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Research Article

A HURDLE APPROACH FOR PRESERVATION OF SEA FOOD PRODUCTS USING DIFFERENT STORAGE FACTORS WITH SYNERGISTIC BACTERIOCIN

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ABSTRACT

Bacteriocin varies from most traditional medicines in being proteinaceous factors that are quickly assimilated by proteases in the human digestive system. They are ribosomal integrated peptides, and this condition develops the chance of improving their properties to induce their activity and range of action. Bacteriocins are complex proteins biologically active with antibacterial action against other microbes, especially closely related bacterial species. Hurdles that have a positive effect by inhibiting microorganisms may have a negative one on other parameters such as nutritional properties or sensory quality, depending on their intensity. In order to lower the preservative level, the hurdle technology concept has been developed, consisting in using combined hurdles to establish an additive antimicrobial effect, and even sometimes a synergetic one, thus improving the safety and the sensory quality of food. In this present study, hurdle technology was efficiently carried out. *Lactobacillus* spp was isolated and produced in the selective media. Effect of bacteriocins and chilling temperatures (-18°C and +4°C); and Effect of bacteriocins and brine salts at various concentrations (5%, 10%, 15%, 20%) were evaluated. Bacteriocins with different chilling temperatures and brine salt concentration showed significant results on compared to control temperatures. Thus, bacteriocins due to their bacteriostatic activity can be efficiently used in hurdle technology which reduces the food spoiling organisms. Thus, bacteriocins in combined with hurdle technology can be used as alternatives for chemical preservatives in preservation techniques.

Keywords: Bacteriocins, Hurdle technology, chilling temperatures, Brine salts, Food spoiling microorganism

INTRODUCTION

Bacteriocins are complex proteins biologically active with antibacterial action against other microbes, especially closely related bacterial species. They are developed by bacteria and are not termed as antibiotics in order to stop confusion and anxiety with curative medicines, which can probably control allergies in humans and other medical conditions[1]. Bacteriocin varies from most traditional medicines in being proteinaceous factors that are quickly assimilated by proteases in the human digestive system. They are ribosomal integrated peptides, and this condition develops the chance of improving their properties to induce their activity and range of action[2]. Bacteriocin generally inhibits the cell wall synthesis, increases the cell membrane permeability and inhibits RNase or DNase activity in the target organisms[3]. Bacteriocins have wide range of applications. Due to their bacteriocidic activity, they can also be used as an alternative for chemical preservatives in preservation techniques.

Hurdles are referred to the protection of food and the sustenance of its quality based on the utilization of protective substances in each type of food. Leroi[4] *et al* (2014) reviewed that examples of hurdles in seafood products are salt, smoke, acids, temperature (high or low) and more recently redox potential. Preservation techniques were studied for several years and several hurdle factors for food products have been described. Such hurdle factors include organic acids, bacteriocins, chitosan, nitrate, lacto-peroxidase, essential oil, modified atmosphere packaging, as well as novel decontamination technologies such as microwave and radio frequency. Hurdle technologies have a significant positive effect by inhibiting the growth of microbes which increases the quality, depending on their concentration. The concept of hurdle technology was developed to reduce the preservatives level. Combined hurdles establish an additive antimicrobial and synergetic activity, which could increase the quality and shelf life of food. Since many techniques are now present for the site directed mutagenesis of bacteriocin structural genes[5] and with the support of genomics and proteomics, the chance of constructing new families of modified peptides with improved antimicrobial activity or stability and particular characteristics has become a genuine probability.

In this present study, hurdle technology was efficiently carried out. *Lactobacillus* spp was isolated and produced in the selective media. Effect of bacteriocins and chilling temperatures (-18°C and +4°C); and Effect of bacteriocins and brine salts at various concentrations (5%, 10%, 15%, 20%) were evaluated.

MATERIALS AND METHODS

The present research work was carried out in Department of Post-Harvest Engineering and Technology, Aligarh Muslim University, Aligarh, India. The research work was performed during the period of May 2018 to September 2018.

