Int.J.Curr.Microbiol.App.Sci (2019) *8*(1): 3202-3211

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 01 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.801.342

Optimization of Carbon and Nitrogen Source for the Production of an Antimicrobial Biopeptide from *Bacillus firmicutes* against Food Borne Pathogens

S. Uday[1] and M.P. Prasad[2]*

[1]Ramaiah College of Arts, Science and Commerce, Bangalore, India
[2]Department of Microbiology/Biotechnology, Sangene Biotech, Bangalore-560071, India

**Corresponding author:*

Keywords

Bacillus firmicutes, Biopeptide/Bacteriocin, Antimicrobial activity, Food borne pathogens, Optimization

Article Info

Accepted:
28 December 2018
Available Online:
10 January 2019

A B S T R A C T

The presence and growth of microorganisms in foods is harmful to human and animal health. The consumption of those foods results in food borne diseases. Thus the major concern is the control of microorganisms to increase the shelf life and prevent harmful microorganisms. Bioactive peptides are known for their ability to inhibit protein-protein interactions due to their small size and specificity. Nature remains the largest source of bioactive peptides since plants, animals, fungi, microbes and their products contain various proteins in them. Currently food preservation by the antimicrobial activity of biopeptides against microorganism growth has been studied. The present study is aimed at optimization of the chemical constituents like carbon and nitrogen sources in the production of the biopeptide from the isolated bacteria identified as *Bacillus firmicutes* based on 16S rRNA sequencing and sequence analysis and its activity against the isolated food pathogens like *E.coli, S aureus, Pseudomonas aeruginosa, Shigella sps, Salmonella sps* and *L. monocytogenes.* Media optimization for the isolate was conducted by varying the carbon (Fructose, Sucrose, Glucose, Maltose, Starch) and nitrogen (Urea, Ammonium nitrate, Ammonium sulphate, Ammonium dihydrogen phosphate, Sodium nitrate) sources. The Maximum antimicrobial activity was observed with 2.0% of glucose media against *E. coli.* Maltose in the medium showed the least inhibitory activity against all the food borne pathogens. The least activity was seen with 0.5% of the concentration of the carbon sources. The maximum zone of inhibition appeared at 2.0% of ammonium nitrate against *E. coli* by the isolate. The least antimicrobial activity was seen against *L. monocytogenes* in both the carbon and nitrogen sources used in the media by the isolate. No antimicrobial activity was observed with 0.5% of the nitrogen source in most of the cases.

Introduction

Food is the substance which gives nutrients and energy material to the living organism for its life and growth. Foods used by human beings contain nutrients like carbohydrates, proteins, fats, vitamins, minerals and other growth factors. Nutritionally, human diet is more complicated than microbial nutrient requirements. Foods used for human

consumption can serve as good source of nutrients for the growth of microorganisms. Presence and growth of microorganisms in foods meant for human or other animal consumption makes them unfit and also serves as potential source of infections to cause a number of food borne diseases. Other microorganisms if allowed to grow in certain food products produce toxic substances that result in food poisoning when the food is ingested. Food borne illness caused by microbial contamination has been a serious issue in recent years, the cost of which is enormous.

Food spoilage by microorganisms can be prevented potentially by the use of biopeptides that possess antimicrobial activity as food additives especially those that preserve foods and enhance food quality.

Microorganisms mainly Gram (+) and Gram negative (-) bacteria produce substances of protein structure possessing antimicrobial activities, called bacteriocins. Although bacteriocins could be categorized as antibiotics but they are not. The major difference between bacteriocins and antibiotics is that bacteriocins restrict their activity to strains of species related to the producing species and particularly to strains of the same species, antibiotics on the other hand have a wider activity spectrum and even if their activity is restricted this does not show any preferential effect on closely related strains (Zacharof and Lovitt, 2012). Bacteriocin, a ribosomally synthesized antagonistic peptides are generally produced by bacteria. This can kill or inhibits the growth of the related bacteria Tagg *et al*., (1976). Recently, three bacteriocin-like peptides named Lichenin, Bacillocin 490 and P40 produced by *B. licheniformis* strain 26 L-10/3RA, 490/5 and P40, respectively, have been reported Pattnaik *et al*., (2001), Martirani *et al*., (2002), Cladera-Olivera *et al*., (2004). The mode of inhibition of bacteriocins depends on the available bio-concentration, and on the nature and the physiological stage of the target strain. In general bacteriocins of *Bacillus* display a bactericidal and bacteriolytic effect, while enterocins for example have only a bactericidal effect Foulquie´ Moreno *et al.*, (2003).

