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OPTIMIZATION OF SUITABLE PHYSIO-CHEMICAL PARAMETERS FOR ENHANCED BACTERIOCIN PRODUCTION BY BACTERIA PRESENT IN DAIRY EFFLUENTS

Y Evangelin[1] and K Kasturi[2*]

[1*]Research Scholar, Department of Bio-Technology, Acharya Nagarjuna University, Guntur-522510, Andhra Pradesh, India
[2]Assistant Professor, Department of Bio-Technology, Acharya Nagarjuna University, Guntur - 522510, Andhra Pradesh, India

***Corresponding Author Email: kasturi.is.kondapalli21@gmail.com**

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^{0}C and incubation time 25hrs-35hrs. Bacteriocins produced by these bacterial isolates (MSB1, MSB2) displayed a wide spectrum of intibition againt pathogens Staphylicoccus aureus, Salmonella typhi employed as test strains.

KEY WORDS

Bacteriocins, MRS broth, Chemical Parameters, Physical parameters, Staphylococcus aureus, Salmonella typhi

INTRODUCTION:

Most of the bacteria present in this dairy effluent 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 [1,4]. The possible use of bacteriocins as food bio preservatives could lead to the replacement of synthetic chemical preservatives which have their antimicrobial action reduced due to the continued appearance of multi resistant 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 known that the composition of media evokes pH changes during growth

and effects the bacteriocin production [2,3,5]. 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 [6,8].

Objective: 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 generals 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 37^0 c for 24hrs. After incubation, cells are removed by centrifugation at 10,000rpm for 10min. 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 600nm. 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 (6000g/10min) followed by neutralization with 2N NaOH and sterilization through membrane filter and serial dilution in each medium. Aliquotes of 100µl,250µl,500µl, were added to 5mm 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 37^0c for 24hrs 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 2mm.

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, beef extract, 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, Ag No3, Kmno4, 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 ata different temperatures like 30^0c, 35^0c, 400c, 45^0c, 50^0c.

Effect of pH on bacteriocin production:

Incubating the cultures at different p^H 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 25hrs, 30hrs, 35hrs,40hrs, and 45hrs respectively.

RESULTS AND DISCUSSION:

In our present study the culture conditions were optimized for better bacteriocin production. Selected isolates capable of producing bacteriocin activity against gram +ve and gram –ve organisms. Among 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 [11]. It also found that at pH 7.5, and temp 35^0c, incubation time of 35hrs 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 [2,10].

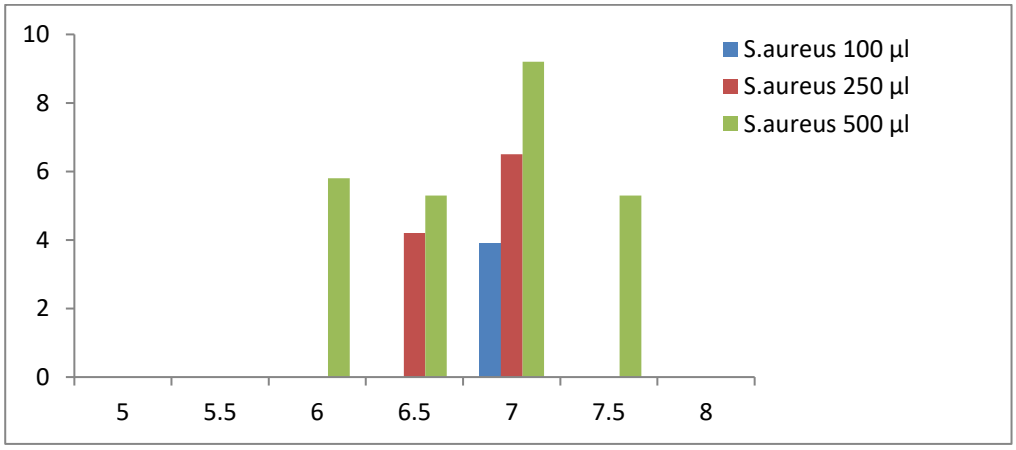
Bacteria present in the dairy effluents are fastidious organisms. They are known to have limited biosynthetic ability, thus requiring multiple amino acids and vitamins for growth. These growth factors are usually supplied by a complex nitrogen sources like yeast extract, Soya peptone and bacteriological 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 [7,9].