Isolation and identification of *Lactobacillus* spp

Nutraceutical bacteriocin producing *Lactobacillus* spp was isolated and identified as per the method described by Sahar Karami[6] et al (2017). The isolated species was used for the production of nutraceutical bacteriocin compounds using the lab scale process. The compounds were extracted and purified using standard bioprocess technology. The production and purification methods are described below.

Production and extraction of bioactive metabolites (bacteriocins) by *Lactobacillus* spp in the selective media[7]

The bioactive metabolite, bacteriocin as extracellular substance from *Lactobacillus* spp was produced in the selective MRS broth. The procedure was explained briefly below about the extraction of bacteriocin from *Lactobacillus* spp used in this present research. About 200 ml of MRS broth was prepared and the selected strains (*Lactobacillus* spp) were inoculated and kept in the incubator shaker for 72 hours with 170rpm and 37°C. The entire 100ml culture was inoculated onto 200ml media and incubated at similar condition. As a scale up process, the 200ml cultured cells were transferred to 500ml production media and incubated for the production of nutraceutical bacteriocins. After incubation the broth was centrifuged and the supernatant containing the bacteriocin was collected in a clean separate flask. The concentrated bacteriocin extracts were re-dissolved in 2ml of sterile distilled water; filter sterilized and stored in sterile Eppendorf tubes until further use.

Identifying the effective hurdle factors that could retard the growth of organisms during different seafood storage conditions

To investigate the effect of bacteriocin along with other factors (chilling temperature, brine salt concentrations and pasteurization temperature) for the growth inhibition of THB and TC was studied. The factors that were selected for the inhibition of the organisms were thus referred to as Hurdle Factors. The effective hurdle factor that could inhibit the increase in number of bacterial gut microbiota in the stored seafood products (Fish) was evaluated in the present research work. Two different hurdle factors with their respective storage conditions were analysed.

1. Bacteriocin + Chilling temperature (°C)

About 400g of fresh marine fish was immersed in bacteriocin solution for 8h at room temperature (30°C). The excess bacteriocin suspension was removed by manual gentle shaking for 30 seconds and packed in a sterile food packing film. A separate pack of fishes (100g) were stored at 4°C in a refrigerator and the other pack of fishes (100g) was frozen at -18°C (freezer). Similarly, another set of fishes without immersing in the bacteriocin (Control) were packed and stored at 4°C and -18°C respectively.

2. Bacteriocin + Brine salt concentrations (%)

To detect the effect of brine salt as one of the hurdle factors, sodium chloride at four different concentrations (5%, 10%, 15% and 20%) was selected. Each concentration of salt solution was prepared, and the marine fishes were immersed with and without bacteriocin suspension for 8h. Difference in the number of cells were enumerated as per microbiological standards and presented separately for total heterotrophic bacteria (THB) and total coliforms (TC).

Table 1: Effect of bacteriocin and chilling temperature at 4°C on Total heterotrophic bacteria (THB)

	1 day	7 days	14 days	21 days	28 days	35 days
Control 4°C	5x10^2 CFU/ml	6x10^2 CFU/ml	7x10^2 CFU/ml	7x10^2 CFU/ml	7x10^2 CFU/ml	8 x10^2 CFU/ml
Bacteriocin 4°C	3 x10^2 CFU/ml	5 x10^2 CFU/ml	5 x10^2 CFU/ml	4 x10^2 CFU/ml	3 x10^2 CFU/ml	4 x10^2 CFU/ml

Table 2: Effect of bacteriocin and chilling temperature at -18°C on Total heterotrophic bacteria (THB)

