ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 7, Issue 2, pp: (308-316), Month: April - June 2019, Available at: www.researchpublish.com

Optimization of production of bacteriocin from *Staphylococcus aureus*

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Abstract: **The aim of the study was to extract the Bacteriocin from *Staphylococcus aureus* and optimize its production using different components and conditions. The research was also designed to know the effect of extracted Bacteriocin on different pathogenic bacteria. The production of Bacteriocin at the commercial level is very cost effective and limited. As many bacteria have the ability to produce Bacteriocin. *Staphylococcus aureus* was used as a source for the production of Bacteriocin. The study was designed to isolate *Staphylococcus aureus* from skin followed by the extraction of Bacteriocin. The effect of extracted Bacteriocin was checked on various pathogenic bacteria such as *Pseudomonas, E.coli, Listeria, Lactobacillus, Klebsiella* and also optimization of the process of Bacteriocin production was performed. The optimization parameters used were temperature, pH, different carbon sources, nitrogen sources and varying salt concentration. The media containing Glucose as a carbon source, Beef extract as a nitrogen source, having 1% salt concentration at pH 6 and at 32 ^{0}C temperature yielded maximum Bacteriocin production. The study concluded that Bacteriocin produced from *Staphylococcus aureus* is effective against pathogenic bacteria and hence can be used as a food preservative.**

***Keywords*: Bacteriocin, BHIB, Optical density, Aureocins.**

I. INTRODUCTION

Bacteriocins are biologically active proteins that show antimicrobial activity against species closely related to the bacteriocin producer. These proteinaceous compounds synthesized by both Gram (+) and Gram (−) bacteria. Bacteriocins produced encoded by either chromosomally or by the plasmid. Plasmid encoded the Bacteriocin vary in size considerably. Some plasmids are found to have genetic information of several Bacteriocins [01]. The production of Bacteriocin and its activity is affected by various physiochemical factors. Bacte riocin production depends on various factors like pH, source of nutrients and temperature Bacteriocin can be used as safe natural bio preservatives as they are sensitive to proteases in the gastrointestinal tract and are effective in controlling foodborne pathogens [02].

Staphylococcus is bacteria belong to the Micrococcaceae family and a genus of Gram + nonspore forming cocci. The antimicrobial substance produced by *Staphylococcus* called Staphylococcins. It can inhibit several bacterial pathogens, therefore, may have practical applications as possible alternatives to antibiotics in medicine and also used as bio preservatives in the food industry [03].

Staphylococcus aureus strains have been investigated to produce different types of Bacteriocin. These bacteria are called Bac+ strains. Some Bacteriocins produced by *Staphylococcus aureus* are Aureocin A70 [04], Aureocin A53 [05], MB92, 146L and 215FN. These Bacteriocins are found to be heat resistant and have the ability to inhibit a variety of pathogenic bacteria e.g. *Listeria monocytogenes.* Aureocin A53 inhibits the growth of pathogens involved in a disease like bovine mastitis [06]. All these Bacteriocin produced are plasmid encoded. The Bacteriocin has known as Bsa (bacteriocin of *Staphylococcus aureus*) also called lantibiotic is encoded by chromosome [07].

The characterization of Bacteriocin performed revealed the effect of pH, heat, detergents, salt and proteolytic enzymes. Greater antimicrobial activity of Bacteriocin has been found at low pH value. [08].

Other Bacteriocins extracted from *Staphylococcus aureus* are staphylococcin C55/BacR1 [09], and 4185 [10]. As per the classification of Bacteriocins, Bacteriocin Bsa and staphylococcin C55 both belong to class lantibiotics. Aureocins A53 and A70 are class II Bacteriocins. Aureocin A70 is heat stable and encoded within a mobilizable 8 kb plasmid known as pRJ6. This Bacteriocin is found effective against *Listeria monocytogenes*. [11] Bsa is identical to the previously identified bacteriocin staphylococcin Au-26, produced by an *S. aureus* strain of vaginal origin. [12] *S.epidermis* and *S. aureus* are the most important Staphylococcin producer investigated so far.

