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OPTIMAL CONDITIONS FOR ACIDOCIN PRODUCTION FROM *LACTOBACILLUSACIDOPHILUS* ISOLATE

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ABSTRACT

The present study was undertaken to determine of optimal conditions for Acidocin production. The medium and conditions for *Lb. acidophilus* was determined and the results showed that the best medium which referred to as Basal Growth Medium (BGM) composed of 2% glucose, 2.5% peptone, 2.5% yeast extract, 2% meat extract, 0.25% K_2HPO_4 and 0.5% tween 80,with pH 6 at temperature 37°C for 24 hours under anaerobic conditions was the suitable medium for acidocin production.

Key words: Acidocin,*Lb.acidophilus*, optimal conditions.

INTRODUCTION

Lactic acid bacteria (LAB) and physiologically related group of gram-positive bacteria produce a variety of compounds with antimicrobial activity, and they are termed bacteriocins. Bacteriocins are generally defined as extracellular released peptide or protein that shows a bactericidal activity against species closely related to the bacteriocin producing strain. Lactic acid bacteria and their metabolites have been shown to play an important role in improving microbiological quality and shelf life of many fermented food products and provide a good example of biopreservation.[1].

Bacteriocins are proteinaceous compound which have inhibitory effects towards sensitive strains produced by both gram-positive and gram-negative bacteria ,Bacteriocins producing lactic acid bacteria are used in food fermentations especially in dairy products. In USA, only

nisin produced by *Lactobacillus lactis*has been permitted as a food preservative , It has also been used in health care products and cosmetics for treatment of acne , They are also being used in toothpaste and mouthwash for the inhibition of dental caries and periodontal diseases [2].

In an attempt to control pathogenic bacteria in food, the production of antimicrobial peptides from bacteria "bacteriocins" has received much consideration, bacteriocins are compounds produced by bacteria that have a biologically active protein moiety and bacteriocidal action, antimicrobial peptides from gram positive organisms such as Lactic acid producing bacteria have attracted much attention and have been the subject of intensive investigation due to their extensive incorporation as bio-preservative ingredient into model foods, particularly in the dairy industry[3].

This study was aimed to determination of optimal conditions to produce acidocin from *Lactobacillusacidophilus.*

MATERIALS AND METHODS

Acidocin Production in Different Media

To determine the best medium for acidocin production, 18-hours old culture of the bacterial isolate was inoculated into different culture media: MRS broth, M17 broth, BHIB, NB, TSB and whey medium. Cultures were incubated an aerobically at 35°C for 24 hours. Antimicrobial activity was determined.

Effect of Medium Composition on Production of Acidocin

Effect of Carbon Source (Sugars)

Different sugars (glucose, lactose, maltose, mannitol, sucrose, fructose and mannose) were used, 2% of each one of them was added to MRS broth without sugar, separately. All media were inoculated with 1% of 18-hours old culture of the bacterial isolate and incubated anaerobically at 35°C for 24 hours. Protein concentration and activity of acidocin was determined.

The optimal concentration of the best carbon source was determined by adding different concentrations of it (1 , 1.5 , 2 , 2.5 and 3) % to MRS broth without glucose , separately, then inoculated with 1% of 18-hours old culture of the selected isolate and incubated anaerobically at 35°C for 24 hours. Protein concentration and activity of acidocin were determined.

Effect of Nitrogen Source

To determine the best nitrogen source and its concentration, three organic nitrogen sources (peptone, meat extract and yeast extract) with different concentrations were used .These concentrations were added to MRS broth without organic nitrogen sources (separately) as shown in table 1.All tubes were inoculated with 1% of 18-hours old culture of the bacterial isolate and incubated anaerobically at 35°C for 24 hours. Protein concentration and activity of acidocin was determined.