	1 day	7 days	14 days	21 days	28 days	35 days
Control - 18°C	**4 x10^2** CFU/ml	**5 x10^2** CFU/ml	**6 x10^2** CFU/ml	**7 x10^2** CFU/ml	**6 x10^2** CFU/ml	**4 x10^2** CFU/ml
Bacteriocin -18°C	**2 x10^2** CFU/ml	**2 x10^2** CFU/ml	**3 x10^2** CFU/ml	**2 x10^2** CFU/ml	**2 x10^2** CFU/ml	**0 x10^2** CFU/ml

Table 3: Effect of bacteriocin and chilling temperature at 4°C on Total Coliforms (TC) by bacteriocin

	1 day	7 days	14 days	21 days	28 days	35 days
Control 4°C	**4 x10^2** CFU/ml	**5 x10^2** CFU/ml	**4 x10^2** CFU/ml	**3 x10^2** CFU/ml	**3 x10^2** CFU/ml	**2 x10^2** CFU/ml
Bacteriocin 4°C	**3 x10^2** CFU/ml	**2 x10^2** CFU/ml	**3 x10^2** CFU/ml	**2 x10^2** CFU/ml	**0 x10^2** CFU/ml	**0 x10^2** CFU/ml

Table 4: Effect of bacteriocin and chilling temperature at -18°C on Total Coliforms (TC) by bacteriocin

	1 day	7 days	14 days	21 days	28 days	35 days
Control -18°C	2 x10^2 CFU/ml	3 x10^2 CFU/ml	3 x10^2 CFU/ml	2 x10^2 CFU/ml	2 x10^2 CFU/ml	0 x10^2 CFU/ml
Bacteriocin - 18°C	2 x10^2 CFU/ml	2 x10^2 CFU/ml	0 x10^2 CFU/ml	0 x10^2 CFU/ml	0 x10^2 CFU/ml	0 x10^2 CFU/ml

Table 5: Effect of bacteriocin and 5% salt Concentration on Total heterotrophic bacteria (THB)

	1 day	7 days	14 days	21 days	28 days	35 days
5% Salt	17 x10^2 CFU/ml	16 x10^2 CFU/ml	15 x10^2 CFU/ml	14 x10^2 CFU/ml	15 x10^2 CFU/ml	16 x10^2 CFU/ml
Bacteriocin + 5% Salt	15 x10^2 CFU/ml	14 x10^2 CFU/ml	13 x10^2 CFU/ml	13 x10^2 CFU/ml	13 x10^2 CFU/ml	14 x10^2 CFU/ml

Table 6: Effect of bacteriocin and 10% salt Concentration on Total heterotrophic bacteria (THB)

	1 day	7 days	14 days	21 days	28 days	35 days
10% Salt	16 x10^2 CFU/ml	17 x10^2 CFU/ml	16 x10^2 CFU/ml	5 x10^2 CFU/ml	14 x10^2 CFU/ml	15 x10^2 CFU/ml
Bacteriocin + 10% Salt	12 x10^2 CFU/ml	14 x10^2 CFU/ml	14 x10^2 CFU/ml	13 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml

Table 7: Effect of bacteriocin and 15% salt Concentration on Total heterotrophic bacteria (THB)

	1 day	7 days	14 days	21 days	28 days	35 days
15% Salt	14 x10^2 CFU/ml	13 x10^2 CFU/ml	14 x10^2 CFU/ml	15 x10^2 CFU/ml	14 x10^2 CFU/ml	13 x10^2 CFU/ml
Bacteriocin + 15% Salt	12 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml	11 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml

Table 8: Effect of bacteriocin and 20% salt Concentration on Total heterotrophic bacteria (THB)

	1 day	7 days	14 days	21 days	28 days	35 days
20% Salt	13 x10^2 CFU/ml	13 x10^2 CFU/ml	14 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml	13 x10^2 CFU/ml
Bacteriocin + 20% Salt	11 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml	10 x10^2 CFU/ml	10 x10^2 CFU/ml

Table 9: Effect of bacteriocin and 5% salt Concentration on Total Coliforms (TC)