The present study has been designed to check the inhibitory effect of Bacteriocin on pathogenic microorganism as well as to optimize the process of production of bacteriocin produced from the *Staphylococcus aureus*.

II. MATERIAL AND METHODS

Isolation of *Staphylococcus aureus*

The Isolation of *Staphylococcus aureus* was done from skin samples using a sterile swab. The sample was inoculated on the selective media Mannitol Salt Agar (MSA) and Brain Heart Infusion Agar and incubated for 24 hr at 37 ^{0}C. The subculturing was done for obtaining a pure culture.

Identification of *Staphylococcus spp.*

The identification of bacterial isolates was done based on morphological and biochemical characteristics following standard tests [13].

Production of Crude Bacteriocin:

For the production of Bacteriocin, 24hr overnight culture of *S. aureus* was taken and centrifuged at 10,000 rpm for 10 min. at 4 ^{0}C. The pellet was discarded and the supernatant was used as crude Bacteriocin [14].

Assay for bacteriocin production

Bacteriocin production by bacterial isolates was done using agar well diffusion method [15]. The Indicator strains used to check the Bacteriocin activity were *Pseudomonas, E.coli, Listeria, Lactobacillus, Klebsiella*. Mueller Hinton Agar plates were inoculated with indicator strain and Bacteriocin was added into the well. After 24 hr formation of Zone of Inhibition was observed onto the plates.

Optimization of Bacteriocin production

The effects of the medium components on the Bacteriocin production was analyzed by growing the strain in different medium components using stranded methods [16], [17].

Effect of pH

To check the effect of pH on Bacteriocin production the Bacteriocin producing strain was inoculated in the 100ml BHI Broth in four different flasks having different pH values. The values of pH taken were 4,5,6,7,8 adjusted with 1 N HCl or 1 N NaOH. Following the inoculation, the culture was incubated at 37 °C for 24 h. After incubation, the absorbance values were determined at 280 nm and the Bacteriocin activity was accessed.

Effect of temperature

To check the effect of temperature on Bacteriocin production the Bacteriocin producing strain was inoculated in the 100ml BHI Broth in different flasks. The incubation of flask was done at different temperature range i.e. 28 °C, 30 °C, 32 °C, 35 °C, 37 °C, 40 °C and 42 °C for 24 hr. After incubation, the absorbance values were determined at 280 nm and the Bacteriocin activity was accessed.

Effect of carbon sources

The effect of Carbon source on the Bacteriocin production was checked using the different carbon sources such as glucose, lactose, sucrose, fructose and maltose 2 % (w/v) in BHI Broth. After the sterilization, the media was inoculated with Bacteriocin producing strain and incubated at 37 °C for 24 h. Bacteriocin value was determined by measuring the absorbance at 280 nm after incubation.

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Effect of Nitrogen sources

The effect of Nitrogen source on the Bacteriocin production was checked using the different nitrogen sources such as yeast Extract, Beef extract, Peptone, Tryptone and Ammonium Chloride 2 % (w/v) in BHI Broth. After the sterilization, the media was inoculated with Bacteriocin producing strain and incubated at 37 °C for 24 h. Bacteriocin value was determined by measuring the absorbance at 280 nm after incubation

Effect of NaCl

The Bacteriocin production also affected by varying salt concentration. The effect was checked by using different salt concentrations like 1%, 2%, 3% and 5% in NHI Broth. The broth was inoculated with Bacteriocin producing strain and incubated at 37 °C for 24 h. Absorbance values were determined at 280 nm.

Statistical analysis

All experiments were carried out in triplicates. Data obtained were analyzed by a one-way analysis of variance (ANOVA). Differences were considered significant at $p<0.05$

III. RESULT AND DISCUSSION

The Bacteriocin is produced from the *Staphylococcus aureus* isolated from the skin. Out of 22 samples of skin swab 5 samples were identified as S. aureus and used for the production of Bacteriocin. The selective media Brain heart infusion broth and Mannitol Salt Agar were used for the isolation of strain (Fig.1). The Gram staining was performed for the identification of isolates showed as round shaped clusters and purple colored colonies. The other biochemical tests used for the identification of the bacteria were Citrate test, catalase test, oxidase test, Methyl red Test.