Lantibiotics or class I bacteriocins that contain unusual amino acids such as lantionines and b-methyl lanthionines. Nisin, the most studied bacteriocin, belongs to this class. Class II of non lantibiotic small, heat stable bacteriocins including Listeria-active peptides (cy-stibiotics), thiol-activated peptides (thiolbiotics) and two peptides complexes. Class III bacteriocins includes large and thermolabile proteins. Members of class IV are complex bacteriocins associated with other chemical moieties. Because bacteriocins are natural products of many microorganisms associated with food, there is currently an enhanced interest in their use as natural preservatives Cleveland *et al*., (2001). The preservation of foods by the antagonistic growth of microorganisms was reviewed by Hurst, (1973). He cited growth of a LAB microflora in milk, sauerkraut and vacuum packaged meats as examples of protective, antagonistic growth. Hurst also considered the role of 'antibiotics' (bacteriocins) such as nisin in the preservation foods that support the growth of LAB. In recent times this has been termed 'biopreservation' to differentiate it from the chemical (artificial) preservation of foods. LAB produces lactic acid or lactic and acetic acids, and they may produce other inhibitory substances such as diacetyl, hydrogen peroxide, reuterin (b-hydroxy-propionaldehyde) and bacteriocins. Bacteriocins are ribosomally-produced, precursor polypeptides or proteins that, in their mature (active) form, exert an antibacterial effect against a narrow spectrum of closely related bacteria Jack *et al*., (1995).

A physiologically diverse range of Gram-positive and Gram-negative bacteria were found to be susceptible to inhibition and inactivation by *Lactoferricin B*, a peptide produced by gastric pepsin. The list of susceptible organisms includes *Escherichia coli, Salmonella enteritidis, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Campylobacter jejuni, Staphylococcus aureus, Streptococcus mutans, Listeria monocytogenes and Clostridium perfringens etc* (Bellamy *et al*., 1992).

The present investigation aims at media optimization with variations in the carbon (Fructose, Lactose, Sucrose, Glucose, Maltose, Starch) and nitrogen sources (Urea, Ammonium nitrate, Ammonium sulphate, Ammonium dihydrogen phosphate, Sodium nitrate) for the production of Biopeptide/ Bacteriocin using agar well diffusion method.

Materials and Methods

Isolation for food borne pathogens

Food samples like canned food, poultry, fish, frozen vegetables and meat products, bakery products, cooked foods, milk and milk products were collected from various super markets and food malls in Bangalore. The microbial populations in the collected samples were quantitatively enumerated by standard serial dilution method using sterile distilled water and 1 gm of the food sample. Dilutions were made from 10^{-1} to 10^{-6}, these dilutions were used in the plating for the isolation of micro-organisms. Spread plate method was used for isolation of the bacteria, 1ml of the food suspension was distributed evenly over the surface of nutrient agar plate using a sterile spreader. Inoculated plates were incubated at 37ºC for 24-48 hours. Colonies developed on the plates were further studied based on the types of colony morphology to differentiate between the types of bacteria.

Further Bacterial identification was done based on standard colony characteristics, Gram staining techniques and biochemical properties of the isolates and growth on specific selective media.

Screening of potential biopeptide/ bacteriocin producing bacterial isolate

The microbial populations in the collected samples were quantitatively enumerated by standard serial dilution method using sterile distilled water with 1 gm of the test sample as mentioned for isolation of Pathogens. Inoculated plates were incubated at 37ºC for 24-48 hours. Colonies developed on the plates were further studied based on the types of colony morphology to differentiate between the types of bacteria.

Further bacterial identification was based on standard colony characteristics, Gram staining techniques and biochemical properties of the isolates. The final test organism was identified based on 16S rRNA sequencing and sequence analysis.

Screening for antimicrobial activity

The isolated microorganisms were streaked on Nutrient agar slants and used for further screening for antimicrobial activity against the selected food borne pathogens like, *E. coli, S aureus, Pseudomonas aeruginosa, Shigella sps, Salmonella sps* and *Listeria monocytogenes*. The test organism for biopeptide production were inoculated in sterile nutrient broth and incubated for 24hrs and was used to streak against the pathogenic test bacteria. Muller Hinton Agar medium was prepared and aseptically poured into sterile petri-plates. After solidification lawn of the pathogenic microorganisms incubated overnight in nutrient broth were made on the agar surface by using sterile cotton swabs. The plates were incubated for 15 minutes in room

temperature inside the laminar air flow. After incubation the isolated biopeptide producing test organism was streaked perpendicular in a straight single streak using a sterile inoculating loop.

The plates were incubated at 37°C for 24-48 hours. After 24 hours of incubation period, microorganisms displaying clear zones of inhibition against the pathogens were recorded if improper growth the results were recorded after 48hours.

