ISSN: 2790-5306 (Print), 2790-5314 (Online)
IRAQI JOURNAL OF AGRICULTURAL RESEARCH - Ministry of Agriculture
Available online at: www.ijarmoa.gov.iq
VOL. 26 NO. (1) 2022

IJAR
IRAQI JOURNAL OF AGRICULTURAL RESEARCH

STUDY OF THE OPTIMUM CONDITIONS FOR THE PRODUCTION OF BACTERIOCIN FROM *Lactobacillus Bulgaricus* *

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Keywords: lactic acid, bacteria, Bacteriocin, *lactobacillus bulgaricus*.

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ABSTRACT

The optimum conditions for the production of bacteriocin from *Lactobacillus bulgaricus* in the liquid MRS medium were studied and NISIN was used as a standard bacteriocin. The concentration was measured on the principle of a spectrophotometer, where it was shown that the best carbon source is glucose at 3% and a better study Conditions from nitrogen sources in terms of ammonium citrate, which showed that the ratio of 0.3% was optimum for production, while for the nitrogen source peptone and meat extract the ratio was 1.5% and the nitrogen source for yeast extract was 0.7% was optimum, while the sources of salts were the optimum conditions for manganese sulfate and magnesium sulfate It was 0.01%, while acid dipotassium phosphate was 0.2%, with a inoculum size of 7×10^7.

INTRODUCTION

During the last two decades, lactic acid bacteriocins were extensively studied, diagnosed and characterized, and the information available about them increased at the molecular and applied levels. The use of bacteriocins in food preservation is limited, and the only bacteriocin used commercially so far is NISIN, which is also of limited use (3). In addition to its wide activity against bacteria, the production of bacteriocins is affected by several factors, including the type of medium and its components, pH number, temperature and sensitivity towards enzymes as evidence of the protein nature of bacteriocins, as well as the duration of incubation and other factors, and that the different factors affect the strains producing bacteriocins (15) The bacteriocins are stable towards temperature and pH. The bacteriocins have low molecular weights, and the bacteriocins maintain the balance of the natural flora (12).

Most bacteriocins are stimulated to produce during the logarithmic phase due to changes in the production environment, such as a decrease in the level of nutrients or their depletion and the occurrence of what can be called physical crowding and lack of space available for cells. Perhaps the entry of the cell into the starvation phase is one of the factors that stimulate it to express the genes responsible for the production of bacteriocins (6).

***Part of M. Sc. Thesis of the first author.**

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Received: June\2022.
Accepted: Aug.\2022.

MATERIALS AND METHODS

Agricultural Media

1-1 MRS liquid medium

It was prepared according to Harrigan and MacCance (5) by dissolving the following ingredients in 1000 ml of distilled water using a water bath

20 g glucosamine, 5 g yeast extract, 10 g meat extract, 10 g peptone, 1 ml Tween80, 5 g sodium acetate, 2 g ammonium citrate, 2 g acid dipotassium phosphate, 0.05 g manganese sulfate, 0.1 g magnesium sulfate, adjusting the initial pH of the medium On (6.5) and sterilized in the osmosis, this medium was used to produce bacteriocin from lactic acid bacteria and to study the effect of environmental conditions on its production.

Preparation of the standard bacteriocin curve (NISIN)

Use NISIN as a standard bacteriocin according to Flores and Alegre (4). NISIN concentrations were prepared by weighing 0.4gm of NISIN and it was dissolved in 1 ml of distilled water to obtain a concentration of (400 mg/ml) and 99 ml of distilled water was added to it to become the concentration (4 mg/ml). Others are (0.25, 0.5, 1, 2, 3, 4) mg/ml as in Table 1 and the absorbance was measured by a spectrometer (from Pye Unicam Company in England) at a wavelength of 595 nm and as shown in Figure 1 where it shows the relationship between bacteriocin concentration and its absorbance.