According to previous studies it has been suggested that production of maximum Bacteriocin has corresponded to the maximum density of cell of producer strain. This is due to the fact that Bacteriocin is the primary metabolite of the microbial cell. [18], [19], [20].

There is an effective reduction in the production of Bacteriocin has been found at concentration 2.5gm and 3.5 gm/100ml of NaCl in culture media. At 37 ^{0}C temperature, there is the highest production of Bacteriocin has been found.

Research performed for the optimization of Bacteriocin has shown the effects of nutrient components and conditions on the production of Bacteriocin. The study revealed that Bacteriocin production was less at low temperature and high salt concentration. This investigation is relevant to our research. [21]

The study has been found that the most effective carbon source is Glucose followed by lactose, sucrose, fructose and Maltose. There is an increase in the production of Bacteriocin has been found in the case of Yeast extract used as a Nitrogen source. Ammonium Chloride was found to be less effective from all other Nitrogen sources i.e. Peptone, Tryptone and beef extract used.

A wide range of pH has been used to check the effect of pH and it has been concluded that Bacteriocin production is high at pH 6- 7. There is a remarkable reduction of Bacteriocin has been found at pH below 6 and above 7.

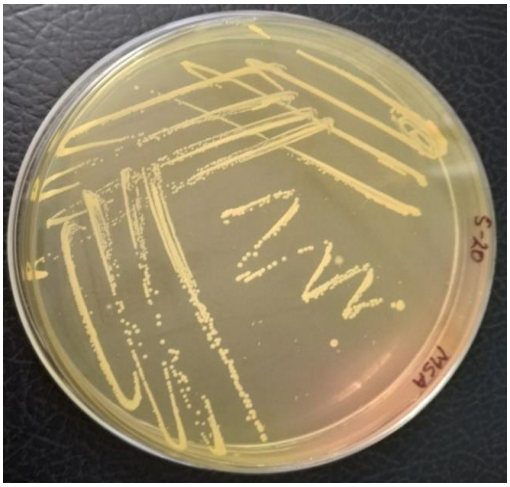

Fig: 1 *Staphylococcus aureus* colonies on Mannitol Salt Agar plate

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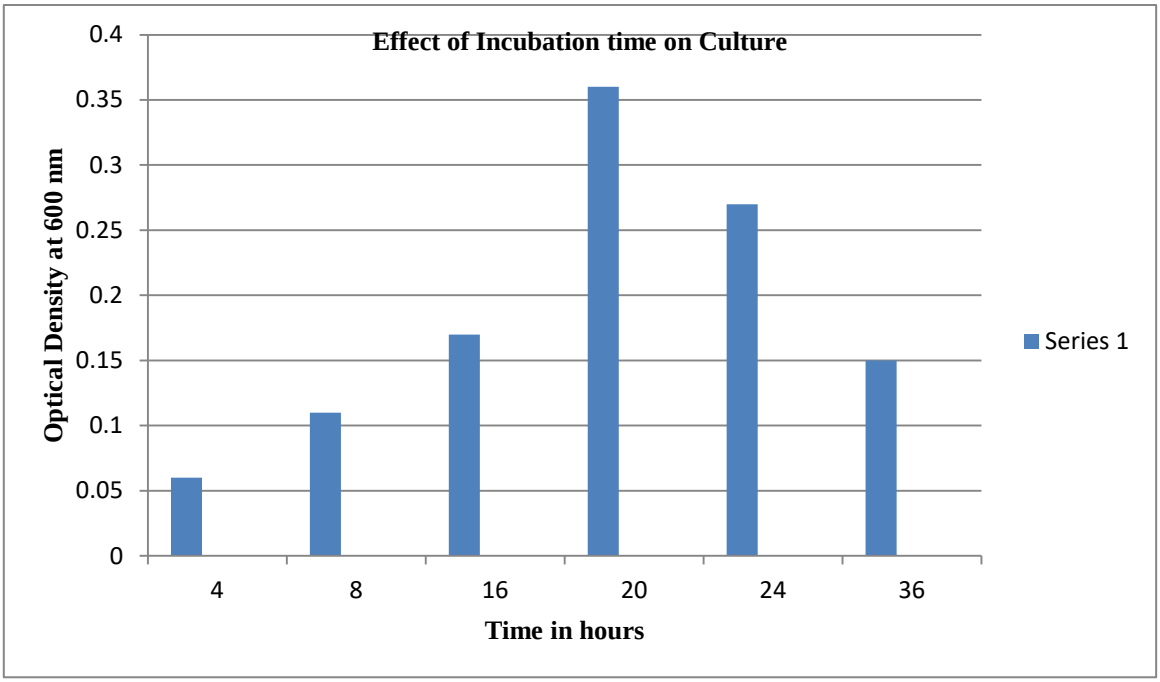
Table-1: Shows Zone of Inhibition found at different concentration of Bacteriocin on different indicator strains