Optimization of chemical parameters for biopeptide/bacteriocin production

The effect of various chemical parameters in the production of Biopeptidee compound for antimicrobial activity was checked using MRS media as it was found to support the growth of the test organism as well as there was an increase in the antimicrobial activity. Optimization with variations in the Carbon and Nitrogen source was done.

Media optimization with variations in the carbon (Fructose, Lactose, Sucrose, Glucose, Maltose, Starch) was conducted for the production of Biopeptide/Bacteriocin using agar well diffusion method. MRS broth was substituted with the different carbon sources keeping the other parameters constant, or the nitrogen was substituted keeping the other compounds and the physical parameters constant. Muller-Hinton agar plates were prepared to evaluate the antimicrobial activity against the selected food borne pathogens viz., *E. coli, S. aureus, Pseudomonas aeruginosa, Shigella sps, Salmonella sps* and *L. monocytogenes*. 100µl inoculum of each selected pathogen was uniformly spread on Muller-Hinton agar plates with the help of a swab. After 5 minutes of incubation, 6 mm diameter well was punched in the plates with the help of sterile cork borer, 80 µl of the inoculum of the test organism was added into the well. The plates were incubated at 37 ºC for 24 hours and after incubation plates were observed for zone of inhibition.

Results and Discussion

Isolation of biopeptide producing bacteria

Different bacterial isolates were screened for the production of biopeptide, the organism exhibiting the maximum zone of inhibition was selected as the final test organism. Based on colony morphology, biochemical characterization and 16S rRNA sequencing and sequence analysis using BLAST, the organism was identified as *Bacillus firmicutes***.**

Optimization of Carbon Source

Carbon source optimization was carried out for the test organism *Bacillus firmicutes* using the following carbon sources; Fructose, Lactose, Sucrose, Glucose, Maltose, Starch substituted in the media and checked for the antimicrobial activity against the food borne pathogens *E. coli, S. aureus, Pseudomonas aeruginosa, Shigella sps, Salmonella sps* and L. *monocytogenes.* Figure 1 shows the images of the plates. The results obtained are presented by bar graph (Figure 2, 3, 4, 5, 6).

The optimization of different carbon source at different concentration was analyzed for the effect of biopeptide/bacterriocin antimicrobial activity of the test organism *Bacillus firmicutes* on different food pathogenic microorganisms. The Maximum antimicrobial activity was observed with 2.0% of glucose media against *E. coli.* The effect of different concentrations was observed with the effect of antimicrobial activity against the pathogenic microorganisms. Maltose in the medium showed the least inhibitory activity against all the food borne pathogens, higher concentrations did not show any activity indicating the inability of organism to

assimilate the carbon source. The least activity was seen with 0.5% concentration of the carbon sources. The least activity was seen against *L. monocytogenes*. The maximum zone of inhibition was found to be 26mm in diameter against *E. coli* with 2.0% of Glucose.

Optimization of nitrogen source

Nitrogen source optimization was carried out for the test organism *Bacillus firmicutes* using the following nitrogen sources; Urea, Ammonium nitrate, Ammonium sulphate, Ammonium dihydrogen phosphate, Sodium nitrate substituted in the media and checked for the antimicrobial activity against the food borne pathogens *E. coli*, *S. aureus*, *Pseudomonas aeruginosa*, *Shigella sps*, *Salmonella sps* and L. *monocytogenes* Figure 7 shows the images of the plates. The results obtained are presented by bar graph (Figure 8, 9, 10, 11, 12).

Fig.1 Antimicrobial activity of *Bacillus firmicutes* at different Carbon sources

Fig.2 Optimization of sucrose concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

