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Optimization of Suitable Physio-Chemical Parameters for Enhanced Bacteriocin Production by Bacteria Present in Dairy Effluents

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ABSTRACT

Bacteriocins are proteinacious toxins produced by bacteria to inhibit the growth of similar (or) closely related bacterial strains. They have received considerable attention during recent years for their preferential use as biopreservative in foods, over chemical preservatives, provoking the formation of an inhospitable environment to microbial survival. Media ingredients, chemical and physical parameters play an important role in the production of bacteriocin. In the present study the influence of growth parameters and various media ingradients on the production of bacteriocins is investigated. Bacteria from dairy effluent produced higher quantities of bacteriocin in MRS (Deman Rogosa Sharpe) broth with dextrose, peptone, ascorbic acid, thiamine HCL, EDTA at pH 7.5, temp 35°C and incubation time 25 hrs-35 hrs. Bacteriocins produced by these bacterial isolates (MSB1, MSB2) displayed a wide spectrum of intibition againt patnogers Staphylicoccus aureus, Salmonella typhi employed as test strains.

Keywords: Bacteriocins; MRS broth; Chemical parameters; Physical parameters; *Staphylococococus aureus*; *Salmonella typhi*

INTRODUCTION

Most of the bacteria present in these dairy effluents are capable of producing a heterogeneous array of molecules that may be inhibiting either for themselves (or) for other Bacteria. These molecules include toxins, primary metabolites, antibiotics and bacteriocins. Antibacterial peptides (or) bacteriocins produced by these bacteria are bactericidal to many gram +ve bacteria causing food spoilage and food borne illness [1,2]. The possible use of bacteriocins as food biopreservatives could lead to the replacement of synthetic chemical preservatives which have their antimicrobial action reduced due to the continued appearance of multiresistant microbial lineages. In addition these molecules present characteristics of resistance to heat, acid, low water activity and oscillations of temp.
Bacteriocins are degraded by the proteolytic enzymes of the gastro intestinal tract and seem to be non-toxic and non-immunogenic to animals. Thus they can be used to enhance the safety and shelf life of many processed foods.
Investigations carried out using complex media demonstrated that bacteriocin production is largely dependent on the medium composition as well as on the qualitative and quantitative nature of the nutrients incorporated in the form of carbon and nitrogen sources. Additionally, it is also know that the composition of media evokes pH changes during growth and effects the bacteriocin production [3-5]. In the present study, different media ingredients and optimized physical, chemical parameters were identified to increase the bacteriocin production by MSB1 and MSB2 strains. The influence of different carbon and nitrogen sources, pH, temperature, incubation time, minerals and vitamins on bacteriocin production by bacterial isolates is revealed [6,7].

Objective

The objective is enhancement of bacteriocin production from bacterial isolates of dairy effluents by altering physio chemical parameters.

MATERIALS AND METHODS

Media and Chemicals

Bacteriological media were obtained from sigma, USA and HiMedia India while general chemicals and solvents of analytical grade were procured from S.D fine chemicals India respectively.

Innoculum Preparation

The strains of MSB1 and MSB2 were grown in MRS both at 37°C for 24 hrs. After incubation, cells are removed by centrifugation at 10,000 rpm for 10 min. The cell pellet was washed with sterile saline solution (0.83%. NaCl) and was resuspended in the same solution to a final optical density of 2.0 at 600 nm. This cell suspension was used as the inoculums for determining the growth pattern.

Analysis of the Samples for Bacteriocin Activity

The bacteriocin activity was measured (determined) by agar well diffusion method. To detect the inhibitory activity in the culture supernatant of two isolates, the culture supernatant fluids were obtained by centrifugation (6000 g/10 min) followed by neutralization with 2 N NaOH and sterilization through membrane filter and serial dilution in each medium. Aliquotes of 100 µl, 250 µl, 500 µl, were added to 5 mm diameter cells made in the MRS medium plates. These plates were spreaded with *Staphylococcus aureus*, *Salmonella typhi* as indicator organisms. The plates were incubated at 37°C for 24 hrs and examined for zone of growth inhibition. The bacteriocin activity unit per ml in a culture both was calculated by multiplying the highest dilution that gave a zone of at least 2 mm.