Table 1 Organic nitrogen sources and their concentrations in MRS broth medium

MRS broth +Organic nitrogen source
MRS without organic nitrogen source
MRS + (0.5 ,1 , 1.5,2,2.5)%Peptone
MRS + (0.5 ,1 , 1.5,2,2.5)% meat extract
MRS + (0.5 ,1 , 1.5,2,2.5)%Yeast extract
MRS + (2 , 2.5)% Peptone + (2,2)% meat extract (respectively)
MRS + (2,2.5)% Peptone + (2.5 , 2.5)% Yeast extract (respectively)
MRS + (2 ,2)% meat extract + (2.5 , 2.5)%Yeast extract (respectively)
MRS + (2,2.5)% Peptone +(2,2)% meat extract +(2.5, 2.5)%Yeast extract

Effect of K_2HPO_4

It was studied by adding K_2HPO_4 at different concentrations (0.1, 0.2, 0.25 and 0.5) % ,separately to MRS broth without potassium phosphate .All tubes were inoculated with 1% of 18-hours old culture of the bacterial isolate and incubated anaerobically at 35°C for 24 hours. Protein concentration and activity of acidocin was determined.

Effect of Tween 80

It was studied by adding different concentrations (0.1, 0.2, 0.25, 0.5, 0.75 and 1) % of tween 80 separately to tubes containing MRS broth without tween 80. All tubes were inoculated with 1% of 18-hours old culture of the bacterial isolate and incubated anaerobically at 35°C for 24 hours. Protein concentration and activity of acidocin was determined.

Basal Growth Medium (BGM)

Depending on results of experiments above, the medium composed of 2-3% glucose+ 2% peptone + 2.5% yeast extract + 2% meat extract + 0.5% K_2HPO_4 + 0.1% tween 80 and 2-3% glucose+ 2.5% peptone + 2.5% yeast extract + 2% meat extract + 0.25% K_2HPO_4 + 0.5%

tween 80 respectively for acidocin production that prepared in this study was used in all subsequent experiments in this study and referred to as Basal Growth Medium (BGM).

Effect of Medium pH on acidocin Production

Volumes of BGM were adjusted to pH 3,3.5,4,4.5,5,5.5,6,6.5 and 7 respectively, with 1N HCl or 1N NaOH and autoclaved.Each tube was inoculated with 2% of an 18-hours old culture of bacterial isolate and incubated anaerobically at 35°C for 24 hours. Protein concentration and activity of acidocin was determined.

Effect of Incubation Temperature on acidocin Production

Volumes of BGM were inoculated with 2% of an 18-hours old culture of bacterial isolate and incubated anaerobically at different temperatures (25, 30, 33, 35, and 37) °C for 24 hours. Protein concentration and activity of acidocin was determined.

Effect of Incubation Periods on acidocin Production

It was studied by inoculating BGM with 2% of an 18-hours old culture of bacterial isolate and incubated anaerobically at 30°C,37°C respectively to acidocin for periods (6,8,12,24 and 48)hours .At the end of each incubation period, Protein concentration and activity of acidocin was determined.

Effect of Aerobic and Anaerobic Conditions on acidocin Production

Volumes of BGM were inoculated with 2% of an 18-hours old culture of bacterial isolate and incubated aerobically and anaerobically at 30°C, 37°C for 12 hours , 24 hours respectively to acidocin .At the end of each incubation period, protein concentration and activity of acidocin was determined.

RESULTS AND DISCUSSION

Optimal Medium for Acidocin Production

The result shown MRS broths were the best media for acidocin production. MRS broth medium recorded the best medium to give the highest inhibition zones againstthe indicator bacteria.Low acidocin production was observed in trypton soya broth (TSB) medium, No production of acidocin was observed in brain heart infusion broth (BHIB), nutrient broth (NB), M17 or whey medium. These results suggest that specific nutrients are required for acidocinproduction , this results also reported by Ali [4].

Optimal Carbon Source

The growth of *Lb.acidophilus*2% glucose resulted in specific activity 36.36 AU/mg protein whereas maltose was the minimum effectual with specific activity 25.97AU/mg protein, as shown in figure (1),Based on these results, glucose which showed the optimal carbon source was considered as the best concentration for Acidocin production.