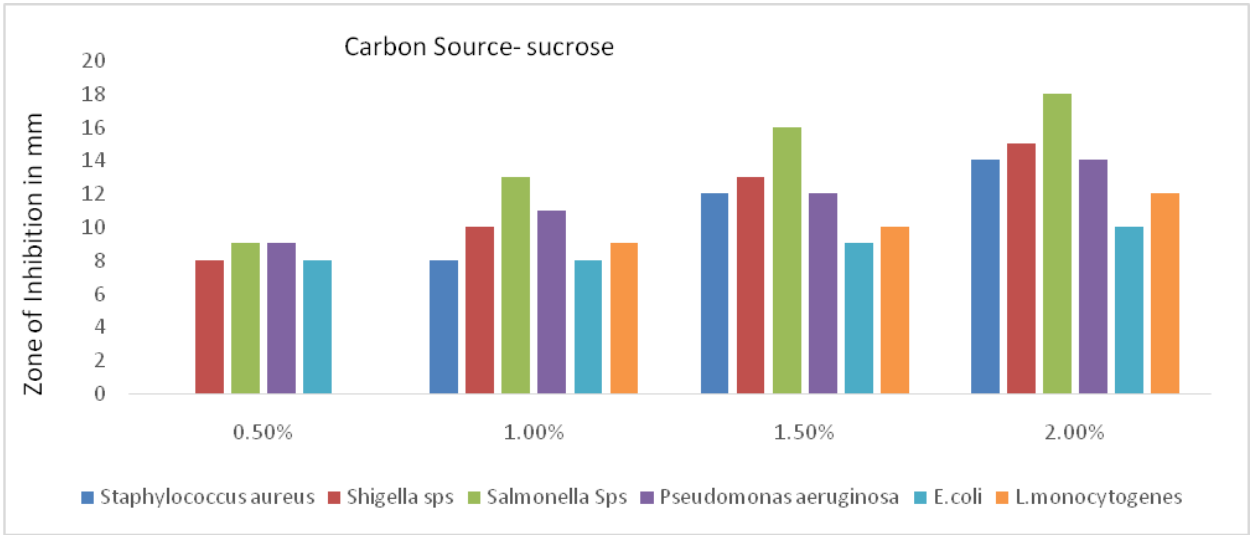


Fig.3 Optimization of glucose concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

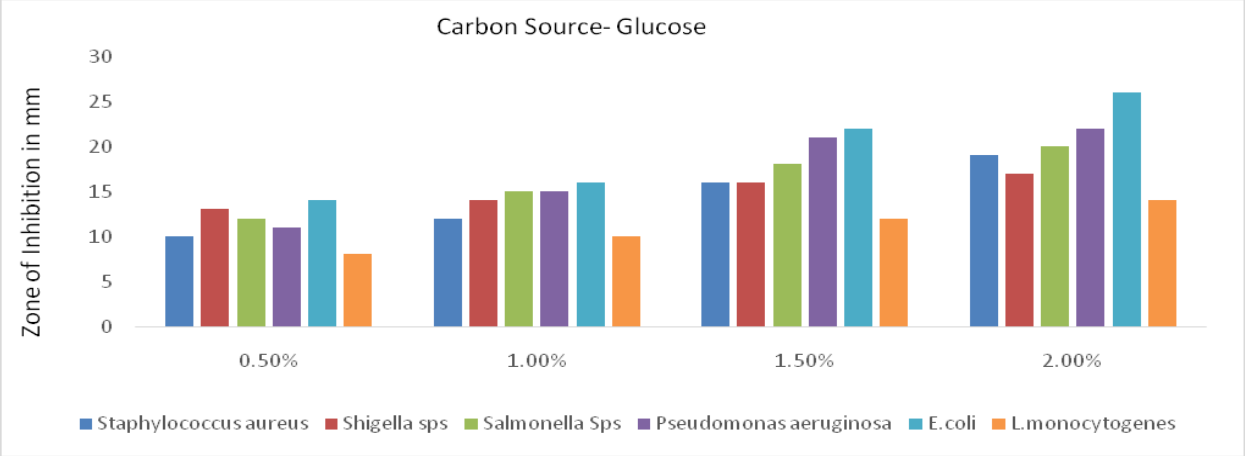


Fig.4 Optimization of maltose concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

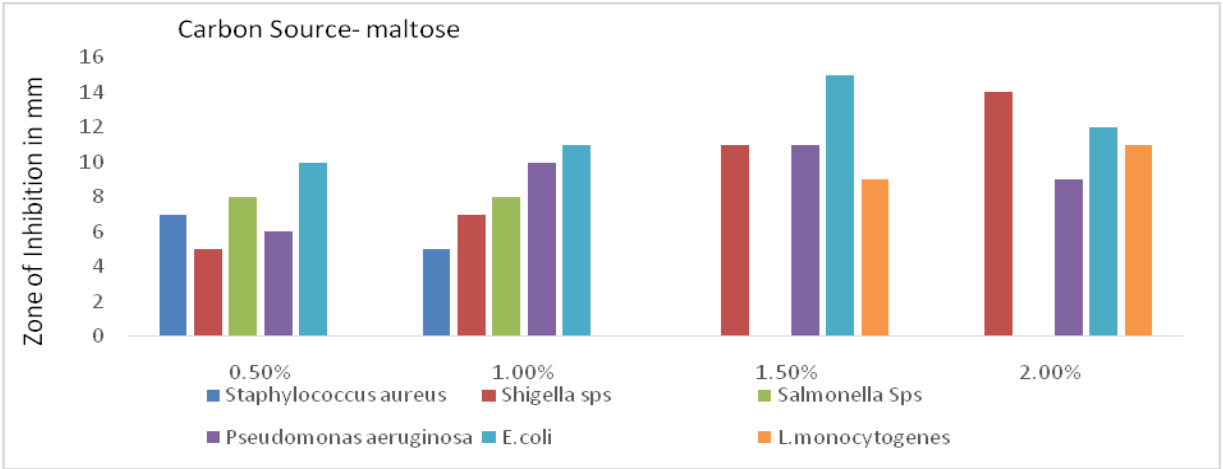


Fig.5 Optimization of fructose concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens using