Table 1: The storage and water NISIN volumes and different NISIN concentrations

Optical absorbance at a wavelength of 595	NISIN concentration (mg/ml)	water volume (ml)	volume of stock NISIN solution (ml)	tube number
0	0	1	0	1
0.002	0.25	2.813	0.187	2
0.20	0.5	2.625	0.375	3
0.28	1	2.25	0.75	4
0.63	2	1.5	1.5	5
0.89	3	0.75	2.25	6
1.5	4	0	3	7

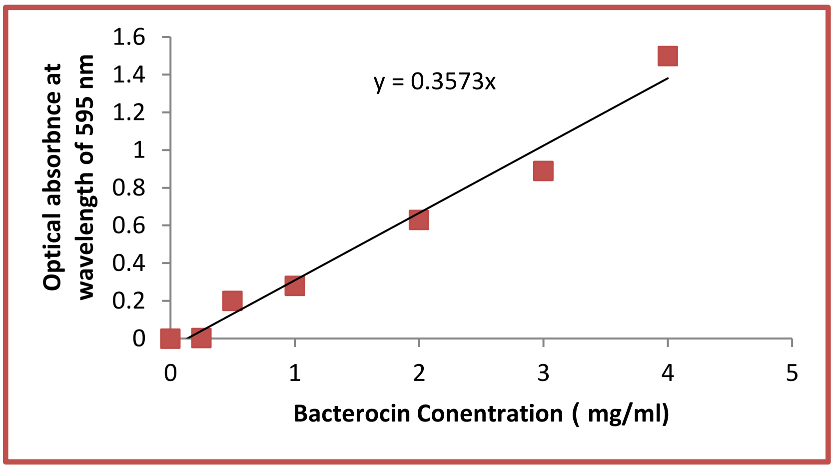


Figure 1: Standard curve of NISIN used as standard bacteriocin.

If the bacteriocin concentration measured from the following equation:

$$\frac{A}{Slope} = C$$

bacteriocin concentration C:
Absorbance A:
0.35733 Slope=

Study of the effect of environmental conditions on bacteriocin production :

A number of factors that affect the production of bacteriocin produced by *Lactobacillus bulgaricus* have been studied using MRS liquid medium prepared in paragraph (1-1)

The effect of carbon sources on production :MRS liquid medium was prepared with its components mentioned in paragraph (1-1) with the use of the carbon source in it, which is sucrose xylose, glucose and lactose each individually and in three concentrations (1, 2, 3) (weight/volume) and the pH was adjusted to (6.5) and it was sterilized with a retardant device.

The media prepared with the above-mentioned carbon sources were inoculumted with an activated culture of *Lactobacillus bulgaricus* in aerobic conditions, at 24 hours of age, with 1% inoculum, and the number of cells (7 x 10^7 W.m.m/ml). The inoculumted media were incubated at a temperature of 37°C for 24 hours. In aerobic conditions, it was subjected to a centrifugation of 6000 x g for 10 minutes at 4 °C, then filtered through microbial filters with a porosity of 0.22 (16).

The effect of nitrogen sources on production :MRS liquid medium was prepared with its components mentioned in paragraph (1-1) with the fixation of the carbon source and its percentage that gave the highest bacteriocin yield. The ratios of nitrogen sources in it were replaced as follows:

Ammonium citrate (0.1, 0.2, 0.3)% , Peptone (0.5, 1, 1.5)% , Meat extract (0.5, 1, 1.5) % Yeast extract (0.3, 0.5, 0.7)%

The ratios were changed for one nitrogen source, and the rest of the ratios of other nitrogen sources were fixed. After choosing the best ratio, it was fixed and the initial pH of the media was adjusted to 6.5 and sterilized with an autoclave device. The prepared media were inoculumted in a previous activated culture at the age of 24 hours in aerobic conditions, with a vaccination rate of 1%, and the number of *Lactobacillus bulgaricus* cells 7 x 10^7 The inoculumted media was incubated at 37 °C for 24 hours, then subjected to centrifugation at 4000 x g for 20 minutes at 4 °C, then filtered through microbial filters with a porosity of 0.22 µm. The activity of bacteriocin was estimated by measuring The absorbance of the filtrate at a wavelength of 600 nm was considered an indication of the amount of bacteriocin produced (15).

Effect of salt on production: MRS liquid medium was prepared with its components in paragraph (1-1) with fixing the proportions of the components that gave the best concentration for bacteriocin production, and the proportions of salts used in the medium were replaced, which are: Manganese sulfate (0.0025, 0.005, 0.01) %, Magnesium sulfate (0.005, 0.01, 0.02) ,%Acid di potassium phosphate (0.1, 0.2, 0.3) %.