Indicator Strain	ZOI (mm)			
	20 µl	**40 µl**	**60 µl**	**80 µl**
Pseudomonas sps	NI	NI	5	14 (HI)
E.coli	4	4	12	17 (VHI)
Listeria sps	NI	2	10	15 (HI)
Klebsiella sps	NI	2	9	14 (HI)

HI: High Inhibition, VHI : Very high Inhibition, NI : No inhibition

Table-2: Shows Optical Density of isolated culture at different Incubation Time

Time (in hr)	Optical Density (at 280nm) (Supernatant)			Mean	Std Dev.
	1	**2**	**3**		
4	0.063	0.058	0.068	0.06	0.01
8	0.105	0.112	0.098	0.11	0.01
16	0.167	0.178	0.154	0.17	0.01
20	0.335	0.289	0.442	0.36	0.08
24	0.268	0.298	0.248	0.27	0.03
36	0.148	0.159	0.136	0.15	0.01
48	0.121	0.132	0.134	0.13	0.01



Graph -1: Shows effect incubation time on culture growth

Table 3: Shows Optical Density of Supernatant at different incubation time

Time (in Hr)	Optical Density (at 280 nm) (Culture)			Mean	Std Dev
	1	**2**	**3**		
4	0.396	0.384	0.398	0.39	0.01
8	1.247	1.254	1.258	1.25	0.01
16	1.199	1.184	1.174	1.19	0.01