	1 day	7 days	14 days	21 days	28 days	35 days
5% Salt	15 x10^2 CFU/ml	17 x10^2 CFU/ml	16 x10^2 CFU/ml	15 x10^2 CFU/ml	14 x10^2 CFU/ml	14 x10^2 CFU/ml
Bacteriocin + 5% Salt	14 x10^2 CFU/ml	13 x10^2 CFU/ml	12 x10^2 CFU/ml	13 x10^2 CFU/ml	13 x10^2 CFU/ml	14 x10^2 CFU/ml

Table 10: Effect of bacteriocin and 10% salt Concentration on Total Coliforms (TC)

	1 day	7 days	14 days	21 days	28 days	35 days
10% Salt	15 x10^2 CFU/ml	16 x10^2 CFU/ml	16 x10^2 CFU/ml	15 x10^2 CFU/ml	14 x10^2 CFU/ml	13 x10^2 CFU/ml
Bacteriocin + 10% Salt	13 x10^2 CFU/ml	13 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml

Table 11: Effect of bacteriocin and 15% salt Concentration on Total Coliforms (TC)

	1 day	7 days	14 days	21 days	28 days	35 days
15% Salt	14 x10^2 CFU/ml	13 x10^2 CFU/ml	13 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml
Bacteriocin + 15% Salt	11 x10^2 CFU/ml	11 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml	13 x10^2 CFU/ml	10 x10^2 CFU/ml

Table 12: Effect of bacteriocin and 20% salt Concentration on Total Coliforms (TC)

	1 day	7 days	14 days	21 days	28 days	35 days
20% Salt	13 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml	11 x10^2 CFU/ml	11 x10^2 CFU/ml	10 x10^2 CFU/ml
Bacteriocin + 20% Salt	11 x10^2 CFU/ml	12 x10^2 CFU/ml	12 x10^2 CFU/ml	10 x10^2 CFU/ml	9 x10^2 CFU/ml	9 x10^2 CFU/ml

RESULTS AND DISCUSSION

Effect of bacteriocin and chilling temperature

The effect of bacteriocin and chilling temperatures for preservation of seafood products is evaluated and the chilling temperatures used are +4°C and -18°C. The inhibitory effect of bacteriocin is compared with the control i.e. the plain sample at the respective temperatures. The results were observed weekly up to six weeks (35 days). The growth of both heterotrophic bacteria and coliforms are evaluated and tabulated.

Table 1 represents the growth of heterotrophic bacteria (THB) at 4°C. At 1st day, the control had 5x10^2CFU per ml which was gradually increased up to 35th day. On 35th day, the growth was observed as 8x10^2CFU/ml. About 6x10^2CFU/ml were observed on 1st week (7th day). The cell count remained constant at 14th, 21st and 28th day (7x10^2CFU/ml). Whereas, bacteriocin incorporated samples showed 3x10^2CFU/ml in the 1st day. The Total Heterotrophic Bacterial count (THB) increased to 5x10^2CFU/ml at the7th day, and then decreased to 4x10^2CFU/ml at 35th day respectively. About 4x10^2CFU/ml were observed at 21st day and 3x10^2CFU/ml at 28th day. Drastic heterotrophic bacterial growth difference was observed between control sample and sample containing bacteriocin.

The growth of heterotrophic bacteria (THB) at -18°C was presented in Table 2. On 1st day, the control had 4x10^2CFU per ml. The growth was observed as 4x10^2CFU/ml at 35th day. The growth was observed as 4x10^2CFU/ml at 35th day. About 5x10^2CFU/ml were observed on 1st week (7th day). The cell count remained constant at 14th and 28th day (6x10^2CFU/ml) and about 7x10^2CFU/ml was observed at 21st day respectively. Whereas in the presence of bacteriocin there were 2 x10^2CFU/ml present in the 1st day. The heterotrophic bacterial count increased to 3x10^2CFU/ml at 14th day. About 2x10^2CFU/ml were observed at 21st day and 2x10^2CFU/ml at 28th day. At 35th day their cell growth declined, it shows that bacteriocin had significant inhibitory effect on seafood than the untreated sample.

