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Research Article

PROFILE OF GROWTH AND THE α-AMYLASE PRODUCTION FROM VARIOUS BACILLUS SPECIES

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ABSTRACT

Objective: In the present research work, three bacteria *Bacillus subtilis*, *Bacillus licheniformis* and *Lactobacillus acidophilus* were subjected to study their growth profile and alpha amylase production from them. **Method:** Nutrient agar and nutrient broth medium were used for their growth. In the nutrient broth, it was found that these bacteria were able to produce α-amylase. Production of α-amylase from these bacteria were studied through Iodine assay and DNSA. **Results:** It was investigated that DNSA technique is better than Iodine assay for the alpha amylase production from Bacillus species bacteria. **Conclusion:** *Bacillus subtilis* has better efficiency to produce alpha-amylase in nutrient medium, in compare to *Bacillus licheniformis*, *Lactobacillus acidophilus*

Keywords: *Bacillus subtilis, Bacillus licheniformis, Lactobacillus acidophilus* and the alpha amylase.

INTRODUCTION

In aged persons, reduced metabolic rate is the major problem. Because of low metabolism, many physiological processes cannot take place. As well as enzyme production rate becomes decreased also. The production of prominent alpha amylase enzyme also becomes low. Starch is the major important constituent of human diet. Alpha-amylase is an enzyme which hydrolyzes starch into maltose/dextrin.

The present research work was carried out to find the alternative of human body's alpha amylase. So, if alpha amylase is not being secreted in the body, in that case, we can take alpha amylase from an external source. For alpha amylase production, bacterial demand has been increased due to their specificity of the reaction, normal conditions for the growth and production, less time for growth production, and less energy consumption.

In the present study, ***Bacillus subtilis, Bacillus licheniformis, and Lactobacillus acidophilus*** were studied. The growth profile and the alpha amylase production from all selected bacteria were also observed.

Bacillus species is fast and easy growing bacteria even in simple and cheaper media and so they secreted enzymes directly into the growth agar medium. This reduces the cost of production. So in the present work, we have selected these bacteria for their growth study and alpha amylase enzyme production study by using Iodine assay and DNSA assay techniques. To avoid sporulation, batch culture system was used for the present research work.

MATERIALS AND METHODS

Culturing of Bacteria

Bacillus subtilis, Bacillus licheniformis, and Lactobacillus acidophilus bacteria were and taken for study; grown separately on nutrient agar plates and slants at 37°C and subcultured at 24h, 48h, 72h and 96hours.

Scaling up of bacteria

The composition of nutrient agar media was (g/l) Peptic digest of animal tissue (Peptone) 10.00**,** Beef Extract 10, Sodium Chloride 5.00, Agar 12.00 and pH was set at 7.0±0.1. Agar is not added in nutrient broth, it was prepared for the production of the enzyme.

For each bacterial species, the following scaling up technique was done separately. The inoculum was prepared by the adding bacteria into sterile distilled water. From this 0.5ml of the suspension was inoculated into 3ml of sterilized nutrient broth and incubated for 24hours. From this 1 ml, the culture was inoculated into 9ml of nutrient broth to make 10 ml cultures and incubated for 24hours. From this 3 ml, the culture was inoculated into 27ml of nutrient broth to make 30 ml cultures and incubated for 24hours. From this 10 ml, culture was inoculated into 90ml of nutrient broth to make 100 ml cultures and incubated at for 24hours.

Growth Profile

Growth absorbance was measured by taking the absorbance of cultures at 600 nm using a Spectrophotometer Model DIGISPEC-200Gl. The growth absorbance was observed using distilled water for adjusting 0% Absorbance at100 % transmission. The uninoculated broth was used as a control, against broth with growth.

Assay for Enzyme study

The activity of the enzyme produced from each species of bacteria in the media was checked separately using enzymatic assay method at regular time intervals of 24h, 48h, 72h, and 96h, to find the time period and the medium with the substrate that showed the highest enzyme production.

The incubated culture broth was centrifuged separately for each bacterial species at 8000 rpm for 20 minutes in a centrifuge. The collected supernatant was used as enzyme extract for assaying the crude enzyme activity estimation by using Iodine assay method and DNSA method.

In the Iodine assay method, at 40°C enzyme solution was warmed in a water bath; aliquots of the substrate stock solution are mixed with the enzyme solution. The reaction mixture was incubated at 25°C and reaction was carried out for 10 minutes. The reaction mixture was taken and added to Iodine reagent to terminate the process. After dilution of this, absorbance was read at 620nm.