The proportion of manganese sulfate has been changed and the proportions of other salts have been fixed according to paragraph (1-1) of the liquid MRS

medium, after choosing the best proportion of it and fixing it, changing the proportions of magnesium sulfate, as well as for acid dipotassium phosphate, and adjusting the pH to 6.5 and sterilizing with the sterilizer device. The media prepared with the three different salt sources were inoculumted with an activated culture of *Lactobacillus bulgaricus* in aerobic conditions at 24 hours of age, 1% inoculum and a number of 7 x 10 cells m.m/ml. The inoculumted media were incubated at a temperature of 37°C for 24 hours. Then it was subjected to a centrifugation xg 4000 for 20 minutes at 4°C and filtered through microbial filters with a porosity of 0.22 µm.

The effectiveness of bacteriocin was estimated by measuring the absorbance of the filtrate containing pectocin at a wavelength of 600 nm, which was taken as an indication of the amount of bacteriocin produced (10).

The effect of the size of the inoculum on production: The liquid MRS medium was prepared as in paragraph (1-1) and the optimum components and ratios were fixed to give the best bacteriocin production. The pH was adjusted to (6.5) and sterilized with osmosis and inoculumted three flasks of inoculum size (7×10^7, 7×10^6, 7×10^5) and incubated at 37°C for 24 hours in aerobic conditions. It was subjected to centrifugation at a speed of 6000 x g for 10 minutes at 4 °C to remove bacterial cells, then filtered through microbial filters with a porosity of 0.22 µm (10).

RESULTS AND DISCUSSION

Optimum conditions for bacteriocin production from *Lactobacillus bulgaricus*: MRS liquid medium was adopted under constant conditions, because the best medium for the production of bacteriocin from *Lactobacillus bulgaricus* is MRS medium (7).

The effect of carbon sources:It can be seen from Figure 2 that shows the effect of carbon sources (sucrose, xylose, glucose and lactose) on the production of bacteriocin from *Lactobacillus bulgaricus* using MRS liquid medium at a temperature of 37 °C for 24 hours. The best carbon source is glucose, at a rate of 3%. The concentration of bacteriocin was 0.59 mg/ml compared to other carbon sources, sucrose, xylose and lactose, and this result contrasts with studies that indic .

The best carbon source for producing bacteriocin from *Lactobacillus bulgaricus* is glucose (19) , also corresponds to. Al-Zahrani *et al*(2) and Sidooski *et al* (18), Glucose is the preferred carbon source for stimulating bacteriocin production. As for the xylose source at a concentration of 2 and 3%, the concentration ratio has decreased because some types of sugars lead to a decrease in bacteriocin production, which can be explained by unfavorable conditions for growth and with osmotic stress that leads to a decrease in the growth rate And that osmotic stress leads to an increase in energy demand and reduces the maximum secretion of bacteriocin, which indicates that the excess energy affects the production of bacteriocin (14).