Influence of Carbon Source on Bacteriocin Production of MSB1 and MSB2

The effect of carbon source on bacteriocin production was evaluated using glucose, dextrose, fructose, sucrose, arabinose, xylose at 1.0% w/v by incorporating them separately along with other MRS ingredients.

Influence of Nitrogen Source on Bacteriocin production

The effect of peptone, tryptone, beefextract, soya and gelatin was evaluated for bacteriocin production by separately adding them to the MRS ingredients.

Influence of Vitamins on Bacteriocin Production

Vitamins like ascorbic acid, thiamine HCL, biotin, nicotinic acid, pyridoxine, and folic acid were added separately to medium to investigate their influence on bacteriocin production.

Influence of Minerals on Bacteriocin Production

Media were added with minerals like EDTA, $AgNO_3$, $KMNO_4$, NAf, B-me, DMSF to evaluate their influence on bacteriocin production of isolates.

Effect of Temperature on Bacteriocin Production

The effect of different temperature on bacteriocin production was carried out by incubating the plates at different temperatures like 30°C, 35°C, 40°C, 45°C, 50°C.

Effect of pH on Bacteriocin Production

Incubating the cultures at different pH values like 5, 5.5, 6, 6.5, 7, 7.5 and 8 will investigate the effect of pH on bacteriocin production of the isolates.

Effect of Incubation Period on Bacteriocin Production

The effect of incubation period on the production of bacteriocin was carried out. Media were incubated for different time periods like 25 hrs, 30 hrs, 35 hrs, 40 hrs, and 45 hrs respectively.

RESULTS AND DISCUSSION

In our present study the culture conditions were optimized for better bacteriocin production. Selected isolates are capable of producing bacteriocin activity against gram +ve and gram –ve organisms. Amoung the various carbon sources tested, dextrose was found to be effective in enhancing the bacteriocin production. Bacteriological peptone was found to be better when various nitrogen sources were tested, while the addition of thiamine HCL, ascorbic acid

and EDTA further increased the production of bacteriocin by the two microbial isolates [8]. It also found that at pH 7.5, and temp 35°C, incubation time of 35 hrs further enhanced the bacteriocin production.

Bacteriocin production by the isolates was studied by measuring the zone of inhibition (Agar well diffusion method) of indicator organisms *Staphyloccocus aureus* and *Salmonella typhi*

The influence of culture medium components on the production of bacteriocin was investigated and found that substantial enhancement in the production of bacteriocin when medium was supplemented with dextrose and bacteriological peptone. Apart from these, bacteriocin production was observed to be temperature and pH dependent [2,9].

Optimization of Medium Conditions for MSB1 (Tables 1-7)

Table 1: pH

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	5	-	-	-	-	-	-
2	5.5	-	-	-	-	-	-
3	6	-	-	5.8	-	-	-
4	6.5	-	4.2	5.3	-	5.1	8.1
5	7	3.9	6.5	9.2	5.1	8.4	9.8
6	7.5	-	-	5.3	5.5	8.9	10.2
7	8	-	-	-	-	-	-