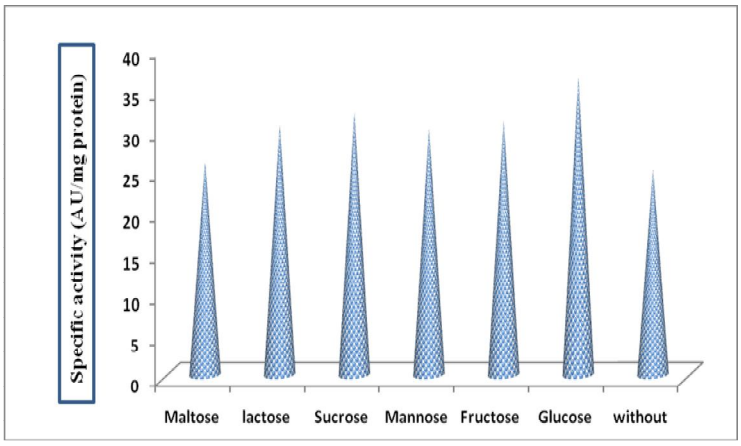


Figure 1 Influence of different carbon sources on production of Acidocin

Optimal Concentration of Glucose

Glucose was added to the media as the best carbon source with different concentration as shown in figure 2, the highest specific activity was 44.44 AU/mg protein at 2% glucose concentration.

The highest specific activity of acidocin was 43.47 AU/mg proteins at 2% and 3% glucose concentration. The specific activity of acidocinwas lowered to (29.41 and 34.48) AU/mg protein when (1 and 1.5) % concentrations of glucose were added, respectively.

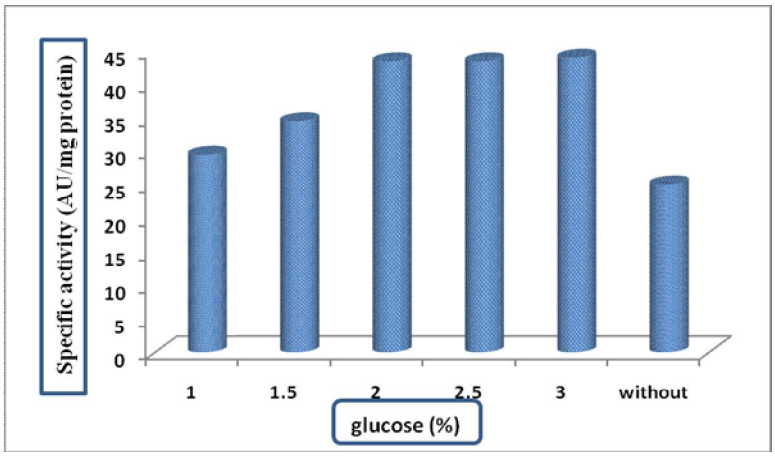


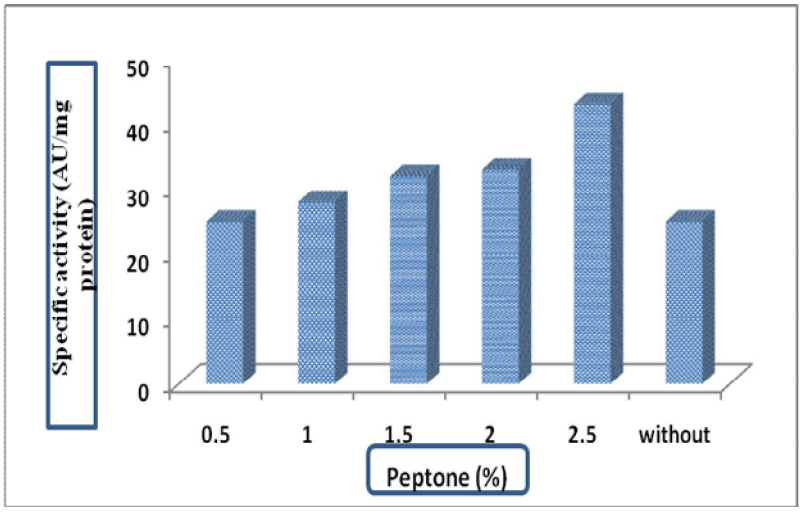
Figure 2 Determination the optimal concentration of glucose for production of acidocin byLactobacillus acidophilus.