Optimization of medium conditions for MSB1

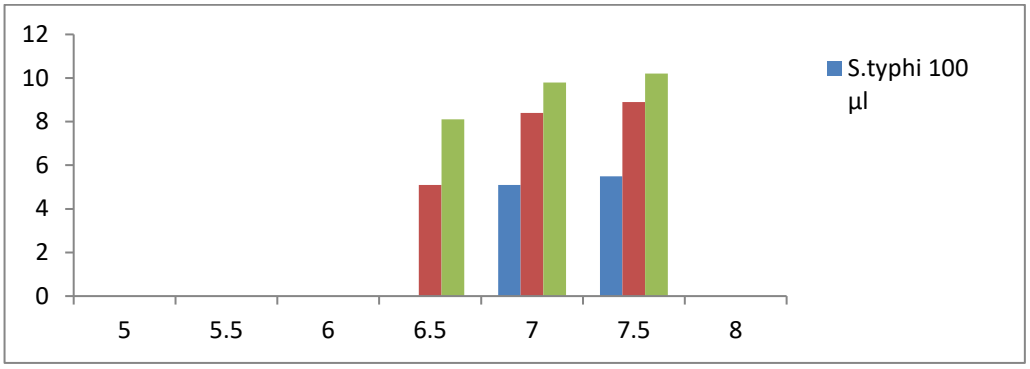
1.pH

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	-	-	-	-	-	-

S.aureus



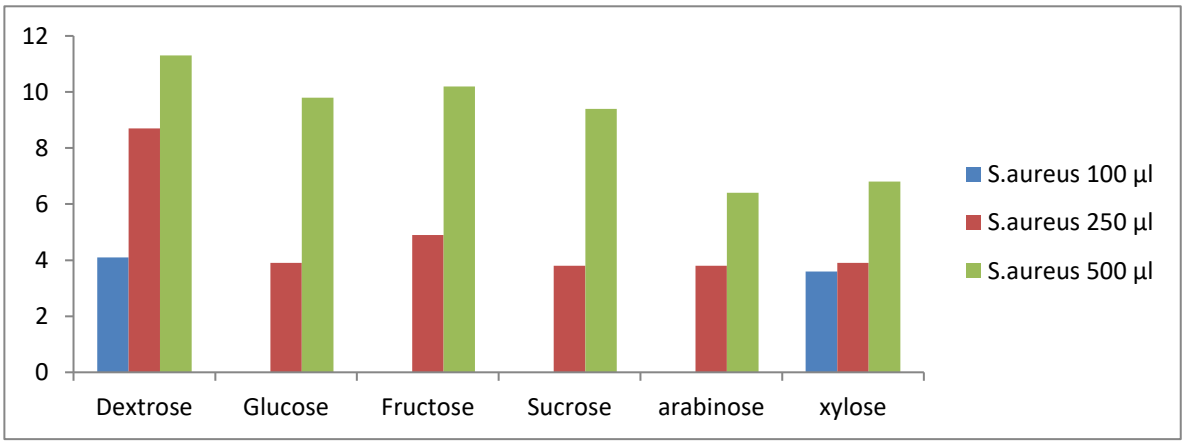
S.typhi



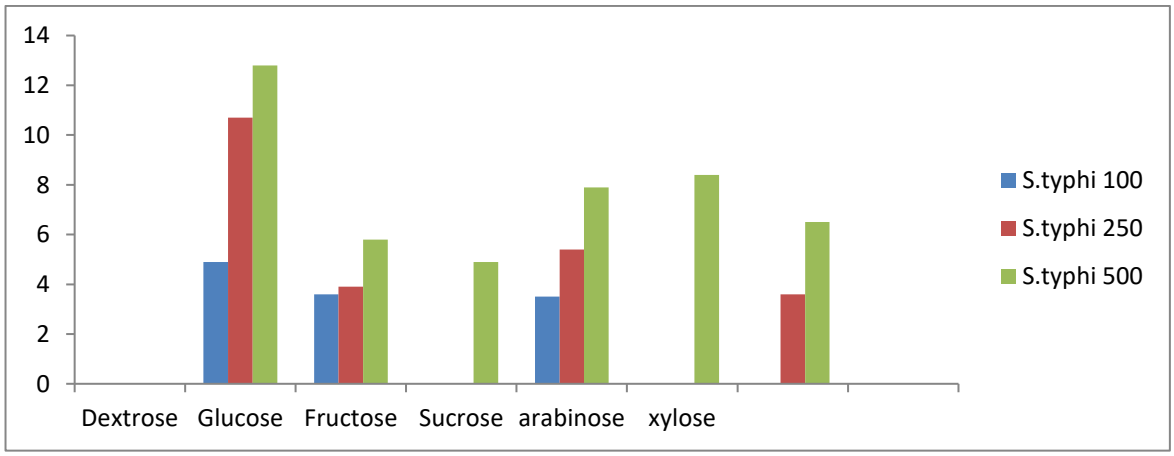
2. Carbon source