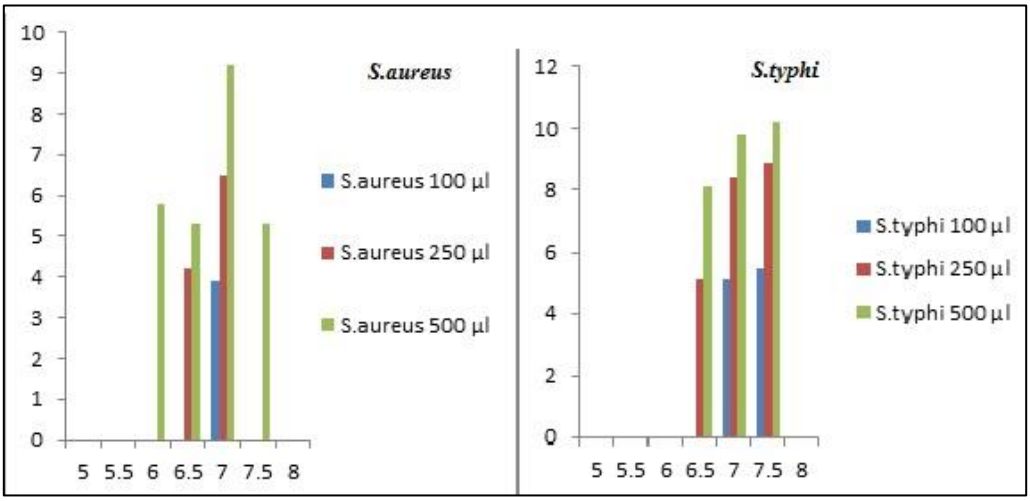


Table 2: Carbon source

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	Dextrose	4.1	8.7	11.3	4.9	10.7	12.8
-2	Glucose	-	3.9	9.8	3.6	3.9	5.8
3	Fructose	-	4.9	10.2	-	-	4.9
4	Sucrose	-	3.8	9.4	3.5	5.4	7.9
5	arabinose	-	3.8	6.4	-	-	8.4
6	xylose	3.6	3.9	6.8	-	3.6	6.5

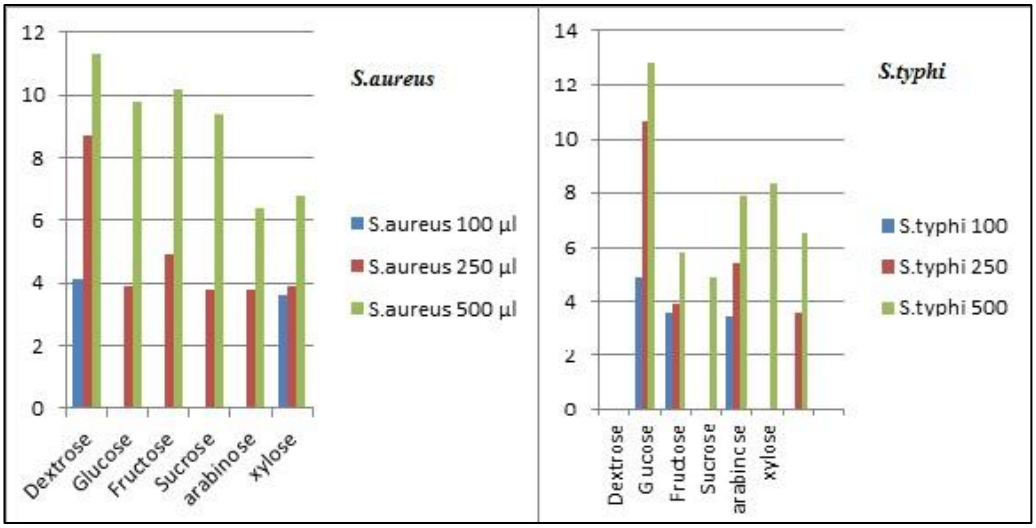


Table 3: Temperature

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	30ºc	-	-	9.1	-	-	4.8
2	35ºc	3.7	5.7	**10.4**	6.8	10.2	**14.1**
3	40ºc	4.2	5.9	8.6	-	-	-
4	45ºc	-	-	-	-	-	-
5	50ºc	-	-	-	-	-	-