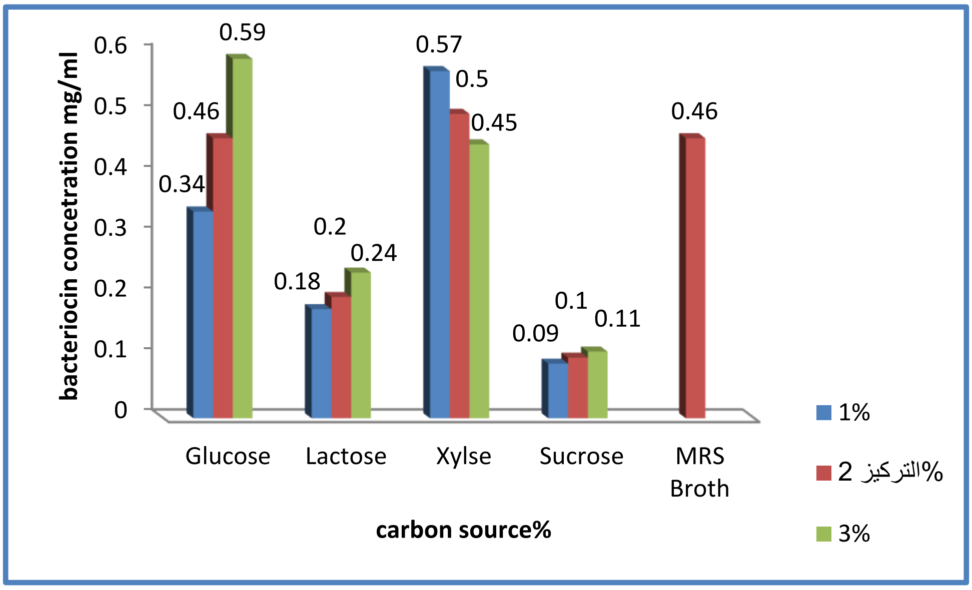


Figure 2: Effect of carbon sources on the concentration of bacteriocin produced from Lactobacillus bulgaricus.

Effect of Nitrogen Sources

The nitrogen sources present in the MRS medium were used (ammonium citrate, peptone, meat extract and yeast extract) with different concentrations to reach the optimum production of bacteriocin. It is noted in Figure 3 the effect of different concentrations of ammonium citrate for the production of bacteriocin from *Lactobacillus bulgaricus* and it was found that the best concentration is 0.83 mg/ ml using 0.3% ammonium citrate.

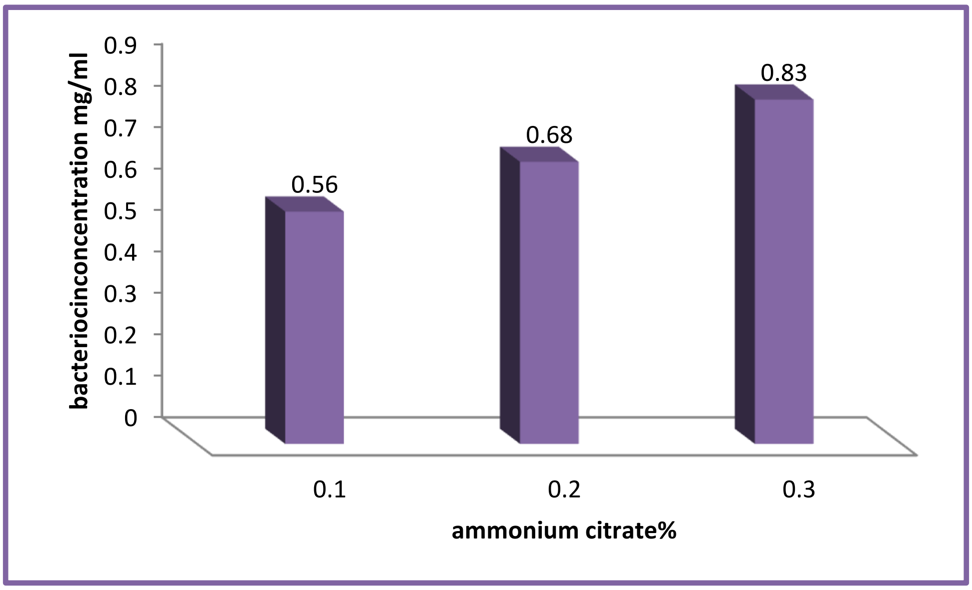


Figure 3: Effect of ammonium citrate concentration on the concentration of bacteriocin.

Produced from *Lactobacillus bulgaricus*

As for the peptone nitrogen source, it noted from Figure 4, which represents the peptone source in the concentration of bacteriocin produced from *Lactobacillus bulgaricus*. The highest concentration of bacteriocin is 0.96 mg/ml, using 1.5% of peptone Vázquez, and Murado (21). Showed that the highest

concentration of peptone It is the best way to produce bacteriocin from lactic acid bacteria.