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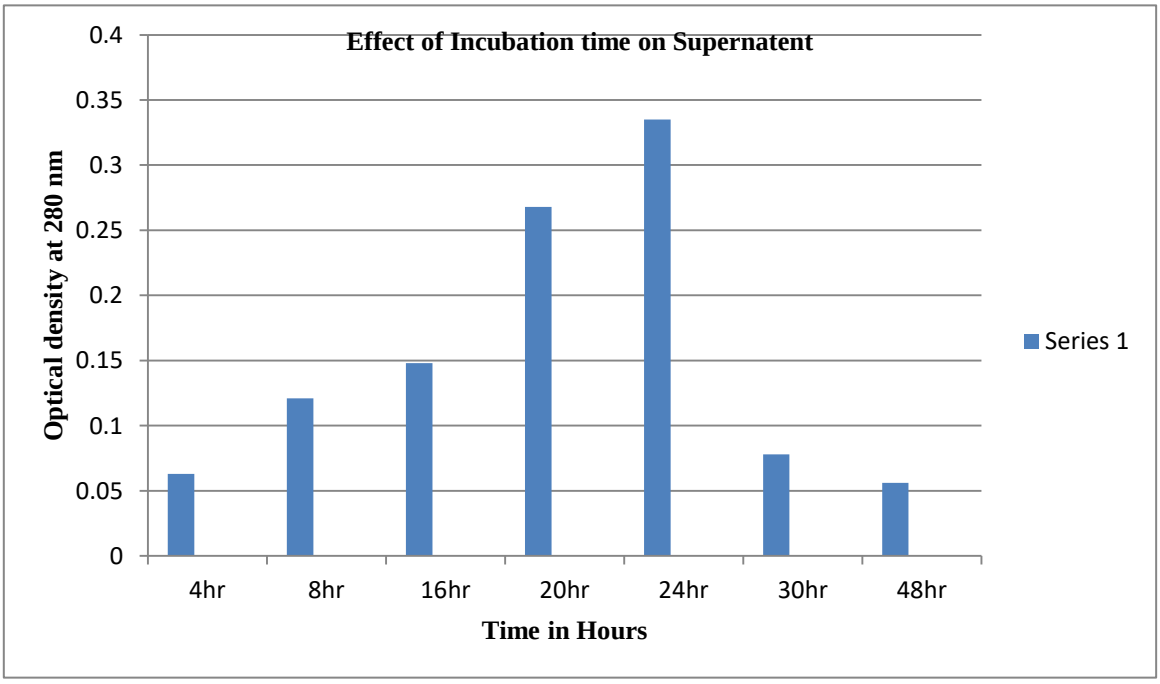
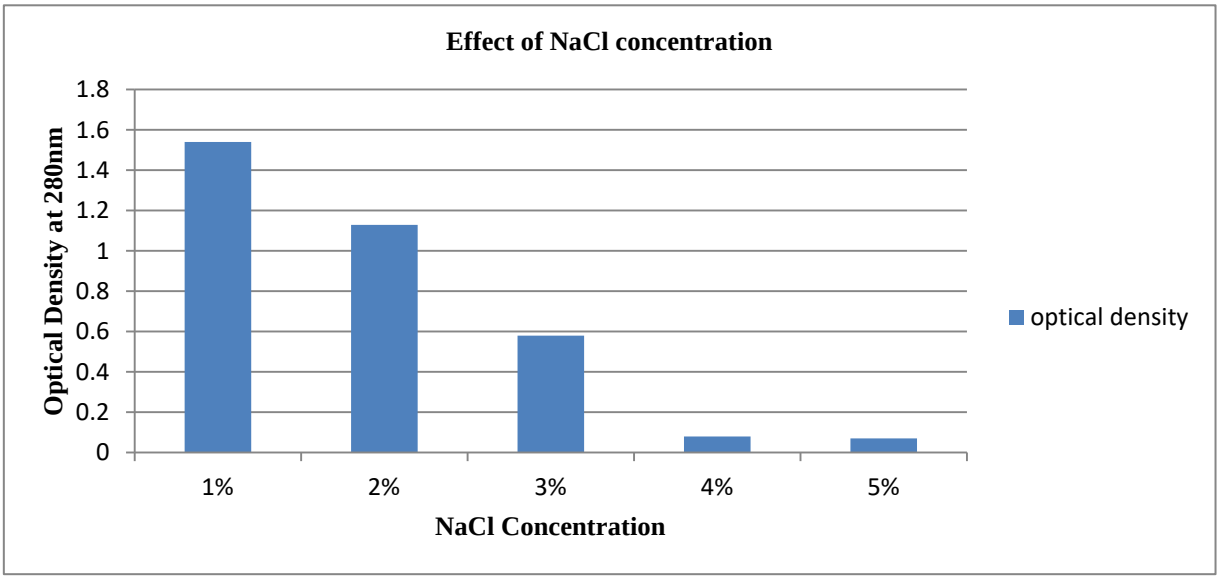
20	1.309	1.313	1.355	1.33	0.03
24	1.455	1.478	1.489	1.47	0.02
36	1.521	1.529	1.538	1.53	0.01
48	1.719	1.726	1.735	1.73	0.01



Graph -2: Shows effect incubation time on Supernatent

Table 4: Effect of NaCl concentration

NaCl concentration	Optical Density			Mean	Std Dev.
1%	1.539	1.546	1.524	1.54	0.02
2%	1.475	1.425	1.492	1.13	0.01
3%	1.178	1.184	1.164	0.58	0.01
4%	0.071	0.092	0.075	0.08	0.01
5%	0.088	0.076	0.052	0.07	0.02