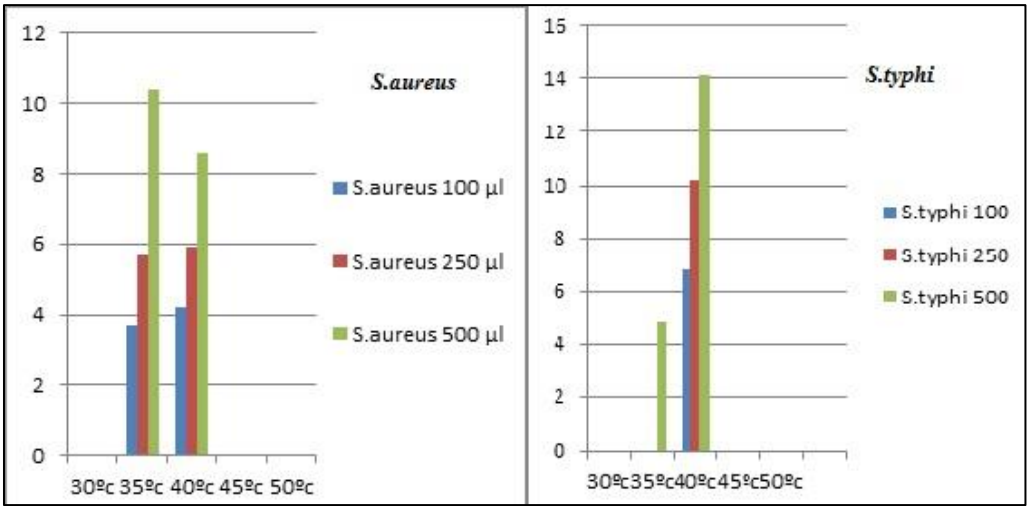


Table 4: Nitrogen source

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	Peptone	3.9	6.7	**12.9**	5.5	8.7	**12.8**
2	Tryptone	-	4.1	6.9	-	5.9	8.9
3	Beef extract	-	4.3	7.1	-	-	6.5
4	Soya	-	-	6.1	3.7	3.9	4.9
5	gelatin	-	-	3.8	-	-	-

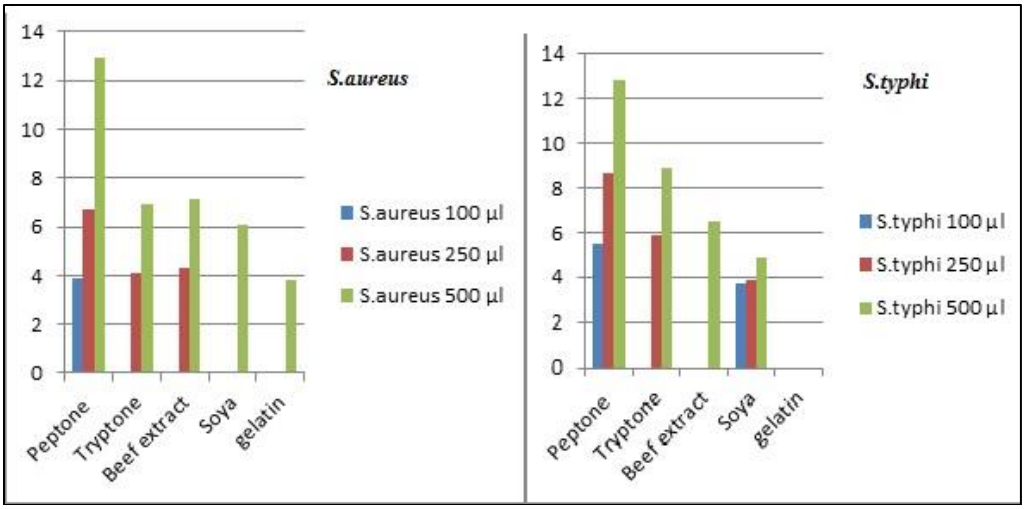


Table 5: Vitamins

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	Ascorbic acid	-	3.9	**10.7**	6.8	8.4	**12.3**
2	Thiamine HCL	-	4.2	9.3	3.9	7.8	11.8
3	Biotin	4.4	7.3	9.1	-	5.3	9.7
4	Nicotinic acid	3.6	6.7	8.7	3.7	5.1	7.3
5	Pyridoxine	3.5	6.5	9.1	-	-	9.2
6	Folic acid	3.7	6.3	8.6	-	4.2	8.1