The DNSA method is to detect the saccharifying action of the α-amylase enzyme. This method was proposed by Bernfield in 1955. In this method, the dinitro salicylic was used. The aliquots of the substrate stock solution are mixed with the enzyme.

It is followed by different time intervals and incubation at room temperature. DNSA reagent is added to the test tube to stop the reaction; the mixture is incubated in boiling water bath for 5 minutes. After cooling in cold water to room temperature, the absorbance of the supernatant at 540 nm is measured.

The absorbance value for the substrate and enzyme mixture are subtracted to the enzyme blanks are analyzed. It gives a measure of the extent of saccharification of starch by α-amylase. This method is also known as dinitro salicylic acid assay.

RESULTS AND DISCUSSION

Isolation of Bacteria

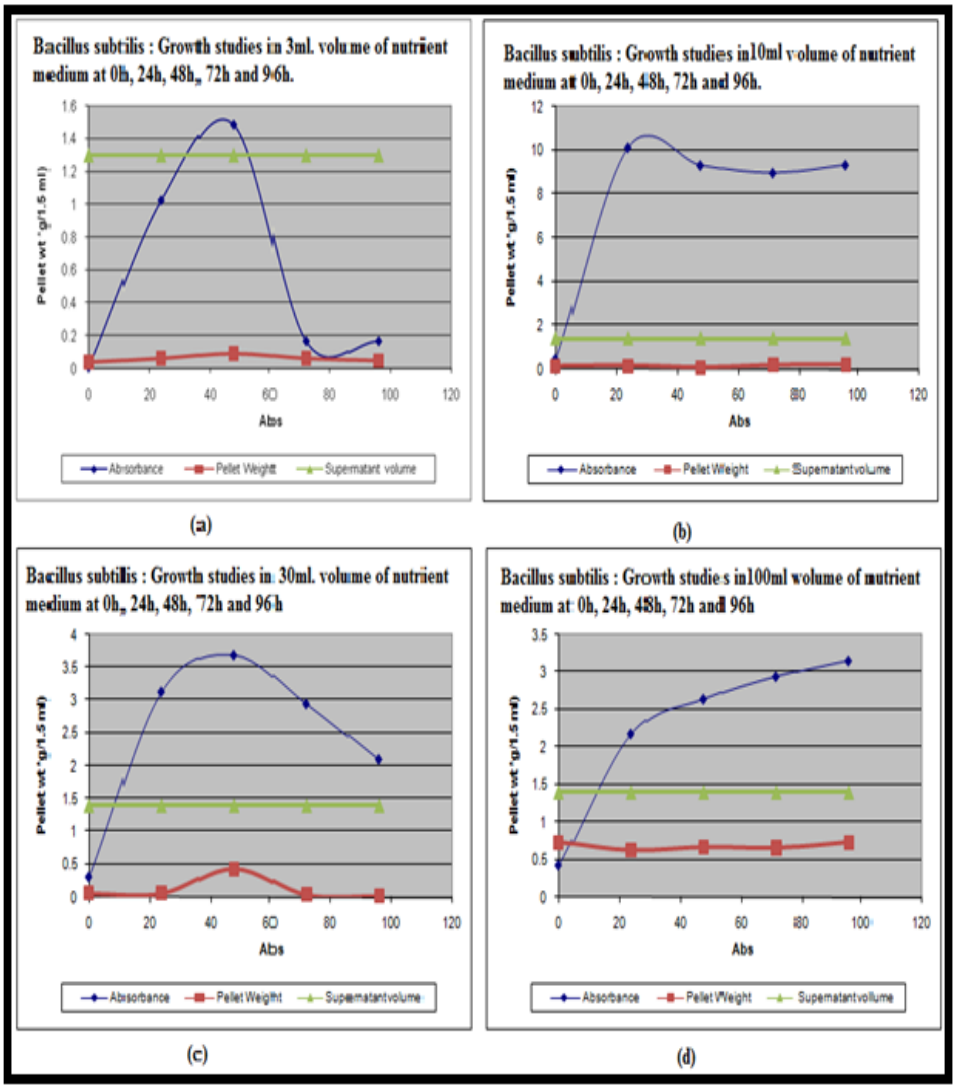
The bacterial species ***Bacillus subtilis, Bacillus licheniformis and Lactobacillus acidophilus*** were isolated by culturing on nutrient agar plates. The results are shown in table 1.