Graph 3: Shows effect of NaCl concentration on Bacteriocin Production

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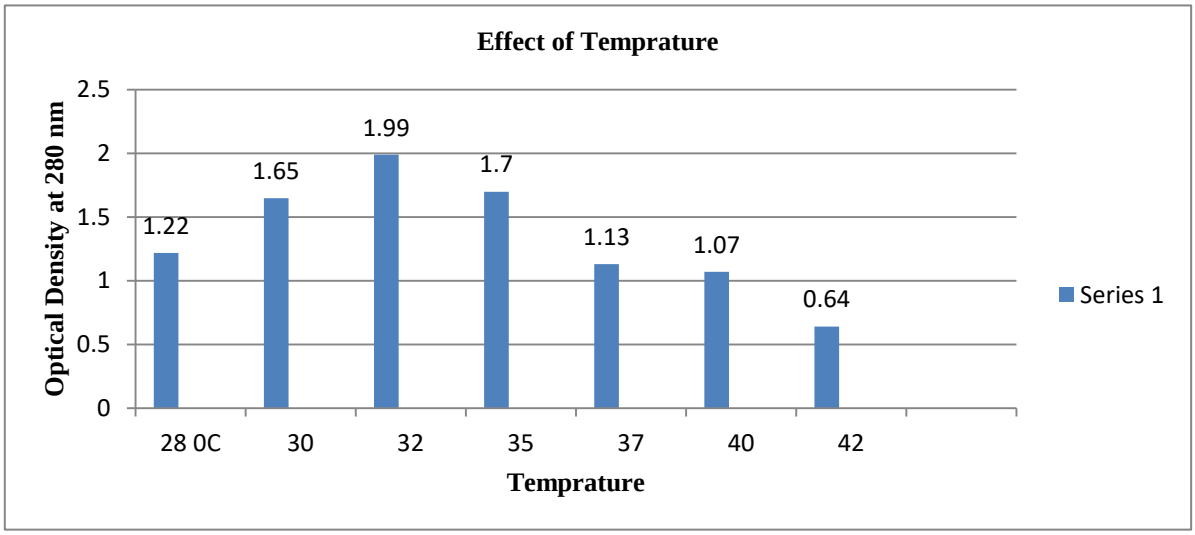
Table- 5: Effect of Temperature

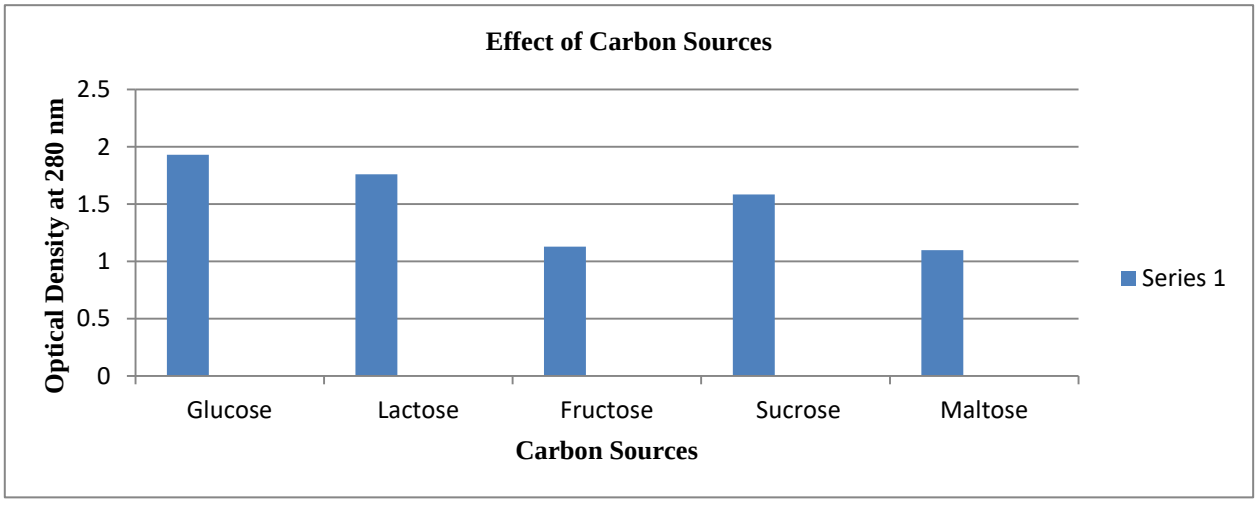
Temperature (^{0}C)	Optical Density			Mean	Std Dev.
	1	2	3		
28	1.210	1.191	1.25	1.22	0.03
30	1.678	1.665	1.598	1.65	0.04
32	1.988	1.968	1.999	1.99	0.02
35	1.704	1.689	1.715	1.70	0.01
37	1.128	1.134	1.121	1.13	0.01
40	1.069	1.058	1.075	1.07	0.01
42	0.598	0.645	0.684	0.64	0.01