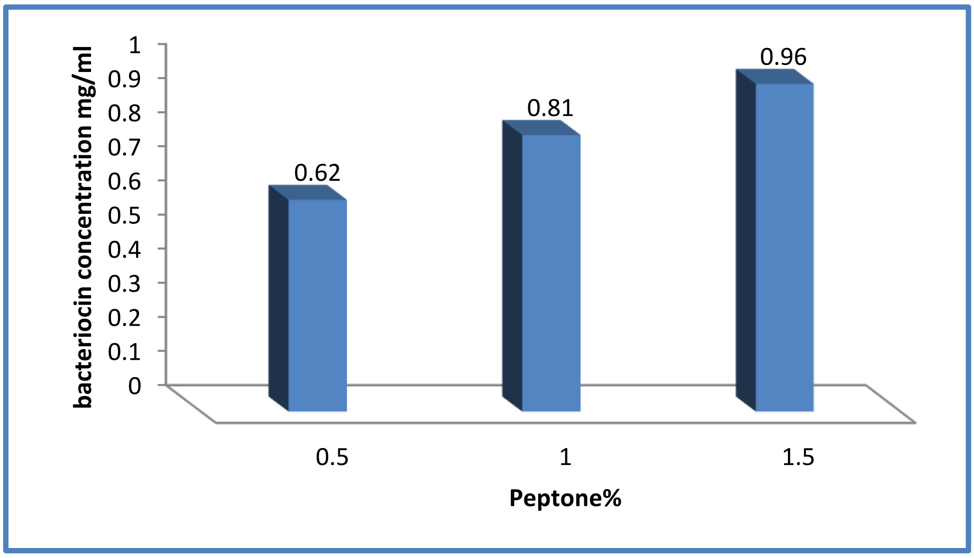


Figure 4: Effect of peptone on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*.

It can be seen from Figure 5 the effect of meat extract on producing bacteriocin from *Lactobacillus bulgaricus*, giving the highest concentration of 0.99 mg/ml using 1.5%. Aasen *et al* (1) explained that replacing half the amount of meat extract led to a reduction in biomass and bacteriocin production from *Lactobacillus Sakei CCUG4687*.

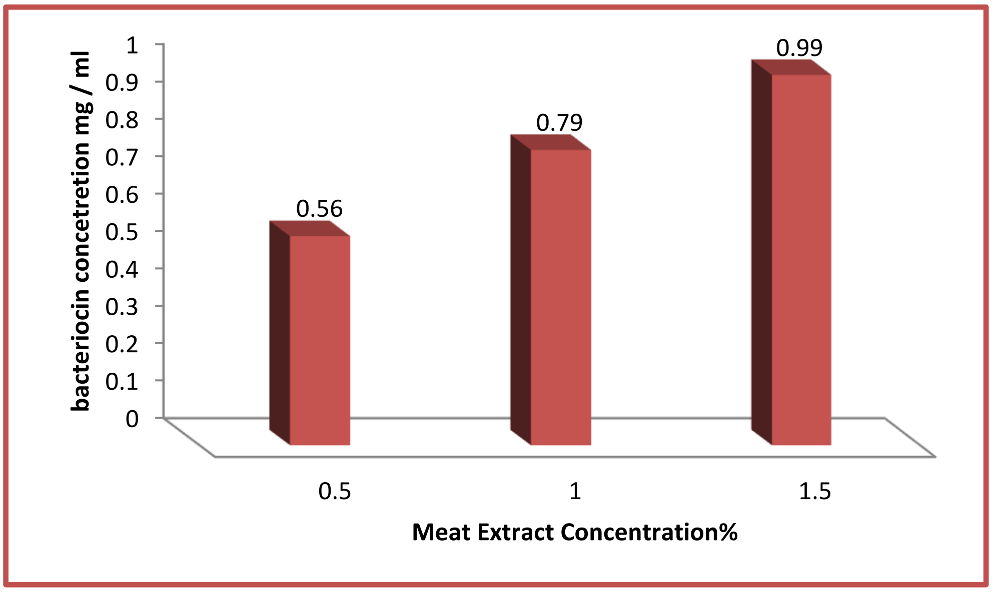


Figure 5: Effect of meat extract on the concentration of bacteriocin produced from *Lactobacillus bulgaricus.*

As for the effect of the nitrogen source for yeast extract, it is noted from Figure 6 that the highest concentration of bacteriocin production from *Lactobacillus bulgaricus* was 1.10 mg/ml using 0.7%.