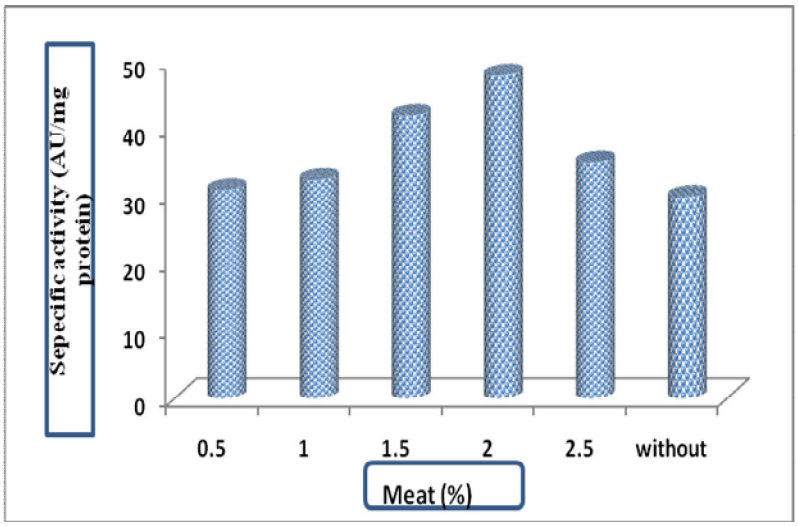
The study of (2) showed that the maximal production of plantaricin obtained in MRS broth containing at 2% glucose. Based on these results, the concentration 2% glucose was considered as the best concentration for acidocin production.

Optimal Concentration of Organic Nitrogen Source

Results of all organic nitrogen sources assayed as shown in figure (3), 2% , 2.5% peptone yielded the highest specific activity of acidocin while at the presence of 2% of meat extract yielded the highest specific activity of acidocin and yeast extract yielded acidocin at 2.5% .

As shown in figure (4) a combination of peptone, yeast extract and meat extract yielded acidocin with specific activity53.33 AU/mg protein .





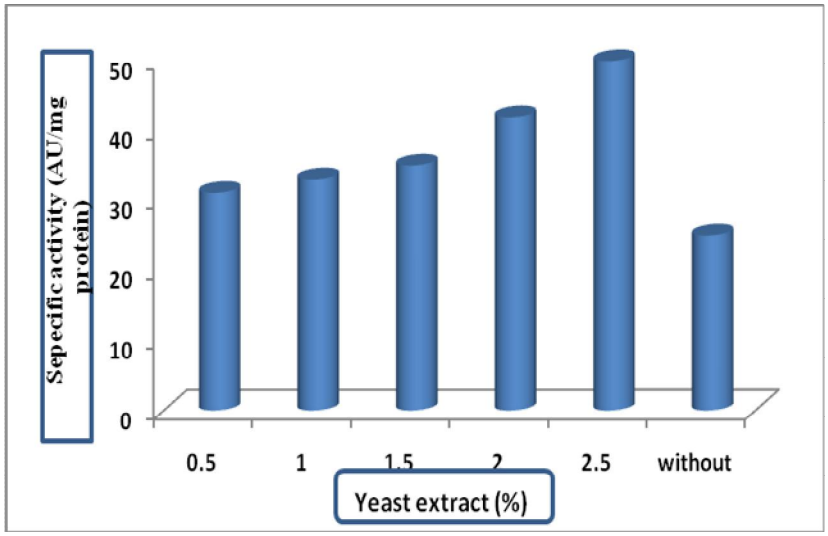


Figure 3 Effect of different organic nitrogen sources on production of acidocin .

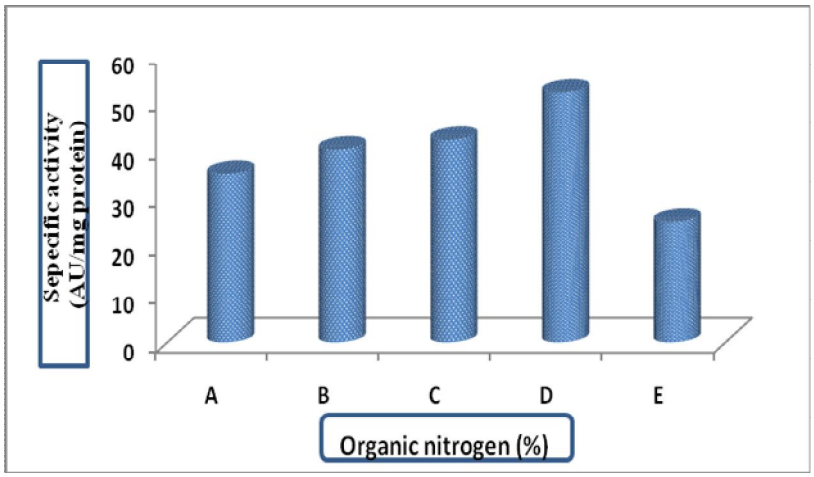


Figure 4 Determination the optimal organic nitrogen source for production of acidocin

A :MRS broth without organic nitrogen source + 2% , 2.5% peptone and 2% , 2% meat extract for plantaricin and acidocin respectively .