Graph -4: Shows effect of Temperature on Bacteriocin Production

Table 6: Effect of Carbon Sources

Carbon Sources	Optical Density			Mean	Std Dev
	1	2	3		
Glucose	1.920	1.930	1.95	1.93	0.04
Lactose	1.744	1.734	1.78	1.76	0.03
Fructose	1.124	1.129	1.121	1.13	0.01
Sucrose	1.583	1.589	1.574	1.58	0.01
Maltose	1.098	1.089	1.099	1.09	0.01



Graph 5: Shows effect of different carbon sources on Bacteriocin Production

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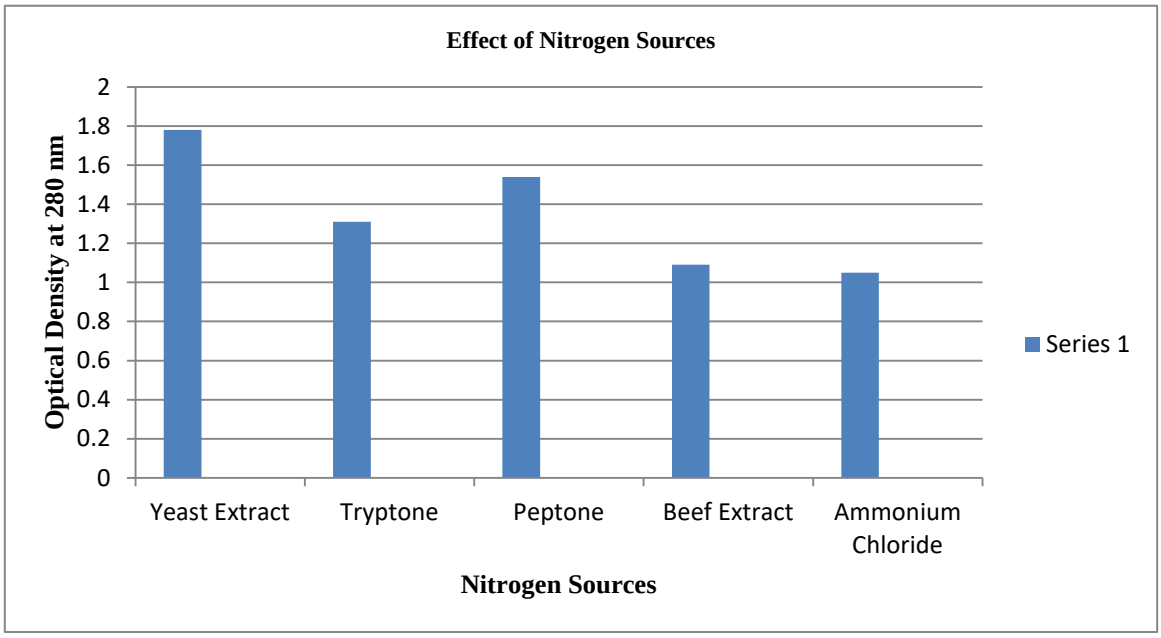
Table -7: Effects of Nitrogen Sources