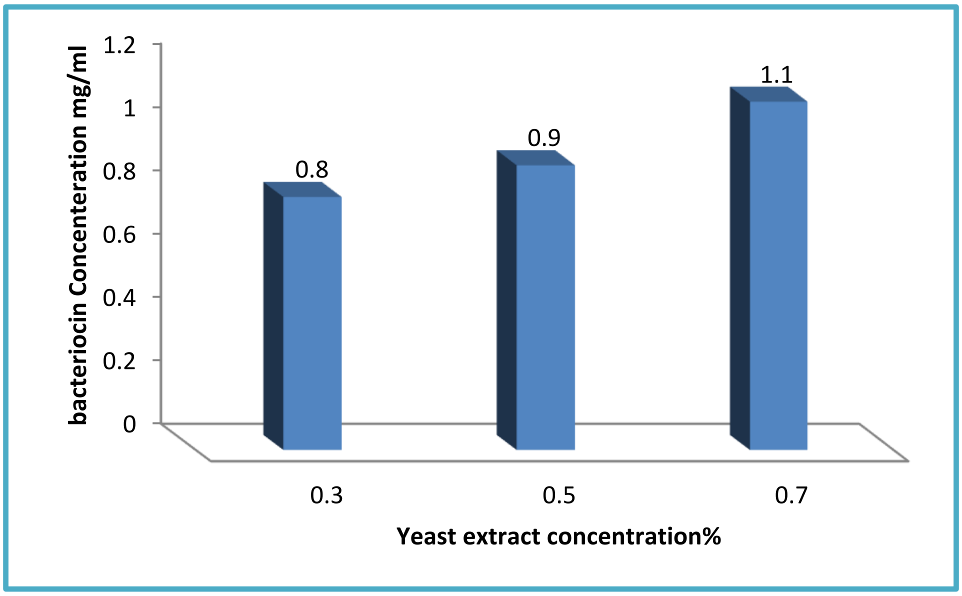


Figure 6: Effect of yeast extract on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*.

Sawatari *et. al.* (17) explained *Lactobacillus bulgaricus* responds to changes in nitrogen availability in its environment resulting from the regulation of the proteolytic system to ensure an adequate nitrogen balance within the cell.

Nitrogen sources have a vital role for effective growth and improvement of microbial strains and play an important role in fermentation processes, as they are included in the synthesis of amino acids because they are considered the basic unit of protein building (9).

Effect of salt on production

Three types of salts were used, which are manganese sulfate, magnesium sulfate and acid dipotassium phosphate, and different ratios were chosen to obtain the best production of *Lactobacillus bulgaricus*.. Figure 7 shows the effect of different concentrations of manganese sulfate on the concentration of bacteriocin produced by *Lactobacillus bulgaricus*. An increase in bacteriocin concentration was observed to 1.3 mg/ml by using 0.01% manganese sulfate from this salt.

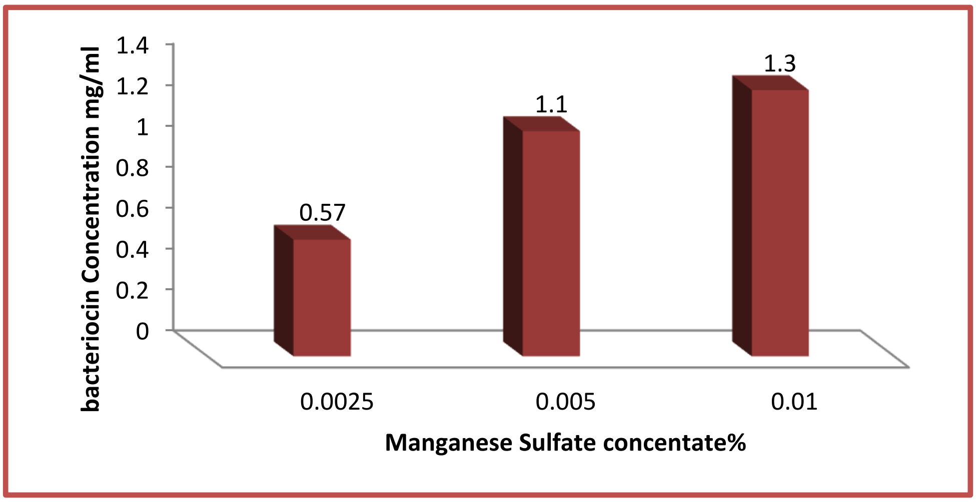


Figure 7: Effect of manganese sulfate on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*.