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

S.aureus



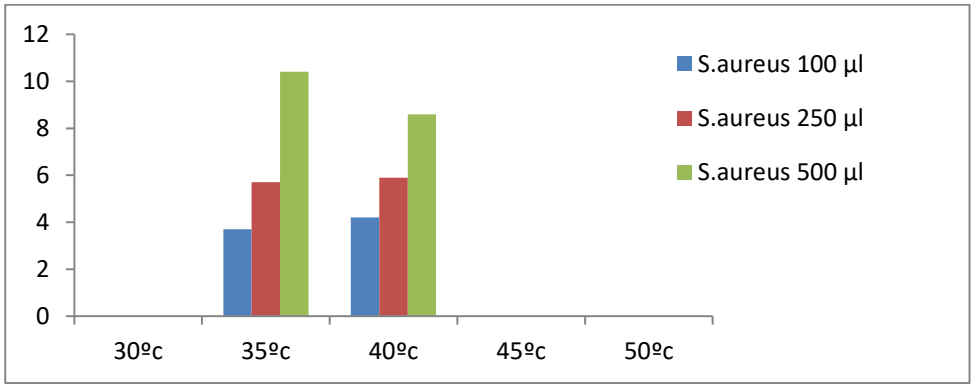
S.typhi



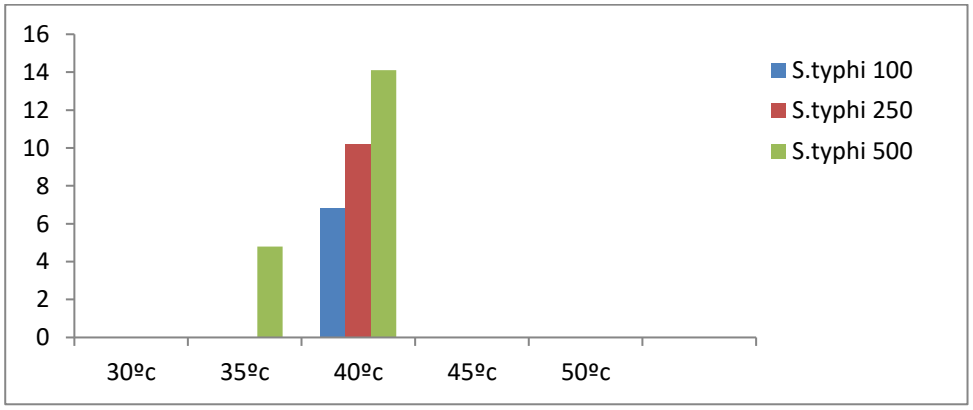
3. Tempertature

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	-	-	-	-	-	-

S.aureus



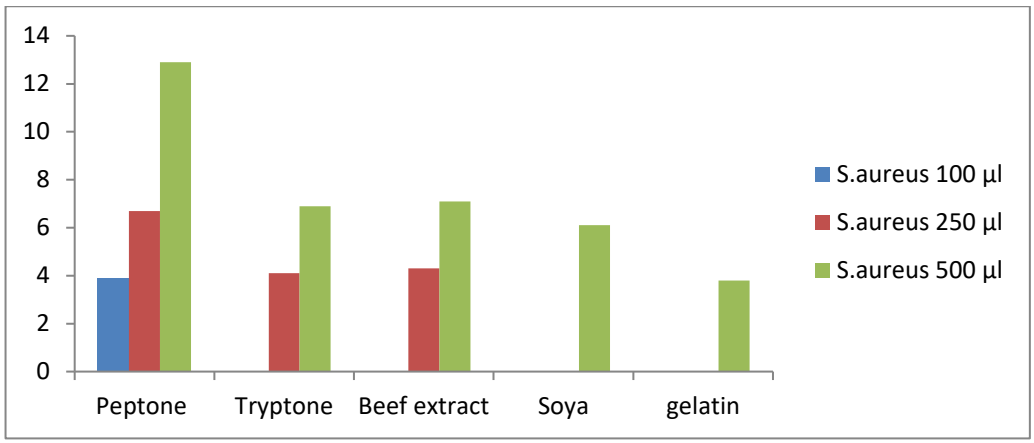
S.Typhi