Growth profile

Separately when all bacterial species ***Bacillus subtilis, Bacillus licheniformis and Lactobacillus acidophilus*** were inoculated on nutrient agar, it remained in lag phase for few hours then it starts depleting. It may be due to depletion of nutrients in the medium and due to the scarcity of the water. All the bacteria isolated were further processed for enzyme activity.

Table 1: Isolation of bacteria

	Bacillus subtilis	***Bacillus licheniformis***	***Lactobacillus acidophilus***
Isolation On Plate			
Diameter size of 1 colony	0.1-5mm	2-4mm	1-1mm
Shape of colony	Round or eclipse	Round to irregular with a rough surface	Round to irregular with margin
Color of colony	Creamish White	White , aged Brown	White shiny



(a)

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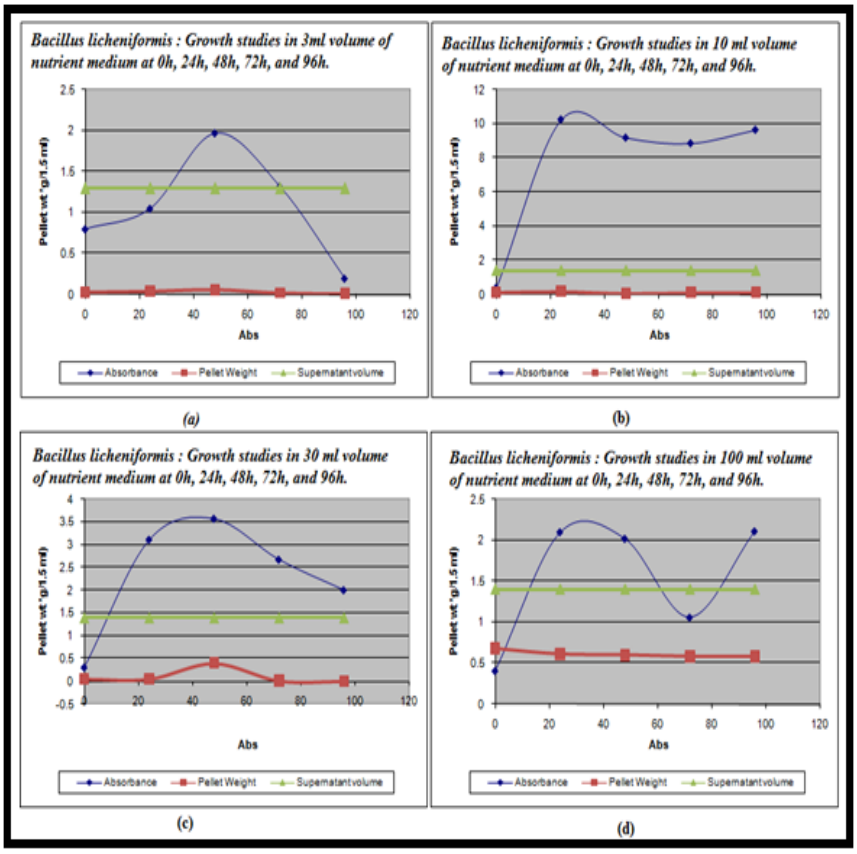

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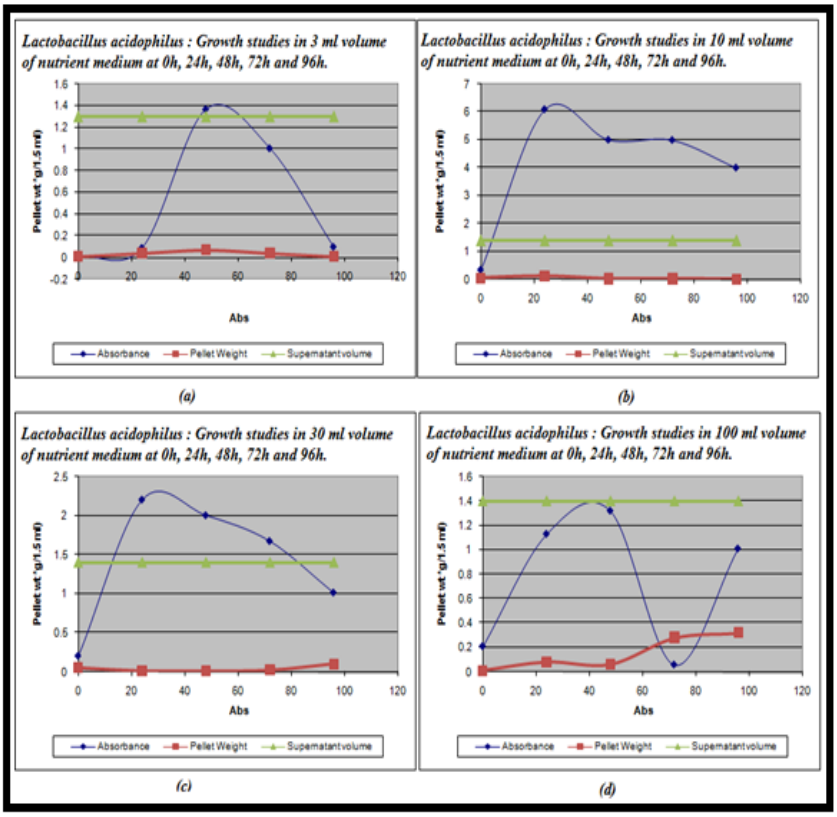