The Total Coliform Growth (TC) on sample incorporated with bacteriocin and untreated at 4°C was presented in Table 3. On 1st day, the control had 4x10^2CFU/ml. The bacterial cell count was increased at 7th day (4x10^2CFU/ml) and started declining in the following weeks. At the 14th day the total coliform count was 4x10^2CFU/ml and the bacterial count remained constant at 21th and 28th day (3x10^2CFU/ml). About 2x10^2CFU/ml was observed at 35th day. Comparatively, the sample incorporated with bacteriocin had 3x10^2CFU/ml at the 1st day and the cell count decreased on following days. About 2x10^2CFU/ml growth was observed at 21st day respectively. There was no cell growth observed at 28th day and declined at 35th day.

Table 4 represents the Total Coliform growth (TC) on control sample and sample incorporated with bacteriocin at -18°C. On 1st day, the control had 2x10^2CFU/ml. The bacterial cell count was increased at 7th day (3x10^2CFU/ml) and started declining in the following weeks. At the 14th day the total coliform count was

$3x10^2$CFU/ml and the bacterial count remained constant at 21th and 28th day ($2x10^2$CFU/ml). No cell growth was obtained at 35th day. Comparatively, the sample incorporated with bacteriocin had $2x10^2$CFU/ml at the 1st day and the cell count decreased on following days. About $2x10^2$CFU/ml growth was observed at 7th day and no cell growth observed from 14th day. The cell growth was completely inhibited from 14th day. As coliforms are pathogenic bacteria which are found less in seafood, preserving under chilling temperatures incorporated with bacteriocin resulted in no growth from 28th day. On comparing to untreated samples, bacteriocin incorporated samples showed better results in both the chilling temperatures (4°C & -18°C).

Nisin is the most extensively identified bacteriocin of antimicrobial proteins produced by LAB. The effect of nisin and pediocin ACCEL was observed on total viable counts of fresh fish fillets at 0°C and 4°C[8]. The growth of the total flora was slightly delayed when the bacteriocin were present in the samples stored at 0°C and 4°C. The efficiency of nisin on quality of fish (*Oncorhynchus mykiss*) was determined. Nisin treated *Oncorhynchus mykiss* showed decreased microbial growth on compared to those treated without nisin and enhanced the quality of the product which was tested up to 16 days at 4 °C.

The effect of *Lactobacillus plantarum* on the preservation of fresh mussels at different temperature was investigated by Genyess[9] *et al*, 2016. Mussels preserved with *L. plantarum*, regardless of the storage temperature, had higher lactic acid bacterial counts. No difference was observed in the count of total heterotrophic bacteria for the different treatments until the 30th day. On day 60th, mussels preserved with *L. plantarum* and stored at both 4°C and -18°C had lower total heterotrophic bacteria count than mussels without *L. plantarum*. For the count of *Vibrio* spp., mussels preserved with *L. plantarum* and stored at -18°C showed lower values on the seventh day, in comparison to the other treatments; and, from the 15th day, mussels preserved with *L. plantarum*, at both temperatures, had lower counts of *Vibrio* spp. The lactic acid bacteria produce bacteriocin that acts as a bio-preservative in controlling the growth of microorganisms on seafoods.

Effect of bacteriocin and brine salts

The effect of bacteriocin with brined samples was examined and optimization was done using different salt concentrations (5%, 10%, 15%, and 20%). The effect of bacteriocin is compared with the control i.e. the brined sample at different salt concentration. The results were observed weekly up to six weeks (35days). The growth of both heterotrophic bacteria and coliforms are evaluated and tabulated.