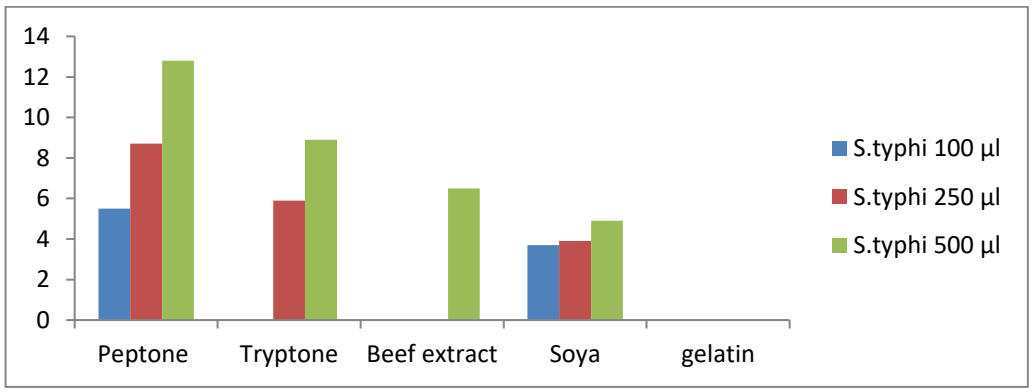
4. Nitrogen source

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	-	-	-

S.Aureus



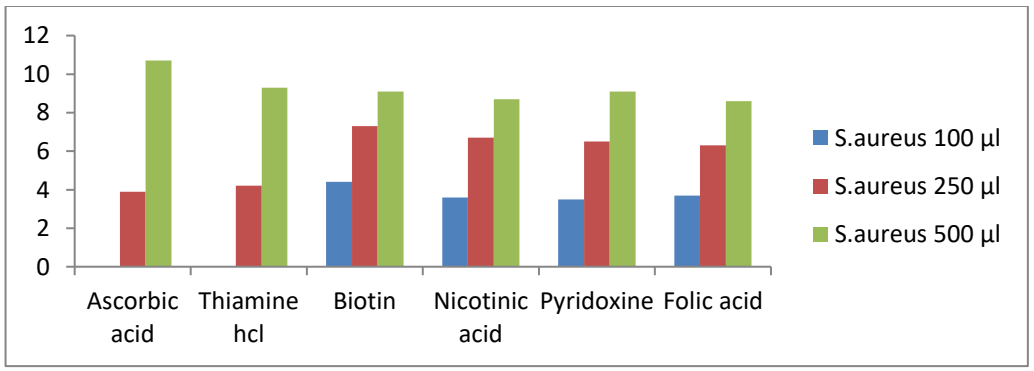
S.Typhi



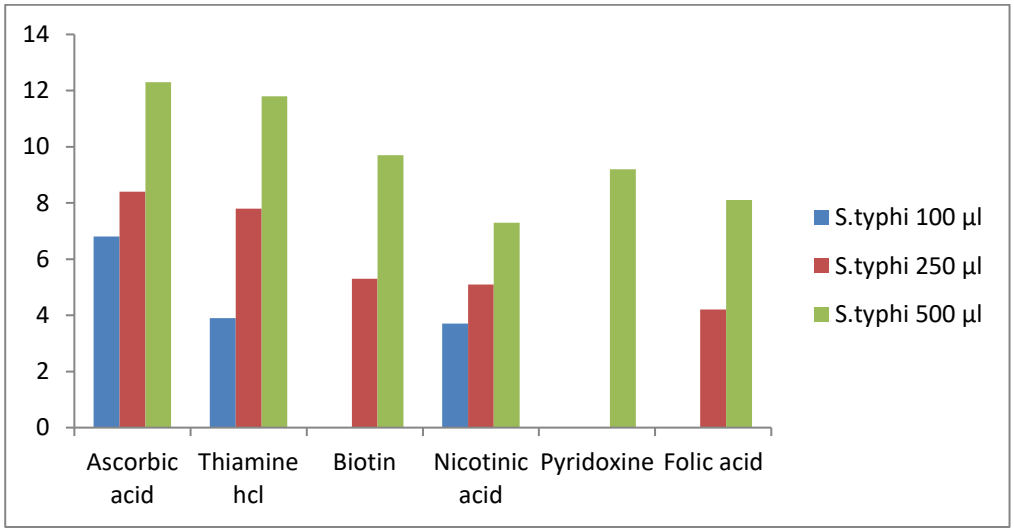
5. Vitamins

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