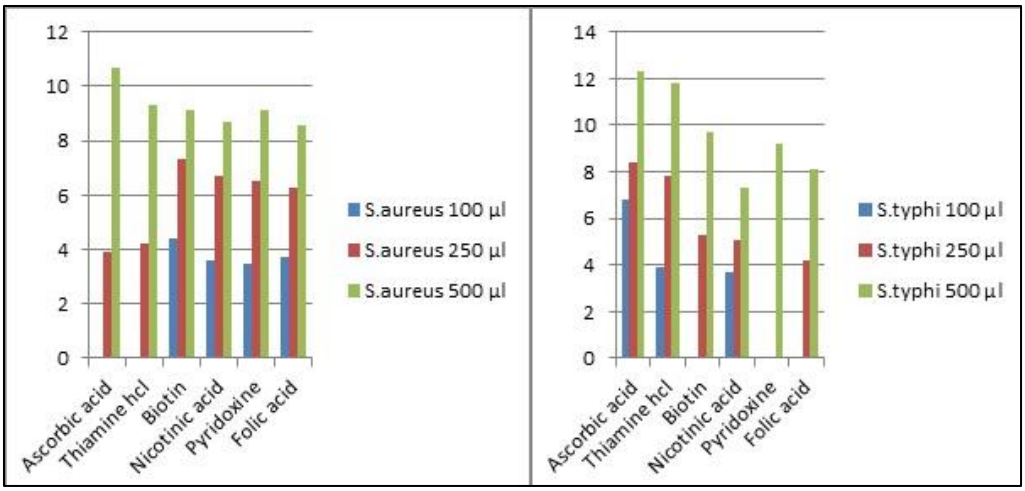


Table 6: Incubation time

S.no	Conditions	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	25hrs	3.6	5.9	**11.8**	5.3	8.9	**10.4**
2	30hrs	3.6	3.8	6.9	-	-	7.5
3	35 hrs	-	3.9	10.8	-	-	8.9
4	40 hrs	-	4.1	10.2	-	-	6.5
5	45 hrs	-	-	-	-	-	-
6	50 hrs						

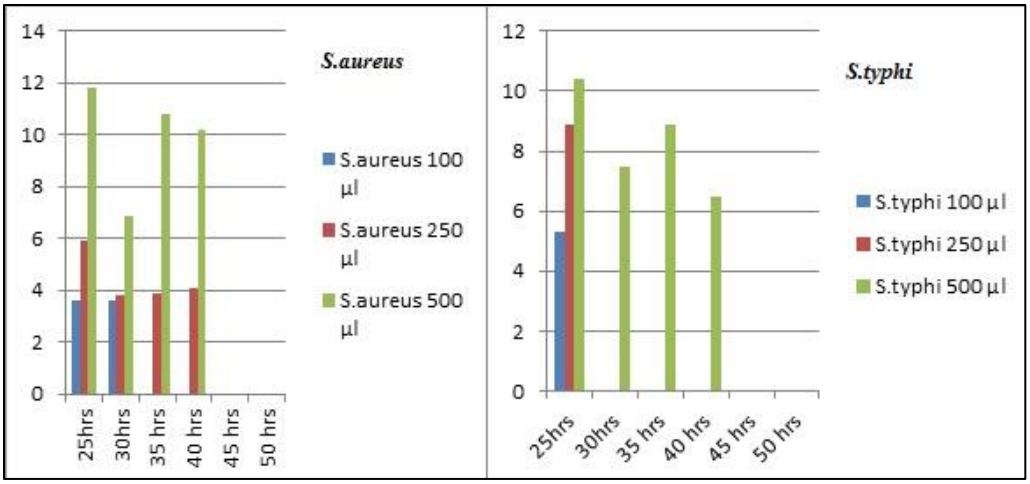
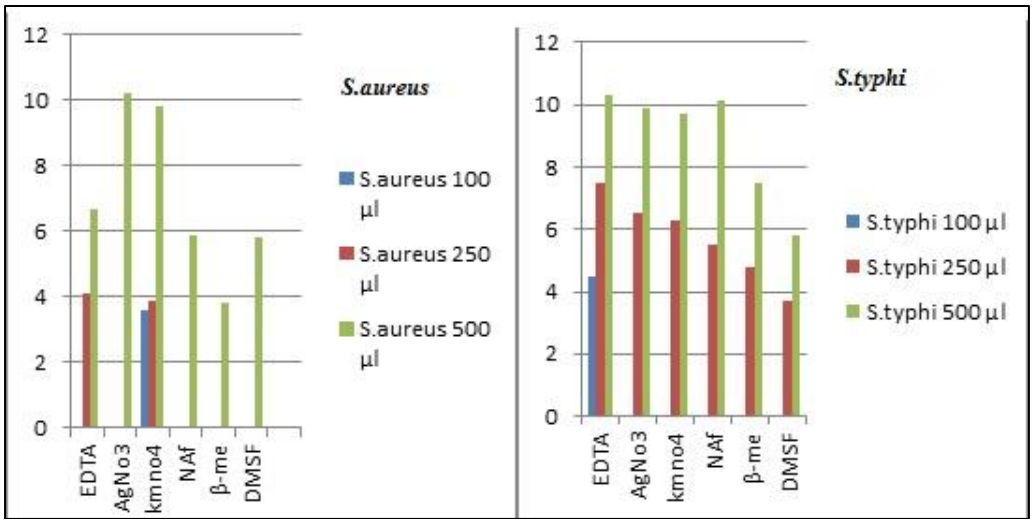