(c)

Figure 1: Growth Absorbance (at 600 nm), Pellet Weight and Supernatant Volume as observed during different growth volume of *(a) Bacillus subtilis, (b) Bacillus licheniformis and (c) Lactobacillus acidophilus* (a) 3ml, (b) 10ml, (c) 30ml and (d) 100ml.

Production of Enzyme

The enzyme when assayed at 24 h, 48 h, 96 h in the 30 ml and 100 ml series of ***Bacillus subtilis, Bacillus licheniformis and Lactobacillus acidophilus*** in nutrient broth showed that the enzyme excreted in higher volume (100 ml), reduced during the period between 24 h to 96 h of growth Liquefying action of α-amylase enzyme. ***Bacillus subtilis*** in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 5773.5 U/ml, 5091.7 U/ml, 5646.2 U/ml and 1068.8 U/ml***, Bacillus licheniformis***in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 5679.2 U/ml, 4961.6 U/ml, 1058.2 U/ml and 968.8 U/ml ***and Lactobacillus acidophilus***in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 1279.2 U/ml, 1661.6 U/ml, 786.2 U/ml and 668.8 U/ml.

Liquefying action of the α-amylase enzyme from *Bacillus subtilis*in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 7343.2 U/ml, 6345.68 U/ml, 5646.27 U/ml, and 1547.09 U/ml respectively. Liquefying action of the α-amylase enzyme from *Bacillus licheniformis*in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 7213.24 U/ml, 6345.56 U/ml, 5266.1 U/ml, and 1547.09 U/ml respectively. Liquefying action of the α-amylase enzyme from *Lactobacillus acidophilus* in 100 ml volume of nutrient broth at 24h, 48h, 72h, and 96h shown 3213.4 U/ml, 3249.8 U/ml, 726.1 U/ml, and 547.9 U/ml respectively.