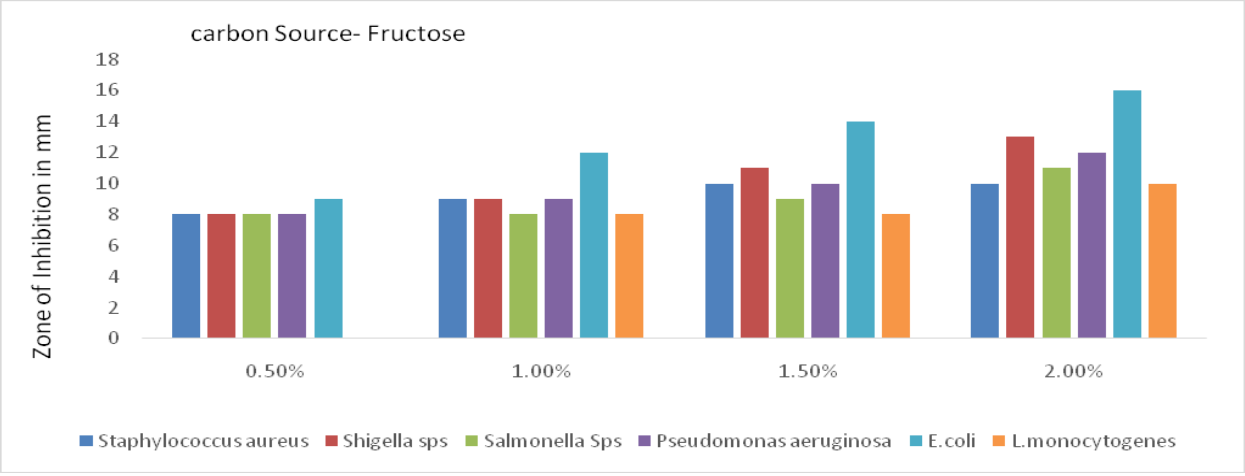


Fig.6 Optimization of starch concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

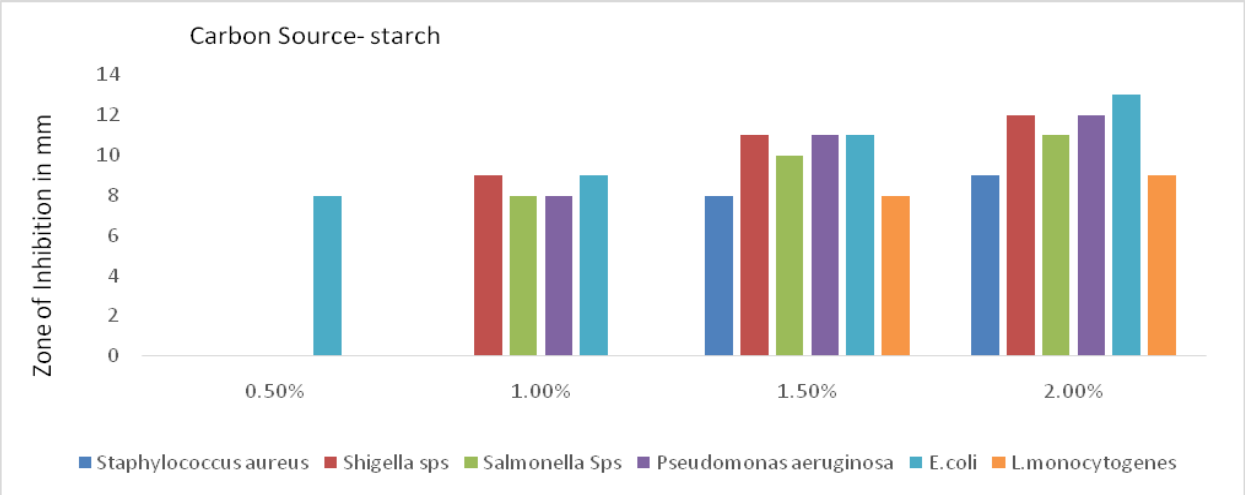


Fig.7 Optimization of urea concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