It can be seen from Figure 8 the effect of magnesium sulfate on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*, and it gave the highest concentration at 1.31 mg/ml at 0.01%.

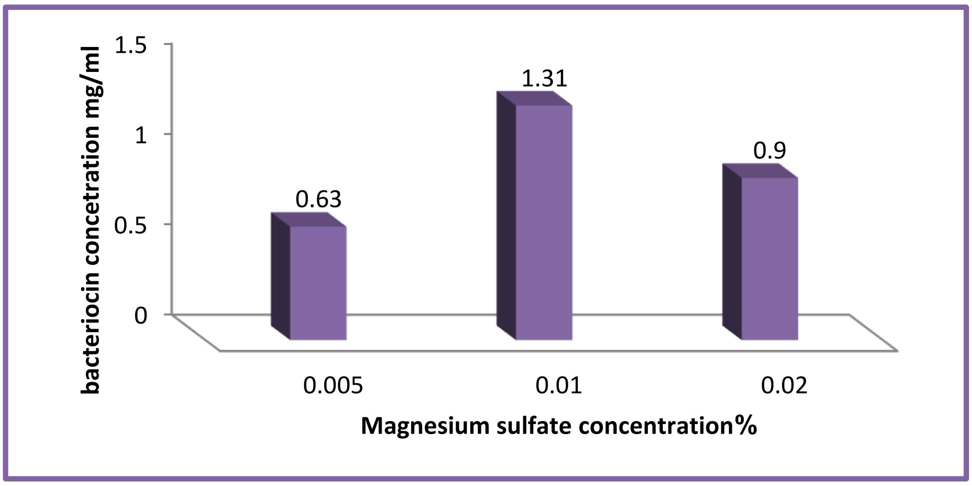


Figure 8: Effect of magnesium sulfate concentration on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*.

As for acid dipotassium phosphate, its effect on the concentration of bacteriocin produced from *Lactobacillus bulgaricus* was observed from Figure (9), which produced the highest bacteriocin concentration of 1.32 mg/ml at 0.2%.