Table 5 represents the growth of heterotrophic bacteria (THB) at 5% salt concentration. On 1st day, the control had $17x10^2$CFU per ml which was gradually decreased up to 21th day. The growth was observed as $16x10^2$CFU/ml at 35th day. About $16x10^2$CFU/ml was observed on 1st week (7th day). The cell count remained constant at 14th and 28th day ($15x10^2$CFU/ml). About $14x10^2$CFU/ml were present at 21st day respectively. Whereas, bacteriocin incorporated samples showed $15x10^2$CFU/ml in the 1st day. The Total Heterotrophic Bacterial count (THB) decreased to $13x10^2$CFU/ml at 14th day. The THB remained constant up to 28th day ($13x10^2$CFU/ml) and about $14x10^2$CFU/ml were observed at the 35th day respectively. Drastic heterotrophic bacterial growth difference was observed between control sample and sample incorporated bacteriocin.

The growth of heterotrophic bacteria (THB) at 10% salt concentration is presented in Table 6. On 1st day, the control had $16x10^2$CFU per ml which was found to be increased in 7th day ($16x10^2$CFU per ml). The growth was observed as $15x10^2$CFU/ml at 21st day and $14x10^2$CFU/ml at 28th day. About $15x10^2$CFU/ml was observed at 35th day respectively. Whereas in the presence of bacteriocin there were 12 $x10^2$CFU/ml present in the 1st day. The heterotrophic bacterial count increased to $14x10^2$CFU/ml at 7th and 14th day. About $13x10^2$CFU/ml was observed at 21st day and $12x10^2$CFU/ml were present at 28th day. On 35th day their cell growth remained constant as they prevented the growth of heterotrophic bacteria.

Table 7 represents the growth of heterotrophic bacteria (THB) at 15% salt concentration. On 1st day, the control had $14x10^2$CFU per ml which was found to be decreased 35th day ($13x10^2$CFU/ml). The growth was observed as $13x10^2$CFU/ml at 35th day. About $13x10^2$CFU/ml was observed on 1st week (7th day). The cell count remained same at 14th and 28th day ($14x10^2$CFU/ml). About $15x10^2$CFU/ml were present at 21st day respectively. Whereas, bacteriocin incorporated samples showed $12x10^2$CFU/ml in the 1st day. The Total Heterotrophic Bacterial count (THB) decreased to $11x10^2$CFU/ml at 14th day. The THB remained constant up to 21st day ($11x10^2$CFU/ml) and $12x10^2$CFU/ml were present at 28th day respectively. On 35th day the THB was found to be $13x10^2$CFU/ml.

The growth of heterotrophic bacteria (THB) at 20% salt concentration is presented in Table 8. On 1st day, the control had $13x10^2$CFU per ml which was found to be increased in 14th day ($14x10^2$CFU per ml). The growth remained constant at 21st and 28th day ($15x10^2$CFU/ml). About $13x10^2$CFU/ml was observed at 35th day respectively. Whereas in the presence of bacteriocin there were 11 $x10^2$CFU/ml present in the 1st day. The heterotrophic bacterial count increased to $12x10^2$CFU/ml at 7th and 14th day. About $11x10^2$CFU/ml was observed at 21st day respectively. The growth was found to be $12x10^2$CFU/ml at 28th and 35th day. From the above results it is clear that, increase in salt concentration increases the inhibitory effect. Moreover, the rate of inhibition of THB was higher in bacteriocin incorporated samples than the plain brined samples and the effective concentration was found to be 20%.

Table 9 represents the growth of Total Coliforms (TC) at 5% salt concentration. On 1st day, the control had $15x10^2$CFU per ml which was gradually increased at 7th day ($17x10^2$CFU/ml). The growth was observed as $16x10^2$CFU/ml at 14th day. About $15x10^2$CFU/ml was observed on 28th day. The cell count remained constant at 28th day and 35th day ($14x10^2$CFU/ml) respectively. Whereas, bacteriocin incorporated samples showed $14x10^2$CFU/ml in the 1st day. The Total Coliforms (TC) decreased to $12x10^2$CFU/ml at 14th day. The TC remained constant at 21st at 28th day ($13x10^2$CFU/ml) and about $14x10^2$CFU/ml was observed at 35th day respectively.