S.Aureus



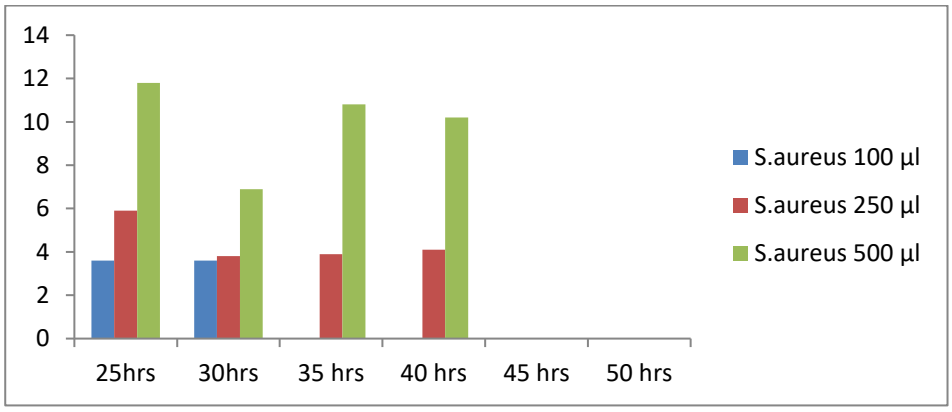
S.Typhi



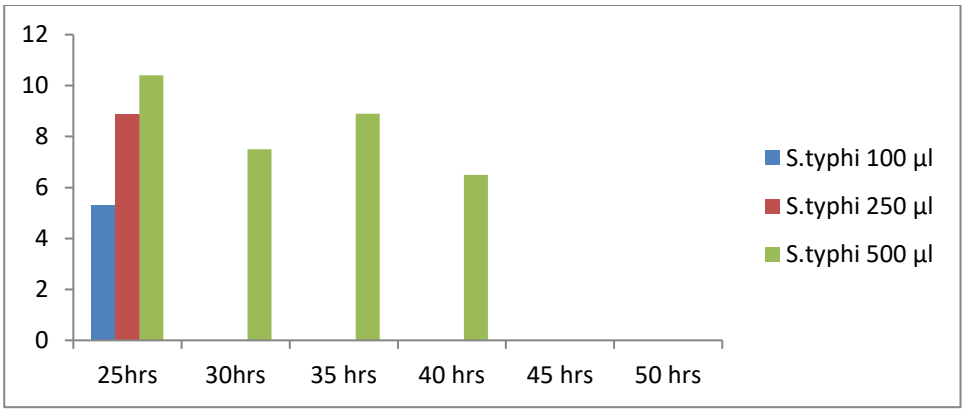
6. Incubation time

S.no	Conditions	Zone of inhibition (in mm)					
		S.aureus			S.typhi		
		100µl	250µl	500µl	100µl	250µl	500µl
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						