Table 7: Minerals

S.no	Condition	Zone of inhibition (in mm)					
	Minerals	*S. aureus*			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	EDTA	-	4.1	6.7	4.5	7.5	**10.3**
2	$AgNO_3$	-	-	**10.2**	-	6.5	9.9
3	$KMNO_4$	3.6	3.9	9.8	-	6.3	9.7
4	NAf	-	-	5.9	-	5.5	10.1
5	β-me	-	-	3.8	-	4.8	7.5
6	DMSF	-	-	5.8	-	3.7	5.8



Optimization of Medium Conditions for MSB2 (Tables 8-14)

Table 8: pH

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	5	-	-	-	-	-	-
2	5.5	-	-	-	-	-	-
3	6	-	5.8	-	-	-	-
4	6.5	-	4.2	5.3	-	5.1	8.1
5	7	3.9	6.5	9.2	5.1	8.4	9.8
6	7.5	-	-	5.3	5.5	8.9	10.2
7	8	-	-	-	-	-	-

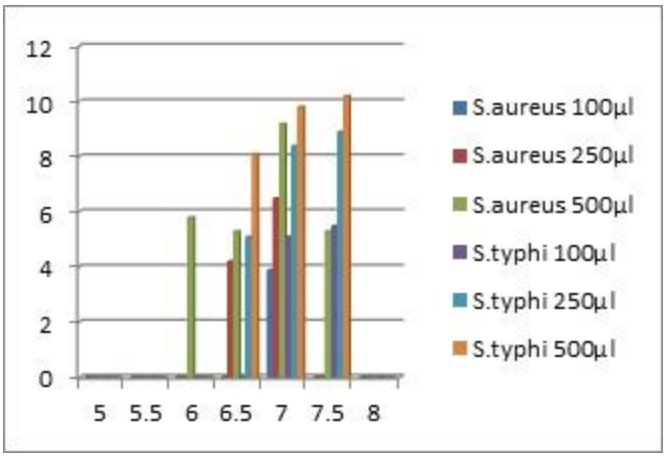


Table 9: Carbon source

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	Dextrose	4.1	8.7	11.3	4.9	10.7	12.8
2	Glucose	-	3.9	9.8	3.6	3.9	5.8
3	Fructose	-	4.9	10.2	-	-	4.9
4	Sucrose	-	3.8	9.4	3.5	5.4	7.9
5	arabinose	-	3.8	6.4	-	-	8.4
6	xylose	3.6	3.9	6.8	-	3.6	6.5