The growth of Total Coliforms (TC) at 10% salt concentration is presented in Table 10. On 1st day, the control had $15x10^2$CFU per ml which was found to be increased in the 7th day ($16x10^2$CFU per ml). The growth was observed as $15x10^2$CFU/ml at 21st day and $14x10^2$CFU/ml at 28th day. The growth was decreased and about $13x10^2$CFU/ml was observed at 35th day respectively. Whereas in the presence of bacteriocin there were 13 $x10^2$CFU/ml present in the 1st day. The bacterial count was $13x10^2$CFU/ml at 7th day and decreased to $12x10^2$CFU/ml at 14th day. About $11x10^2$CFU/ml was observed at 21st day and $12x10^2$CFU/ml at 28th day. On 35th day the total number of coliforms was found to be $11x10^2$CFU/ml.

Table 11 represents the growth of Total Coliforms (TC) at 15% salt concentration. On 1st day, the control had $14x10^2$CFU per ml which was found to be decreased at 7th day ($13x10^2$CFU/ml). The growth was observed as $13x10^2$CFU/ml at 14th day. The cell count remained same at 21st and 28th day ($12x10^2$CFU/ml) and about $11x10^2$CFU/ml was observed at 21st day respectively. Whereas, bacteriocin incorporated samples showed $11x10^2$CFU/ml in the 1st day. The bacterial count was $11x10^2$CFU/ml at 7th day and increased to $12x10^2$CFU/ml at 14th and 21st day. About $12x10^2$CFU/ml was observed at 21st and 28th day. On 35th day the total number of coliforms was found to be $11x10^2$CFU/ml.

The growth of Total Coliforms (TC) at 20% salt concentration is presented in Table 12. On 1st day, the control had $13x10^2$CFU per ml which was found to be decreased in 7th day ($12x10^2$CFU per ml). The growth remained constant at 21st and 28th day ($11x10^2$CFU/ml). About $10x10^2$CFU/ml was observed at 35th day respectively. Whereas in the presence of bacteriocin there were $11x10^2$ CFU/ml present in the 1st day. The heterotrophic bacterial count increased to $12x10^2$CFU/ml at 7th and 14th day. About $10x10^2$CFU/ml was observed at 21st day respectively. The growth was found to be $9x10^2$CFU/ml at 28th and 35th day. From the above results it is clear that, the rate of inhibition of TC was higher in bacteriocin incorporated samples than the plain brined samples and the effective concentration was found to be 20%.

Salting is one of the oldest food preservation methods. Salting of seafood is done with salt. Chlorine and sodium ions are carried from brine to fish, and water dipoles are carried from fish to the environment. The effects of brine and dry salting methods on the nutritional composition of chub and the changes on microbial that arise during storage were investigated by Binici and kaya[10], 2017. An increase in bacterial and yeast growth during storage was observed and the growth of coliforms were reduced.

The Effect of bacteriocins and chilling temperatures (-18°C and +4°C) and effects of bacteriocin and brine salts at different concentration (5%, 10%, 15%, 20%) were studied. Bacteriocins with different chilling temperatures showed significant results on compared to control temperatures. Similarly, bacteriocin with brine salts at different concentration showed better results than the plain sample containing brine salts.

CONCLUSION

Lactobacillus spp was isolated and produced in the selective media. Effect of bacteriocins and chilling temperature (-18°C and +4°C); and Effect of bacteriocins and brine salts at various concentrations (5%, 10%, 15%, 20%) were evaluated. Bacteriocins with different chilling temperatures and brine salt concentration showed significant results on compared to control temperatures. Thus, bacteriocins due to their bacteriostatic activity can be efficiently used in hurdle technology which reduces the food spoiling organisms. Thus, bacteriocins in combined with hurdle technology can be used as alternatives for chemical preservatives in preservation techniques.

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