S.Aureus



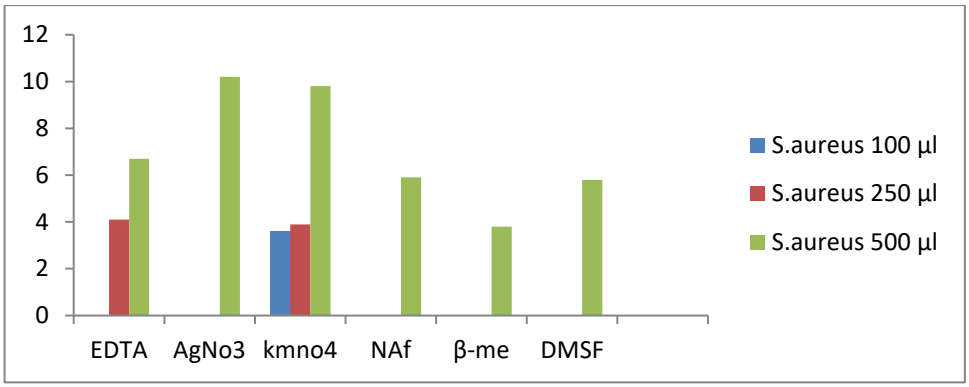
S.Typhi



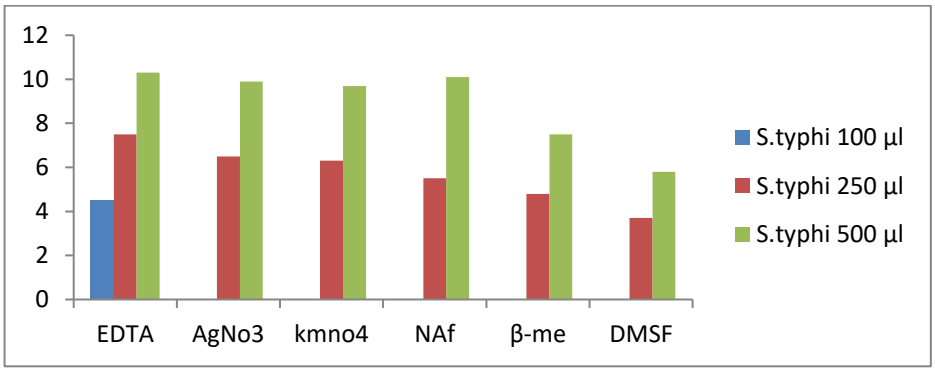
7. Minerals

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

S.Aureus



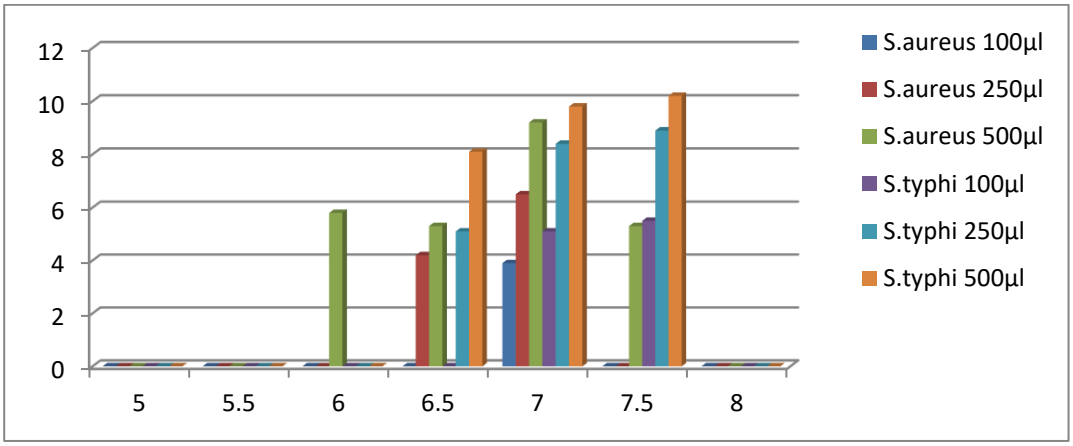
S.Typhi



Optimization of medium conditions for MSB2

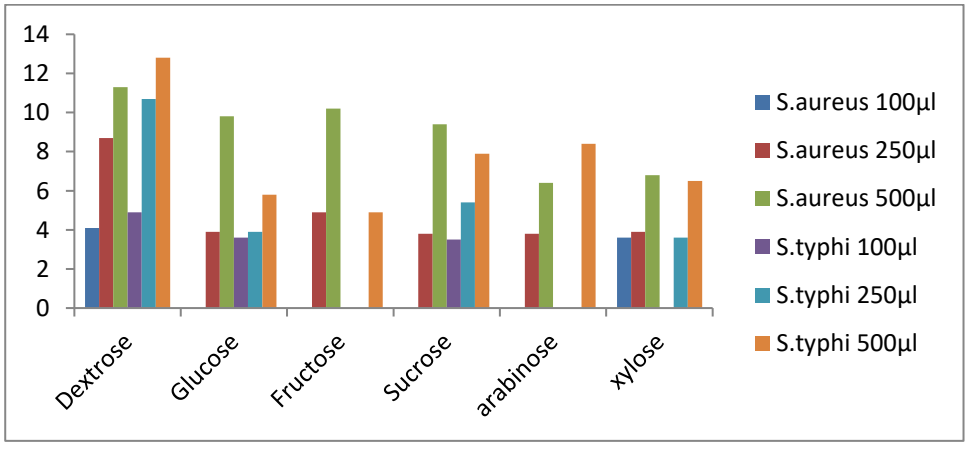
1.pH

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	-	-	-	-	-	-



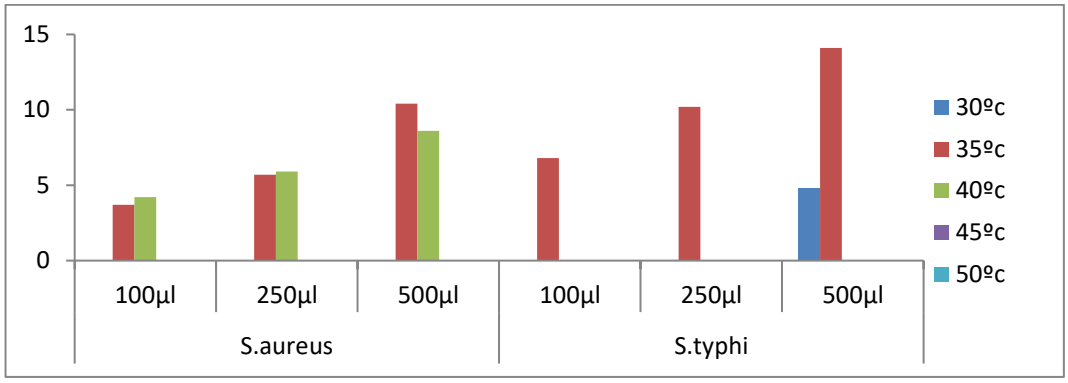
2.Carbon Source:

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



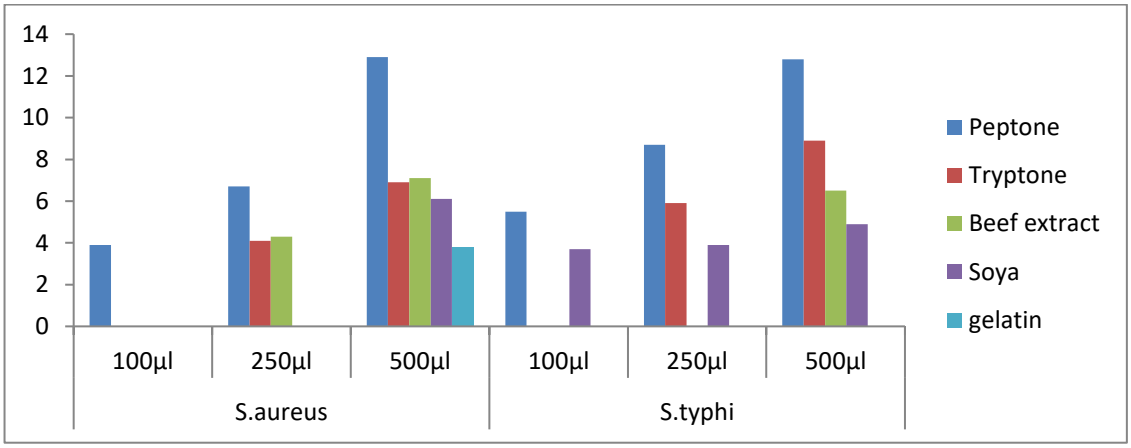
3.Temperature:

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	-	-	-	-	-	-



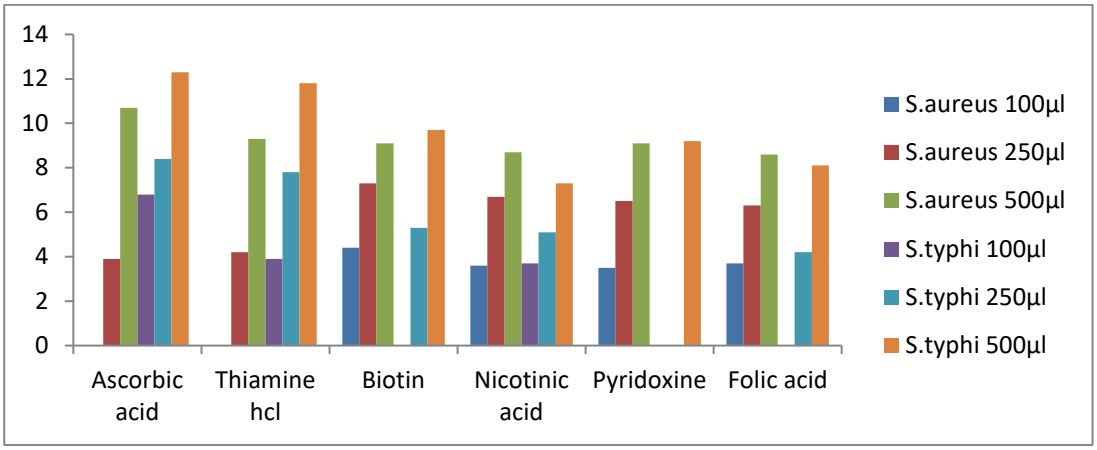
4.Nitrogen Source

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	-	-	6.4	3.7	3.9	4.9
5	gelatin	-	-	3.8	-	-	-