B : MRS broth without organic nitrogen source + 2% , 2.5 % peptone and 2.5% , 2.5 % yeast extract for plantaricin and acidocin respectively

C : MRS broth without organic nitrogen source + 2% , 2% meat extract and 2.5% , 2.5% yeast extract for plantaricin and acidocin respectively

D : MRS broth without organic nitrogen source + 2% , 2.5 % peptone, 2% , 2% meat extract and 2.5% , 2.5% yeast extract.

E : MRS broth without organic nitrogen source .

Most of the bacteriocin producing organisms requires stabilizers or a unique medium composition for bacteriocin synthesis . It is probable that the yeast extract may in part serve

to inactivate an inhibitor of bacteriocin synthesis [5]. Todorov et al. [6], reported that yeast extract is the most effective organic nitrogen compound for bacteriocin production.

Effect of Potassium Phosphate on AcidocinProduction

As shown in figure (5), acidocin production was differed according to the presence of different concentrations of K_2HPO_4 , the optimal acidocin production was recorded in the presence 0.25% $K_2HPO_4$with specific activity 58.82 AU/mg protein. The mineral salts were known to increase bacteriocin production by LAB, K_2HPO_4 contains phosphorus source which is very important for cell growth and thus bacteriocin production. Similar results have been recorded by Powell et al.,[7].

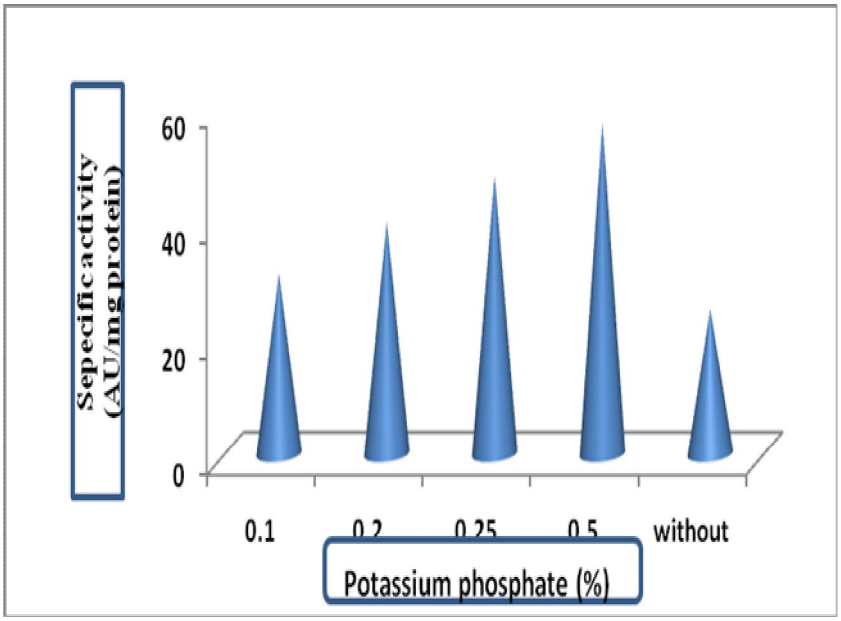


Figure 5 Influence of different concentrations of potassium phosphate on production of acidocin.

Effect of Tween 80 on Acidocin Production

The additions of (0.5%) tween 80 concentrations yielded Acidocin with specific activity 72.72 AU/mg protein. When tween 80 was omitted from the broth medium, the specific activity significantly reduced to 24.53 AU/mg protein, as shown in the figure 6. The addition of tween 80 to the growth medium caused increased in the production of bacteriocins by more than 50% [8]. The presence of 0.1% of tween 80 in the growth medium increased the bacteriocin production [5] , similar results were recorded for nisin [9].