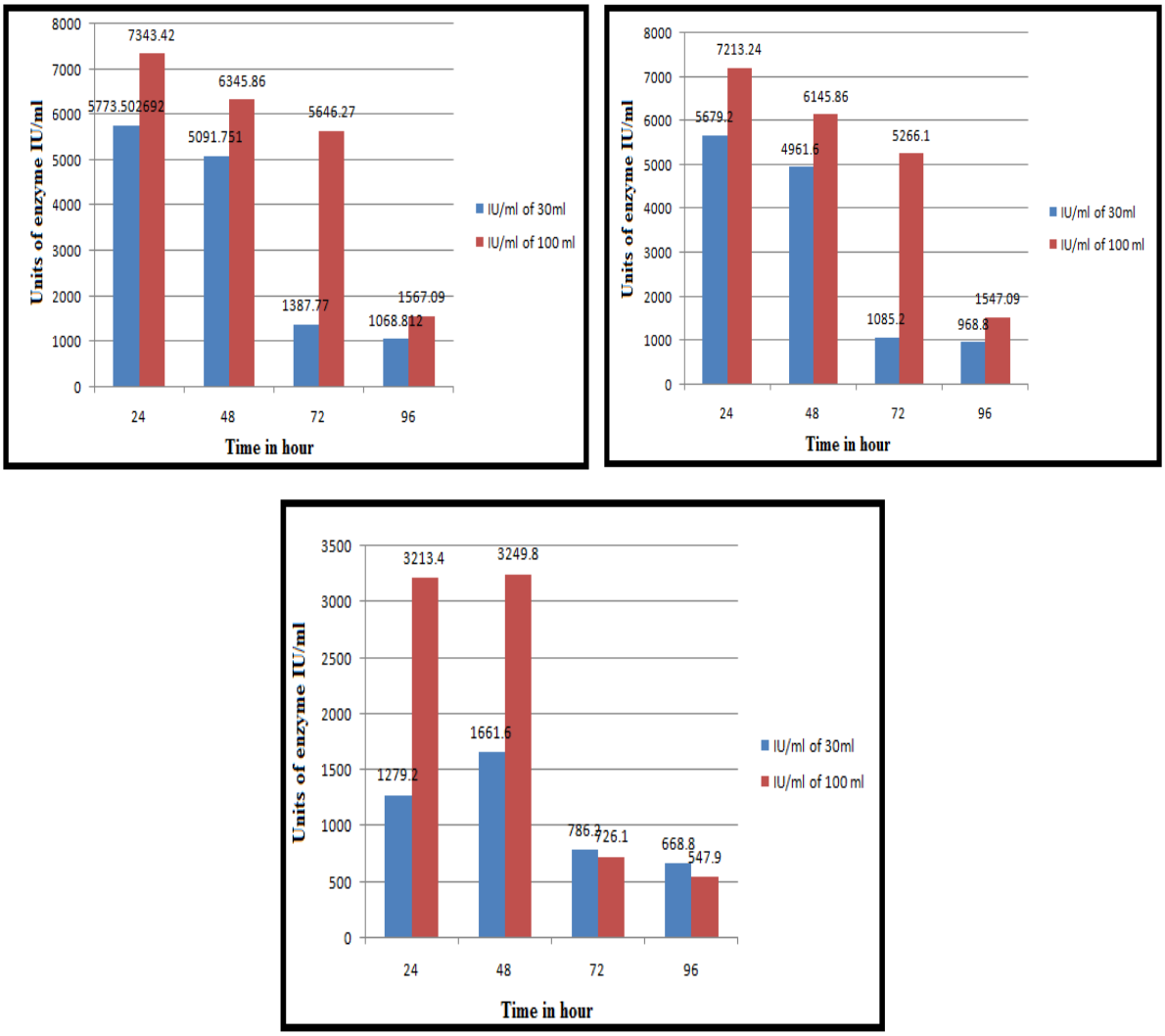

Figure 2: Enzyme α-amylase (assayed as liquefying activity) secreted during growth of (a)*Bacillus subtilis (b)Bacillus licheniformis (c)Lactobacillus acidophilus* in 30 ml and 100ml volumes of the Nutrient broth.

Saccharifying action of α- amylase enzyme in nutrient broth showed that the enzyme excreted in higher volume (100 ml), reduced during the period between 24 h to 96 h of growth saccharifying action of α-amylase enzyme from

Bacillus subtilis in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 28.83 U/ml, 32.02 U/ml, 98.97 U/ml and 72.75 U/ml; Liquefying action of α-amylase enzyme from *Bacillus subtilis*in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 99.4 U/ml, 166.56 U/ml, 172.99 U/ml, and 167.24 U/ml respectively.

Bacillus licheniformis in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 26.83 U/ml, 30.12 U/ml, 96.75 U/ml and 70.75 U/ml; Liquefying action of α-amylase enzyme from *Bacillus licheniformis* in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 96.4 U/ml, 160.65 U/ml, 170.99 U/ml, and 165.24 U/ml respectively. *Lactobacillus acidophilus* in 30 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown from 10.83 U/ml, 12.11 U/ml, 18.17 U/ml and 19.75 U/ml; Liquefying action of α-amylase enzyme from *Lactobacillus acidophilus* in 100 ml volume of nutrient broth at 24h, 48h, 72h and 96h shown 15.4 U/ml, 95.56 U/ml, 96.99 U/ml, and 90.24U/ml respectively.