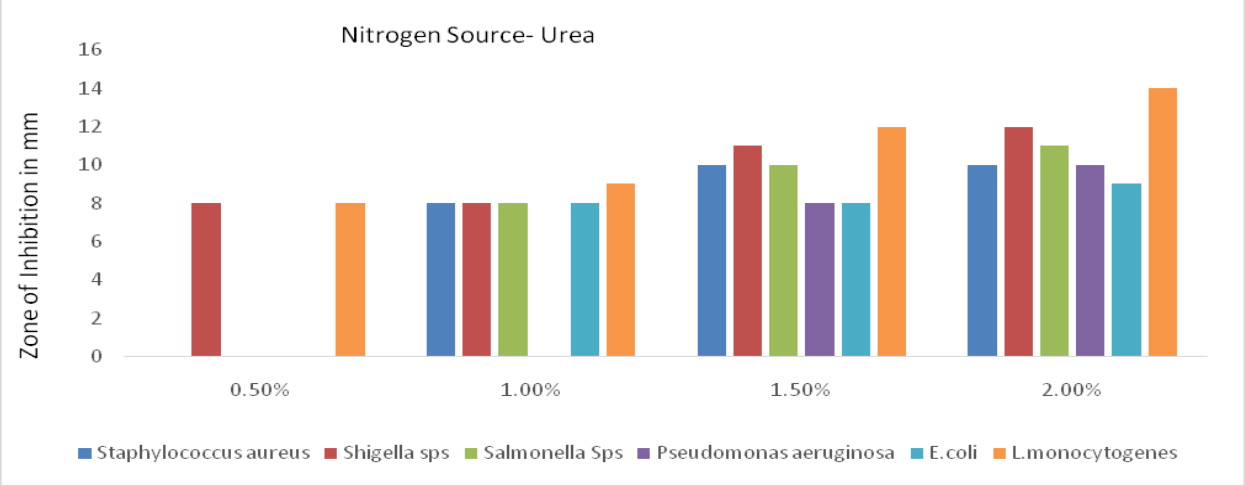


Fig.8 Optimization of ammonium sulphate concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

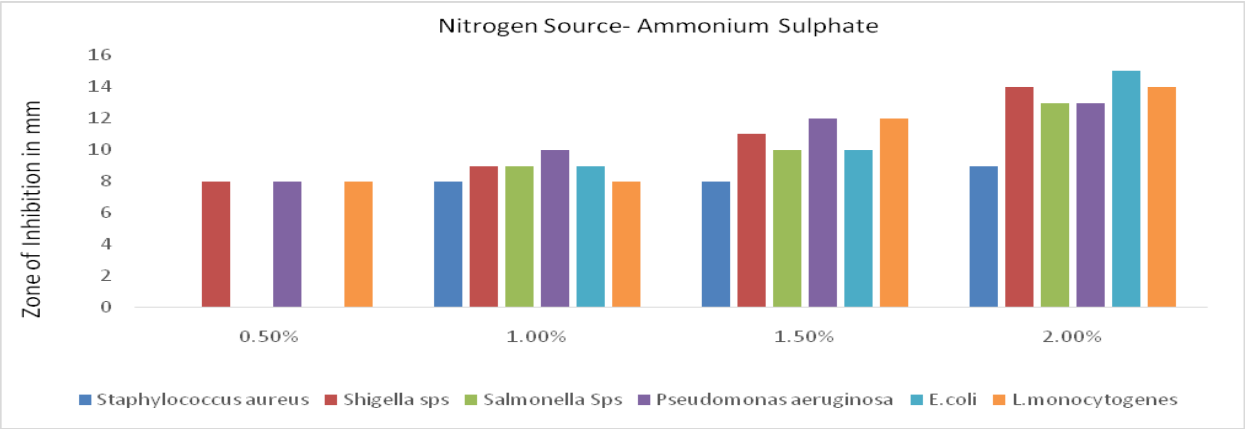


Fig.9 Optimization of ammonium nitrate concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