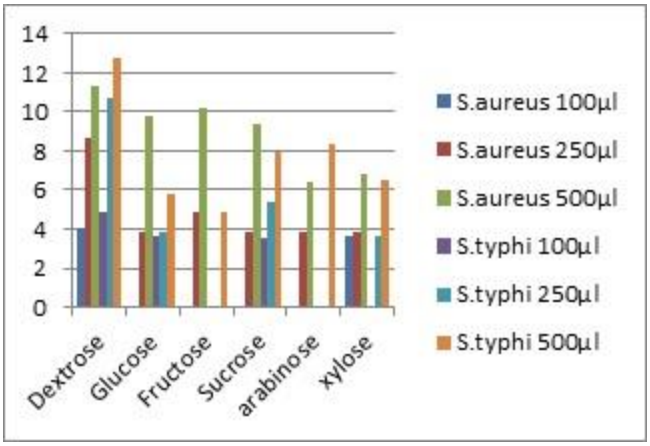


Table 10: Temperature

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	30°C	-	-	9.1	-	-	4.8
2	35°C	3.7	5.7	10.4	6.8	10.2	14.1
3	40°C	4.2	5.9	8.6	-	-	-
4	45°C	-	-	-	-	-	-
5	50°C	-	-	-	-	-	-

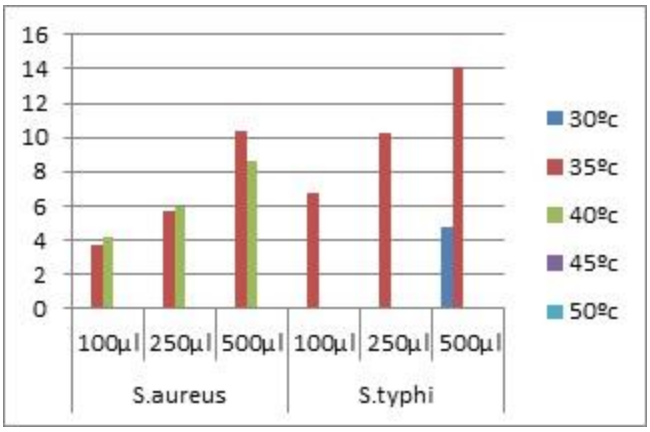


Table 11: Nitrogen source

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	Peptone	3.9	6.7	12.9	5.5	8.7	12.8
2	Tryptone	-	4.1	6.9	-	5.9	8.9
3	Beef extract	-	4.3	7.1	-	-	6.5
4	Soya	-	-	6.4	3.7	3.9	4.9
5	gelatin	-	-	3.8	-	-	-

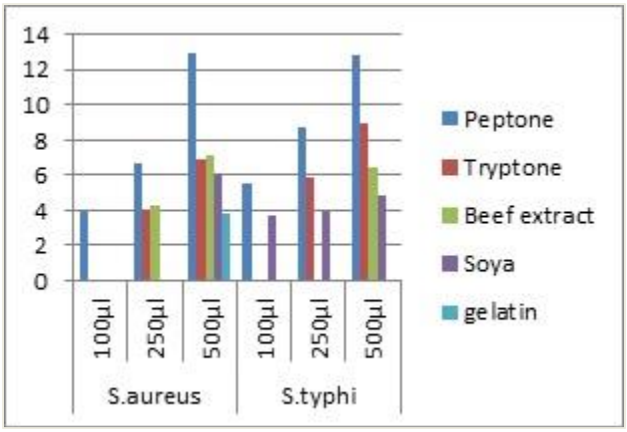


Table 12: Vitamins

S.no	Condition	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	Ascorbic acid	-	3.9	10.7	6.8	8.4	12.3
2	Thiamine HCL	-	4.2	9.3	3.9	7.8	11.8
3	Biotin	4.4	7.3	9.1	-	5.3	9.7
4	Nicotinic acid	3.6	6.7	8.7	3.7	5.1	7.3
5	Pyridoxine	3.5	6.5	9.1	-	-	9.2
6	Folic acid	3.7	6.3	8.6	-	4.2	8.1