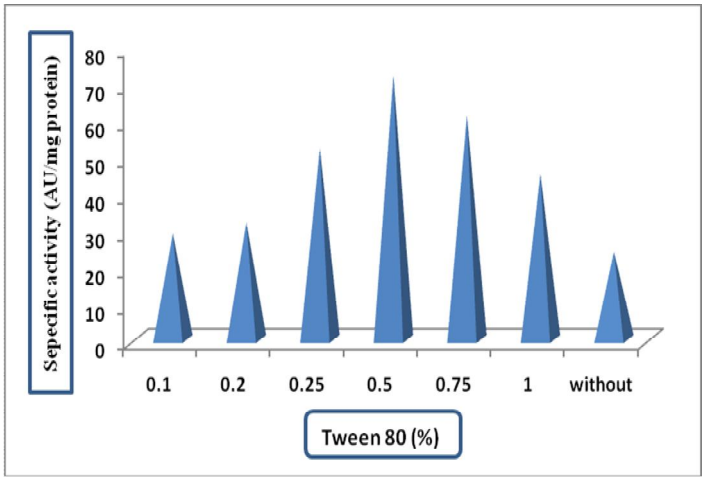


Figure 6 Influence of different concentrations of tween 80 on acidocin production.

Optimal pH for Acidocin Production

Results showed the pH 6 was the best value for Acidocin production when the specific activity recorded 94.11 AU/mg protein , (figure 7).

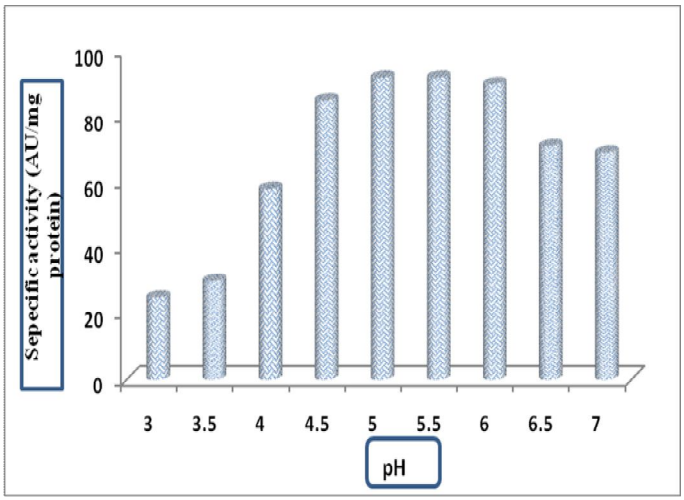


Figure 7 Influence of different pH values on acidocin production .

The activity of bacteriocin elaborated by the test isolates was also pHdependent, the highest antibacterial activity was exhibited in an acidic pH range [10].

Optimal Temperature for acidocin Production

As shown in figure (8), the maximum specific activity (97.56AU/mg protein) was recorded for acidocin at 30°C, at temperature (37°C) the specific activity decreased to 68.37 AU/mg protein, while in case of acidocinspecific activity (98.15 AU/mg protein) was recorded for acidocin at 37°C, lower temperature (25°C) the specific activity decreased to 35.08 AU/mg protein.

The reduction in the bacteriocin production at higher and lower temperature referred to slow growth that led to retardation of bacteriocin production .The temperature affects microorganism through its effect on oxygen solubility in the media, on kinetic energy of molecules and on reaction velocity in the cell, and these affect on bacteriocin production [11].

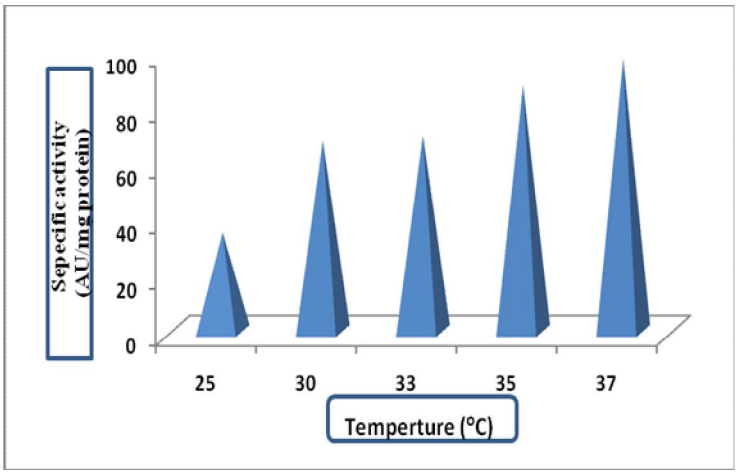


Figure 8 Effect of different temperatures on production of acidocin produced. Riaz*et al*.[12] showed the optimum temperature for growth of *L.acidophilus*was 35°C and pH was 6.0