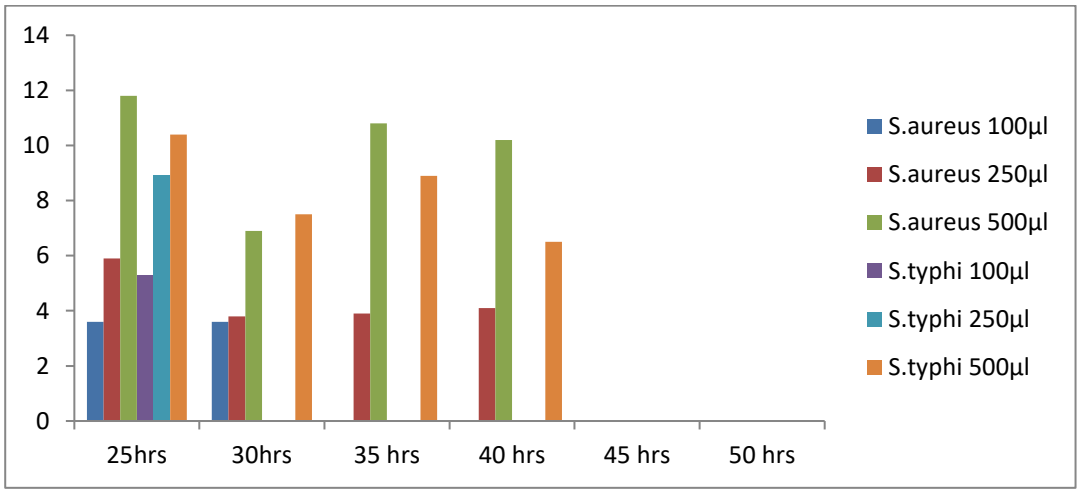
5.Vitamins

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



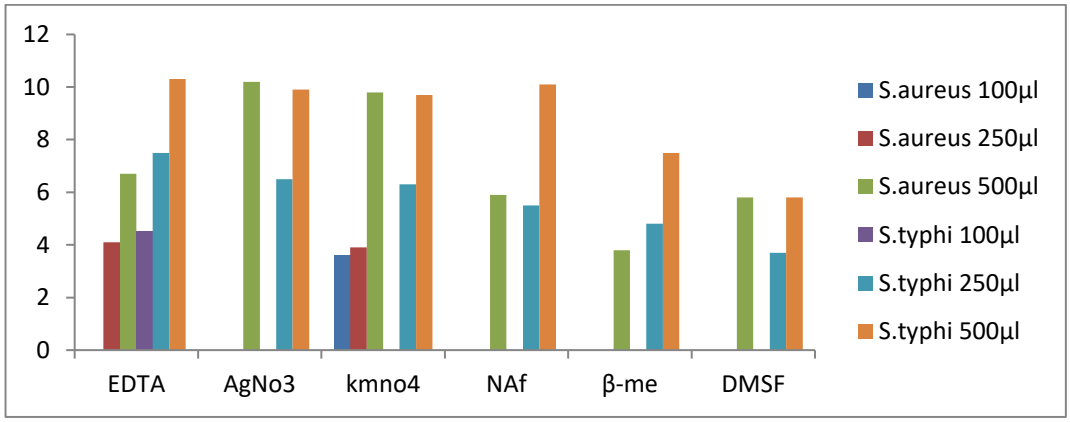
6.Incubation Time

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	50hrs	-	-	-	-	-	-



7. 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



REFERENCES:

[1] Klaenhammer T R, "*Bacteriocins of lactic acid bacteria*", Biochimie 70: [1988] pp.337-349.

[2] Parente E, Ricciardi A, "*Influence of PH on the production of enterocin 1146 during batch Fermentation" Lett* Appl Microbiology 19: [1994] pp.12-15.

[3] Tagg J R, *"Mc Given A R assay system for bacteriocin"* Appl microbiol 21: [1971] pp.125.

[4] Vignolo G M, Kariuz M N, Ruiz – Holgado AAP, Oliver G, *"Influence of growth conditions on the production of lactocin 705, a bacteriocin production by Lactobacillus casei CRL:705."* J Appl bacteriol 78: [1995] pp.5-10

[5] Torodov S, Gotcheva B, Dousset X, Onno B, Ivanova I, "*Influence of growth medium on Bacteriocin production*".

[6] Bala subranayam B V, Varadaraj M C. *"Ciltural conditions for the production bacteriocin by a native isolate of L. Delbruck, SSP, Bulgaricus CFR 2028 in milk medium".* J. appl. Microbiology 84: [1998] pp.97-102.

[7] Data H, Lacrocrix C, Hang J, Simard R E, "*Influence of growth conditions on production and activity of mesenteric 5 by a strain of Leuconostoc mesenteries".* Appl Microbiol. Biotechnol. 39: [1993], pp.166 – 173.

[8] Graciela M, Vignolo M, dekaruz M, Aida de ruiz H, Oliver G *"Influence of growth conditions on the production of lactocin 705, a bacteriocin produced by L.casei CRL 705",*J.appl.bacteriol. 78: [1995] 5-10.

[9] Ogunbanwo S T, Sanni A I, Onilude A A, *"Influence of cultural conditions on the production of Bacteriocin by lactobacillus brevis OGI".* Afr.J. biotechnol 2: [2003] pp.179-184.

[10] Aasen I M, Moretro T. Katla, T. Axelsson L. *"Influence of complex nutrients, temp and p^H on Bacteriocin production by lactobacillus sakei CCUG 47687".* Appl. Microbial. Biot 53: [2000] pp. 159-166.

[11] Sourav B and Arijit D. *"Study of physical and cultural parameters on the bacteriocin produced by lactic acid bacteria isolated from traditional Indian fermented foods",* American Jounal of Food Technology 2010, 5(2) pp.111-120.

***Corresponding Author:**
Y Evangelin*
Email: evangelin_y@yahoo.com