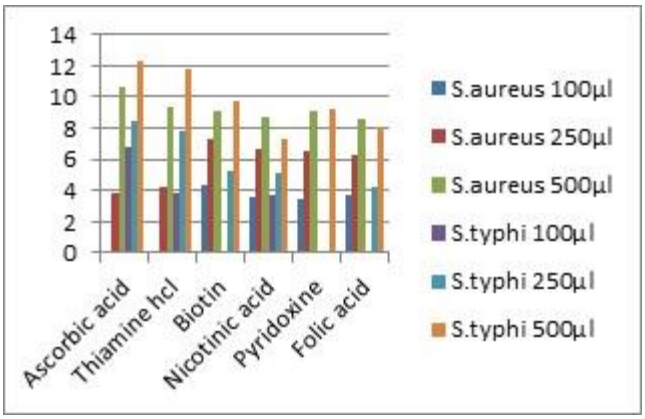


Table 13: Incubation time

S.no	Conditions	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	25 hrs	3.6	5.9	11.8	5.3	8.9	10.4
2	30 hrs	3.6	3.8	6.9	-	-	7.5
3	35 hrs	-	3.9	10.8	-	-	8.9
4	40 hrs	-	4.1	10.2	-	-	6.5
5	45 hrs	-	-	-	-	-	-
6	50hrs	-	-	-	-	-	-

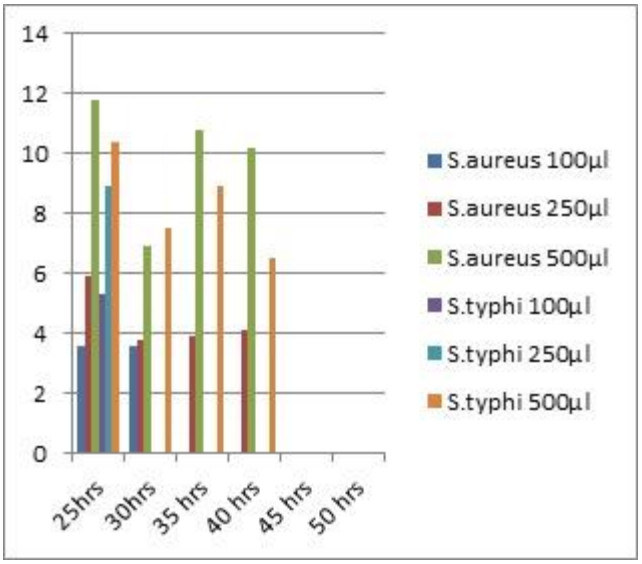
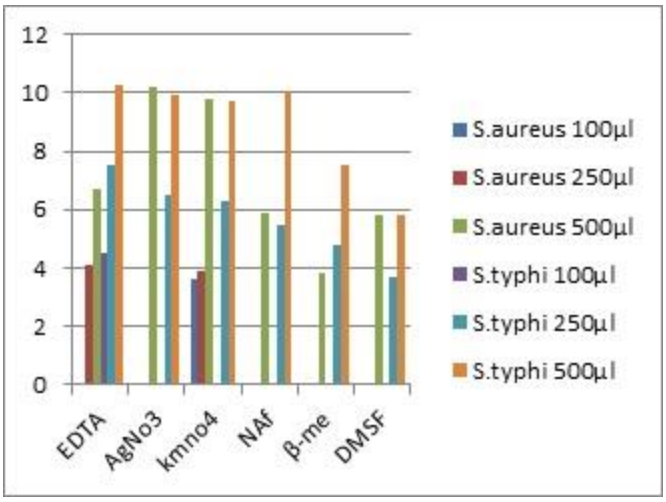


Table 14: Minerals

S.no	Condition Minerals	Zone of inhibition (in mm)					
		S. aureus			*S. typhi*		
		100 µl	250 µl	500 µl	100 µl	250 µl	500 µl
1	EDTA	-	4.1	6.7	4.5	7.5	10.3
2	$AgNO_3$	-	-	10.2	-	6.5	9.9
3	$KMNO_4$	3.6	3.9	9.8	-	6.3	9.7
4	NAf	-	-	5.9	-	5.5	10.1
5	β-me	-	-	3.8	-	4.8	7.5
6	DMSF	-	-	5.8	-	3.7	5.8



CONCLUSION

Bacteria present in the dairy effluents are fastidious organisms. They are known to have limited biosynthetic ability, thus requiring multiple amino acids and vitamins for growth. These growth factors are usually supplied by a complex nitrogen sources like yeast extract, Soya peptone and bacteriological peptone. Bacteriocin production is strongly dependent on pH, temperature, and incubation time. Our results showed that carbon as well as nitrogen sources played a major role in increasing the bacteriocin production [10,11].

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