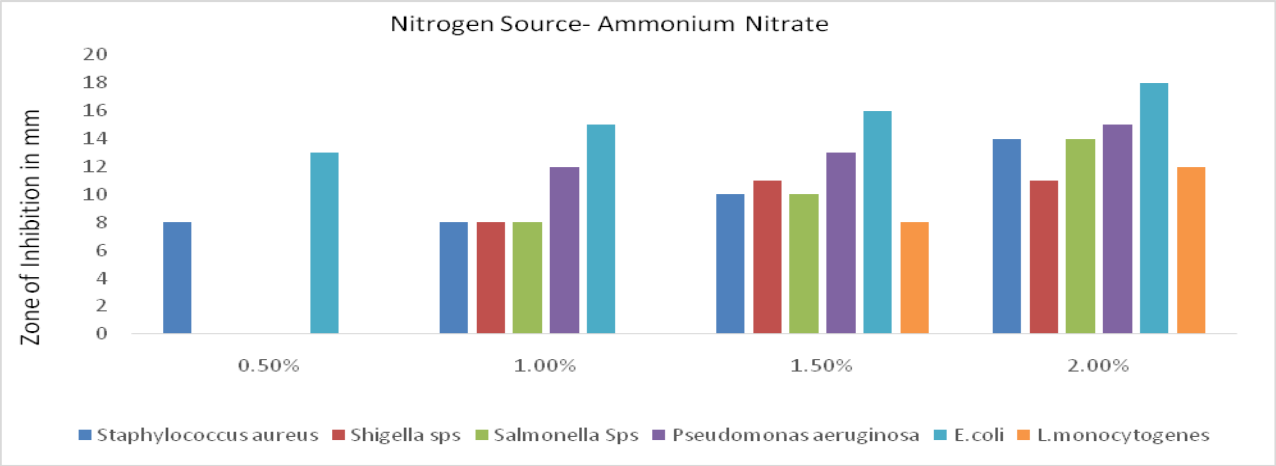


Fig.10 Optimization of ammonium dihydrogen phosphate concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens

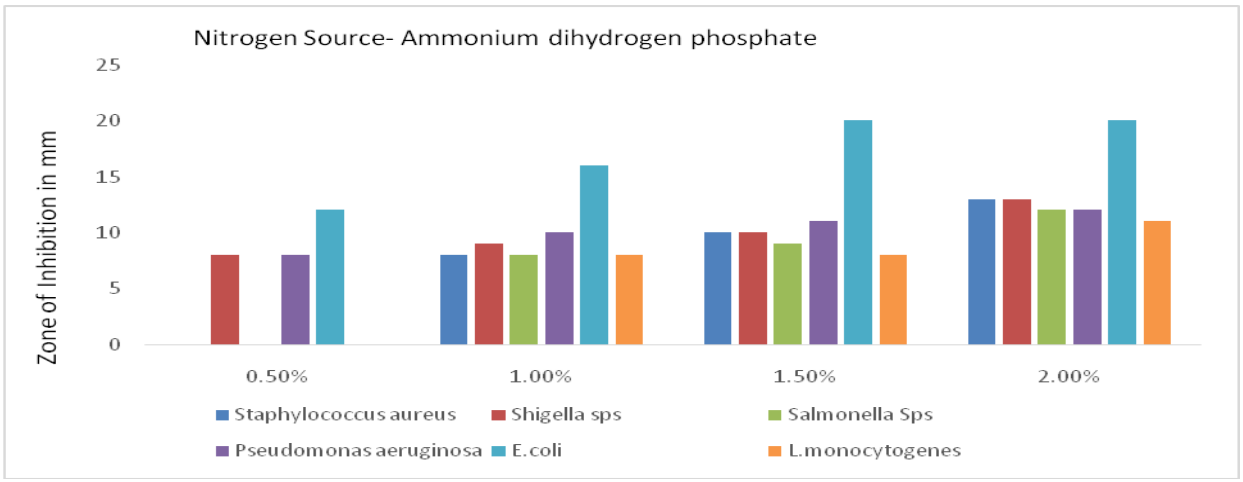
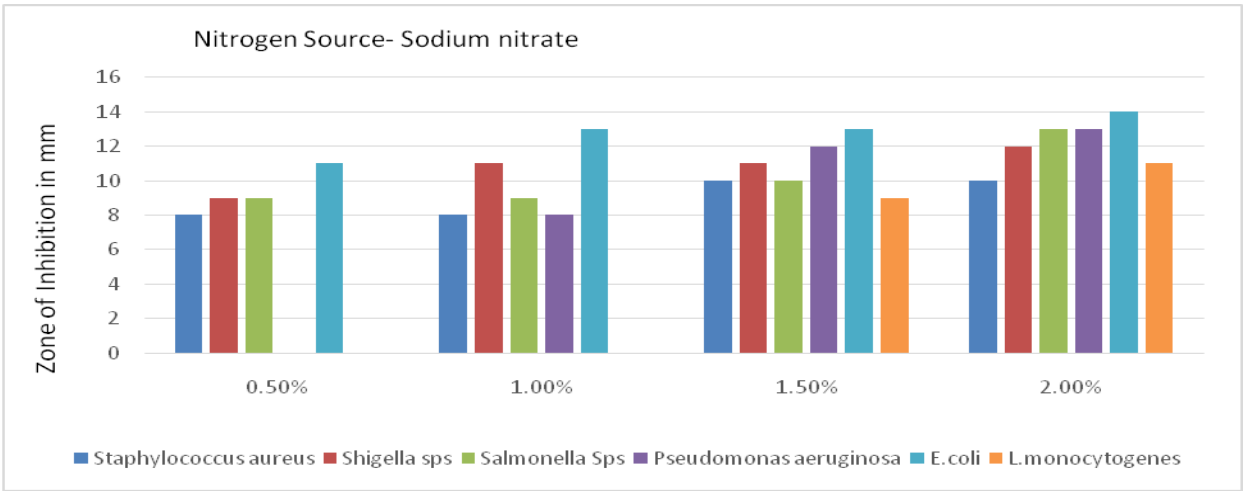