Optimal Incubation Period for Production

The evolution of the acidoin production was followed up at different incubation periods; maximum production of acidoin was observed at 24 hours of incubation with specific activity 114.28 AU/mg protein . Gradual decreasing of specific activity was recorded after 24 and 48 hours of incubation, as shown in figure (9) .maximum bacteriocin yield in a culture may occur at different phases of growth cycle, depending on the type of bacteria.

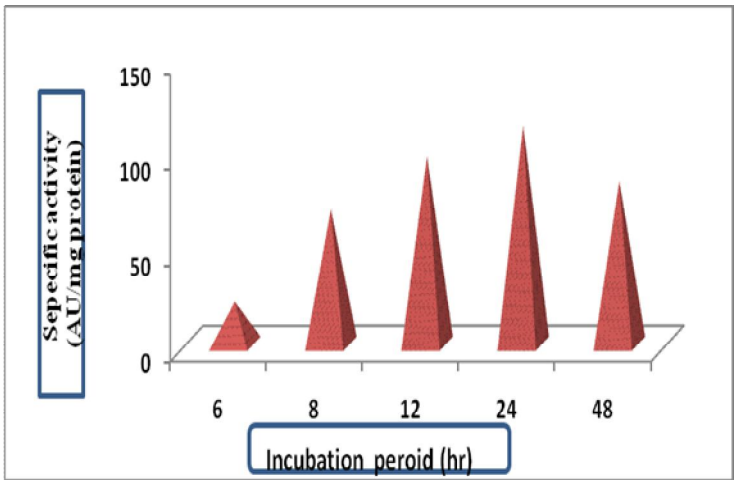


Figure 9 Production of acidocin at different incubation periods.

Effect of Incubation Conditions on Acidocin Production

The specific activity of acidocin recoded 114.28 AU/mg protein, respectively under anaerobic conditions , under aerobic conditions the production of acidocin recorded 60.6 AU/mg protein.These results indicated that anaerobic conditions are favored foracidocin production than aerobic conditions, as shown in figure (10).

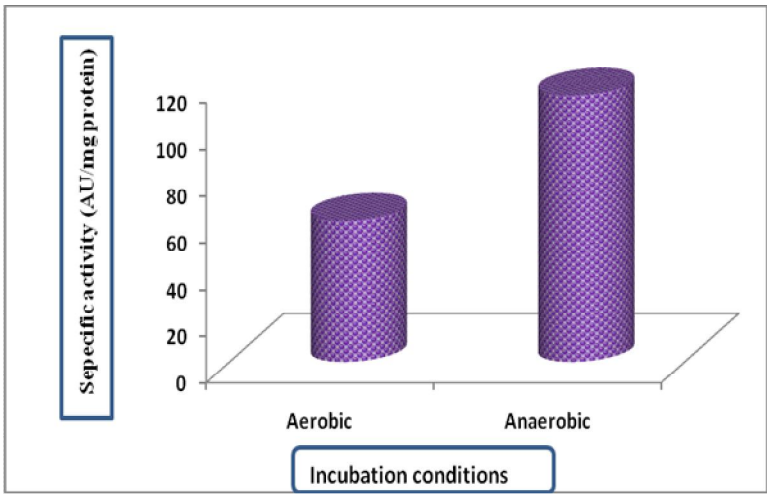


Figure 10 Influence of incubation conditions (aerobic and anaerobic) on production of acidocin produced

Bacteriocinwhich produce by *Lactobacillus*was active under limited or reduced oxygen in the medium as indicated by [13].

Thus, based on results above, the medium composed of 2% glucose, 2.5% peptone, 2.5% yeast extract, 2% meat extract, 0.25% K_2HPO_4 and 0.5% tween 80, referred to as Basal Growth Medium (BGM) that prepared in this study, with initial pH 6 , at 37°C incubation temperature for 24 hours under anaerobic conditions recorded the best production of Acidocin by *Lb.acidophilus.*

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