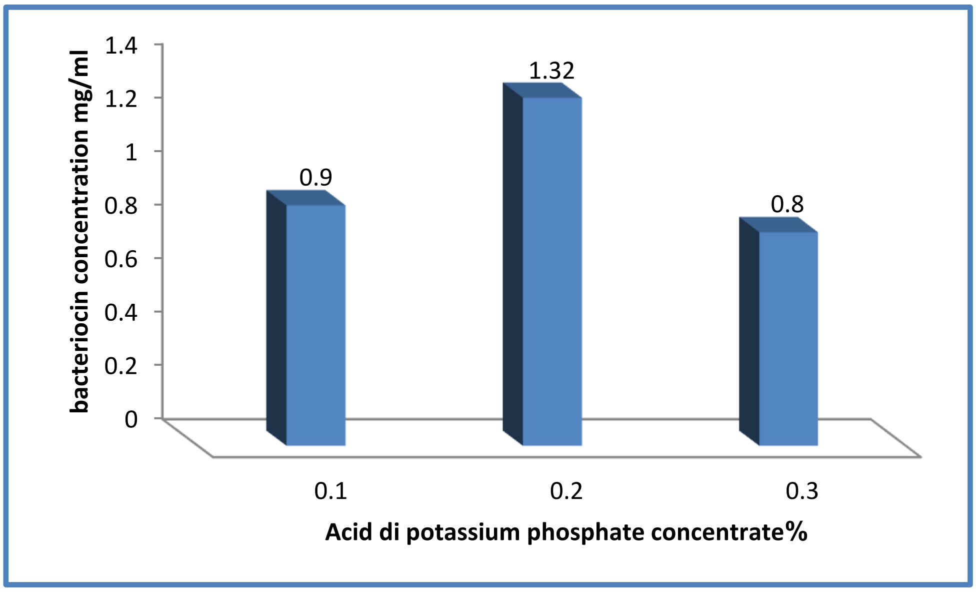


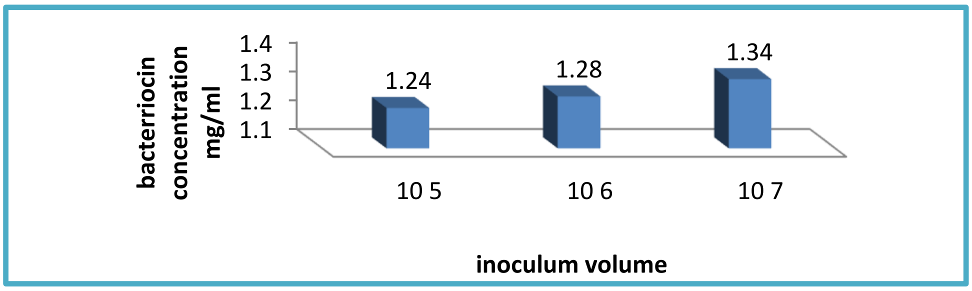
Figure 9: Effect of acid di potassium phosphate concentration on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*.

These results are in agreement with what was stated by Kumar and Arumugam(8) and Mezher(11) , where they indicated that the addition of glucose, nitrogen sources and salts have an effect on bacteriocin production. Magnesium and acid di potassium phosphate and it was concluded that they have no effect on the production of bacteriocins. The difference in the quantities produced from bacteriocin depends on the variation in the concentration of the components of the medium, while Upendra *et al*. (20), used acid dipotassium phosphate and magnesium sulfate to produce bacteriocin.

The production of bacteriocins, in general, depends on the growth and physiological activity of the types of bacteria produced, and the amount of bacteriocins depends on the environment and the materials in which they grow. It is in logarithmic growth and reaches its highest after 24 and 48 hours of incubation

The effect of the size of the inoculumtion

Figure 10 shows the effect of three concentrations of the inoculum on the production of bacteriocin produced by *Lactobacillus bulgaricus* (7 x 10^5, 7 x 10^6, 7 x 10^7) and m/mL using liquid MRS at a temperature of 37°C. For 24 hours, it appears that the best concentration was 1.34 mg/ml in a inoculum volume (7 x 10^7).



10^5 10^6 10^7

Figure 10: Effect of the size of the inoculum on the concentration of bacteriocin produced from *Lactobacillus bulgaricus*.

Used a cell count to produce bacteriocin from lactic acid bacteria at a concentration of (10^7-10^8) (13).

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ISSN: 2790-5306 (Print), 2790-5314 (Online)
مجلة الزراعة العراقية البحثية ـ وزارة الزراعة
متاح على الانترنت: www.ijarmoa.gov.iq
مجلد 26 العدد (1) 2022

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دراسة الظروف المثلى لإنتاج البكتريوسين من *Lactobacillus bulgaricus**

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الملخص

تم تحديد ظروف النمو المثلى لإنتاج البكتريوسين من بكتريا ***Lactobacillus bulgaricus*** وقد تمت دراسة الظروف المثلى لإنتاج البكتريوسين في الوسط **MRS** السائل و استخدم **NISIN** لانه يُعدّ بكتريوسيناً قياسياً وتم قياس التركيز على مبدأ جهاز المطياف الضوئي **Spectrophotometer**، إذ اظهر ان افضل مصدراً كاربونياً هو الكلوكوز بنسبة **3%** ودراسة افضل ظروفاً من مصادر النتروجين من حيث سترات الامونيوم التي اظهرت ان نسبة **0.3%** كانت هي المثلى للإنتاج بينما للمصدر النيتروجيني الببتون ومستخلص اللحم كانت بنسبة **1.5%** هي المثلى والمصدر النتروجيني مستخلص الخميرة كانت بنسبة **0.7%** هي المثلى بينما مصادر الاملاح كانت الظروف المثلى لكبريتات المنغنيز وكبريتات المغنسيوم فكانت بنسبة **0.01%** أما فوسفات ثنائي البوتاسيوم الحامضي كانت بنسبة **0.2%** وبحجم لقاح 7×10^7 .

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* جزء من رسالة ماجستير للباحث الأول.

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تاريخ تسلم البحث :حزيران /**2022**.
تاريخ قبول البحث :آب /**2022**.