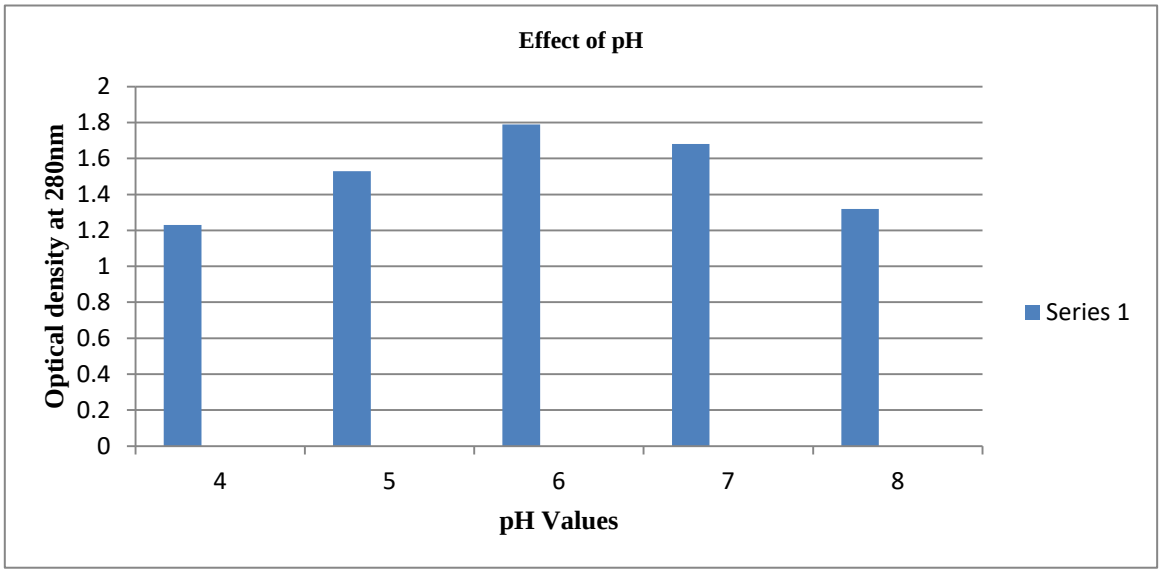
Nitrogen Sources	Optical Density			Mean	Std Dev.
Yeast Extract	1.784	1.780	1.776	1.78	0.00
Tryptone	1.312	1.309	1.319	1.31	0.01
Peptone	1.534	1.542	1.529	1.54	0.01
Beef Extract	1.091	1.078	1.098	1.09	0.01
Ammonium Chloride	1.053	1.041	1.068	1.05	0.02



Graph 6: Shows effect of different Nitrogen sources on Bacteriocin Production

Table 8: Effects of different pH

pH	Optical Density			Mean	Std Dev
4	1.223	1.219	1.241	1.23	0.02
5	1.529	1.534	1.524	1.53	0.01
6	1.788	1.794	1.776	1.79	0.01
7	1.666	1.662	1.689	1.68	0.02
8	1.220	1.290	1.350	1.32	0.04



Graph 7: Shows effect of pH on Bacteriocin Production

IV. CONCLUSION

The research has concluded that *Staphylococcus aureus* is Bacteriocin producing microorganism and this Bacteriocin is found to be affective against pathogenic microorganism. Hence can be used as food preservative and also in food packaging. It is predicted that research in bacteriocin production and combination treatment for food preservation and enteropathogens will be an advantageous step for both the producer and the consumer of the food industry [22].

ACKNOWLEDGEMENT

The author wants to thank Mr. Nitin Sharma, Orbit biotech Mohali for providing guidance and technical support for doing research work.

CONFLICT OF INTERST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflict of interest.

AUTHOR CONTRIBUTION

All authors contributed equally in the research work.

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