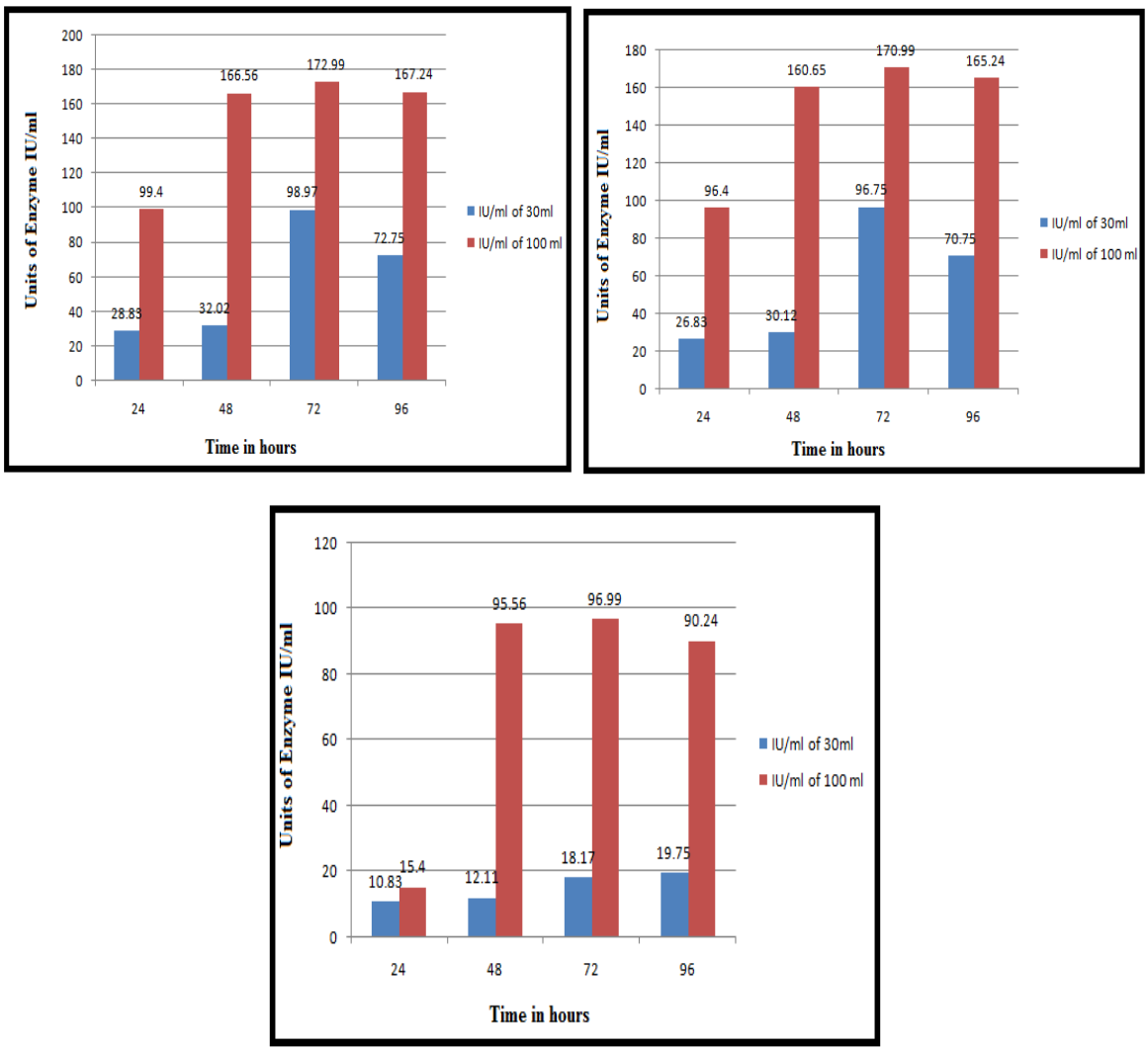

Figure 3. Enzyme α-amylase (assayed as Saccharifying activity) secreted during growth of (a)*Bacillus subtilis (b)Bacillus licheniformis (c)Lactobacillus acidophilus* in 30 ml and 100ml volumes of the Nutrient broth.

The enzyme excreted in lower volume (30 ml), did not reduce to the extent as in higher volume (100 ml) during the period between 24 h to 72 h of growth and the reason that may attributed for this behavior by the organism is the excretion of the other metabolites (like proteases in higher levels in higher volume of broth) and that heterologous proteins (amylases) are often rapidly degraded in the presence of such extracellular proteases. However, confirmation to this reasoning needs to be performed and proven in this study yet.

CONCLUSIONS

It was investigated that DNSA technique is better than Iodine assay for the alpha amylase production from ***Bacillus species*** bacteria. *Bacillus subtilis*has better efficiency to produce alpha-amylase in a nutrient medium, in compare to *Bacillus licheniform is, Lactobacillus acidophilus.*

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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