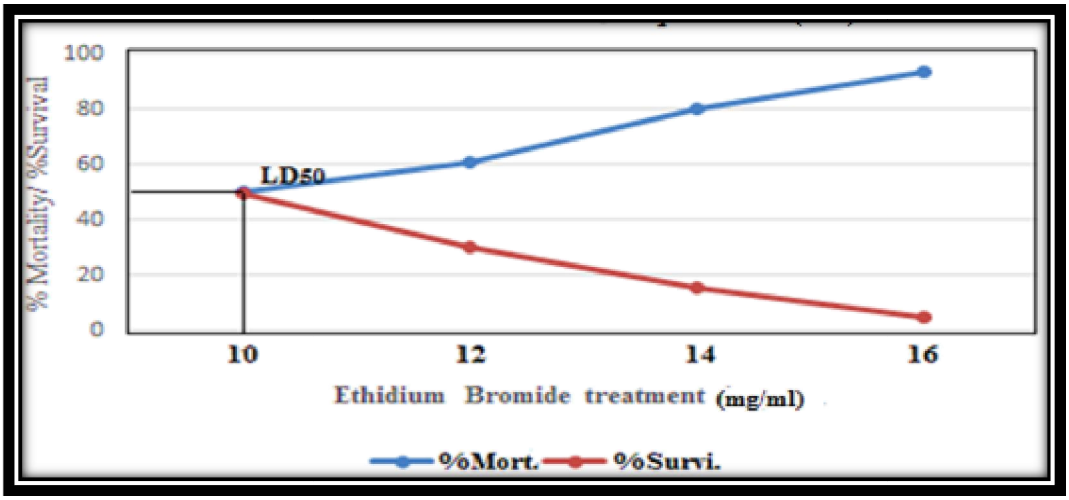

Figure 1: Survival curve of Ethidium Bromide treated *Lactobacillus rhamnosus L43*

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Table 2: Antibiotic sensitivity test of strain *Lactobacillus rhamnosus L43* before plasmid curing and after plasmid curing

Sr. No.	Name of Antibiotics (mcg)	Antibiotic sensitivity	
		Before plasmid curing	After plasmid curing
1	Streptomycin 25	R	S
2	Fusidic acid 30	R	R
3	Gentamycin 10	R	S
4	Oxacillin 5	R	R
5	Bacitracin 10	R	R
6	Erythromycin 15	R	S
7	Cefprozil 30	R	R
8	Imipenem 10	R	R
9	Cefpadoxine 10	R	R
10	Teicoplanin 30	R	R
11	Cefpadoxivel/Clavulanic acid 10/5	R	S
12	Fosfomycin 200	R	R
13	Vancomycin 30	R	R
14	Rifamycin 15	R	S
15	Vancomycin 30	R	S
16	Roxitromycin 30	R	R
17	Cefuroxime 30	R	S
18	Meropenem 10	R	R
19	Nalidixic acid 30	R	S
20	Piperacillin 100	R	R
21	Cefuroxime 30	R	R
22	Co-Trimoxazale 25	R	S

Table 3: Comparative analysis of antibacterial activity of *Lactobacillus* rhamnosus L43 after plasmid curing

Sr. No.	Name of pathogens (Jagatap pathology laboratory)	Antibacterial activity of B25 (mm)	
		Before plasmid curing	After plasmid curing
1	*K. pneumoniae* ssp. *Pneumonia*	16.5	NE
2	*Escherichia coli*	11	NE
3	*Pseudomonas aeruginosa*	13.5	NE
4	*Enterococcus casseliflavus*	12	NE

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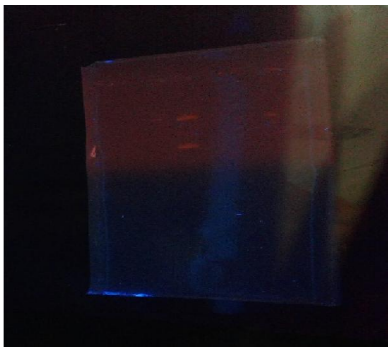
5	*Staphylococcus sciuri*	10.5	NE
6	*Klebsiella pneumoniae* 535	18.5	NE

NE: Not Effective

PHOTOPLATE 1

Antibacterial activity of *Lactobacillus rhamnosus L43* against pathogens before and after plasmid curing

PHOTOPLATE 2



Isolation of Plasmid from *Lactobacillus rhamnosus* L43

IV. CONCLUSION

According to the present study, *Lactobacillus rhamnosus* L43 is a plasmid bearing bacteria which is having antibacterial activity and bacteriocin producing marker available on the selected isolate.

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