Fig.11 Optimization of sodium nitrate concentration for the antimicrobial activity of *Bacillus firmicutes* against the food borne pathogens



The optimization of the media for the isolate with different nitrogen sources was carried out. The zone of inhibition was seen to increase with increase in the concentration of the nitrogen source. The maximum zone of inhibition was seen at 2.0% of ammonium nitrate against *E. coli* by the isolate. The effect of nitrogen was mainly seen against *E. coli* by the isolate. The least antimicrobial activity was seen against *L. monocytogenes* by the isolate. The antimicrobial activity was not seen with 0.5% of the nitrogen source in most of the cases.

In conclusion, the current research investigated the media optimization with respect to carbon and nitrogen source for the production of biopeptide from *Bacillus firmicutes* isolated from natural sources against food borne pathogen. All over maximum microbial activity was observed against *E. coli* pathogen which was corroborated by 26 mm inhibition zone. Herein the media contained 2% glucose. Maltose showed negligible activity even at higher concentration. For all the study least activity was observed against *L.monocytogenes.* Optimization of nitrogen source exhibited an increase in inhibition zone with respect to nitrogen concentration in media. This result also showed maximum activity against *E. coli* at the presence of 2% ammonium nitrate whereas in presence of different nitrogen source, activity of *L.monocytogenes* could not be inhibited by the synthesized biopeptide. Thus overall study substantiated the importance of carbon and nitrogen source for the synthesis of antimicrobial biopeptide against food borne pathogen.

References

Zacharof M. P, Lovitt R. W. 2012. Bacteriocins Produced by Lactic Acid Bacteria A Review Article. APCBEE *Procedia*. 2: 50 –56.

Tagg, J.R., Dayani, A.S., Wannamaker, L.W. 1976. Bacteriocins of Gram-positive bacteria. *Bacteriological. Reviews*. 40: 722–756.

Pattnaik, P., Kaushik, J.K., Grover, S., Batish, V.K. 2001. Purification and characterization of a bacteriocin compound (Lichenin) produced anaerobically by *Bacillus licheniformis* isolated from water buffalo. *Journal of Applied Microbiology*. 91: 636–645.

Martirani, L., Varcamonti, M., Naclerio, G., De Felice, M., 2002. Purification and partial characterization of bacillocin 490, a novel bacteriocin produced by a thermophilic strain of *Bacillus licheniformis*. *Microbial Cell.* Fact. 1: 1–5.

Cladera-Olivera, F., Caron, G.R., Brandelli, A. 2004. Bacteriocin-like peptide production by *Bacillus licheniformis* strain P40, Lett. Appl. Microbiol. 38, 251.

Foulquie Moreno, M.R., Callewaert, R., Devreese, B., Van Beeumen, J., DeVuyst, L. 2003. Isolation and biochemical characterization of enterococci from different sources. *Journal of Applied Microbiology*. 94: 214–2 29.

Cleveland, J., Montville, T. J., Nes, I. F., Chichindas, M. L. 2001. Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology*. 71: 1–20.

Hurst, A. 1981. Nisin. *Adv. Appl. Microbiol* 27: 85–123.

Jack, R.W., Tagg, J.R., Ray, B. 1995. Bacteriocins of Gram positive bacteria. *Microbiological Reviews*. 59: 171–200.

Bellamy W, Takase M, Wakabayashi H, Kawase K, Tomita M. 1992. Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. *Journal of Applied Bacteriology*. 73(6): 472-479.

How to cite this article:

Uday, S. and Prasad, M.P. 2019. Optimization of Carbon and Nitrogen Source for the Production of an Antimicrobial Biopeptide from *Bacillus firmicutes* against Food Borne Pathogens. *Int.J.Curr.Microbiol.App.Sci.* 8(01): 3202-3211.
doi: https://doi.org/10.20546/ijcmas.2019.801.342