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Original Article

STUDY ON GROWTH PROFILEAND THE PRODUCTION OF A-AMYLASE FROM *LACTOBACILLUS ACIDOPHILUS* ISOLATED FROM THE CURD

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ABSTRACT

Curdisused in general as probiotic agent since ancient times. In Indian market, curdis the most common fermented milk product. It is the coagulated milk product. But its scientific use as probiotic drink is not soclear till now. But severalstudies have been carried on this aspect.***Lactobacillus*** is the bacteria which converts milk sugar lactose into lactic acid by the process of fermentation. In the present research work, ***Lactobacillus acidophilus*** was isolated from the curd . It was studied that the isolated strain was Gram positive, cocco-bacilli and non-motile in nature. Bacteria were subjected to study its growth profile. Nutrient medium was used for their growth. In the nutrient broth, itwas observed that the isolated bacteria were also capable to produce α-amylase . Production of α-amylase from ***Lactobacillus acidophilus*** has been investigated under submerged fermentation. The optimum temperature, pH and fermentation duration was investigated on the growth of bacteria for enzyme production.

Keywords: *Lactobacillus acidophilus, submerged fermentation*

INTRODUCTION

With the age, metabolic rate in human body becomes reduced. Due to reduced metabolic rate, many enzymes production rate decreases also. One of the major enzyme alpha amylase is also associated with this fact. Alpha amylase is an enzyme that hydrolyzes complex sugar like starch into simple sugar like maltose. Starch is the major important constituent of human diet. For this purpose, it is processed enzymatically into simple and digestible form. This food starch is hydrolyzed by the action of alpha amylase into absorbable form. As with the age, the production rate of the enzymes becomes less so in old aged people, the problem associated with food specially starch digestion starts.

In general, after the food epecially after eating rice, potato or other starch containing food people like to take curd or buttermilk. Many researchers have suggested that curd contains many ***Lactobacillus***bacteria.

Now days, for alpha amylase production, demand ofbacteria has been increased due to their specificity of reaction, normal conditions for the growth and production, less time for growth-production, and less energy consumption. In industries and many other sectors ***Bacillus species,***are being extensively used to produce alpha amylase.

On the basis of previous studies, authors have come to know that ***Lactobacillus acidophilus*** secretes alpha amylase. So, authors have revealed in the present study that curd is used for carbohydrate digestion in many local areas in India, because curd contains ***Lactobacillus acidophilus*** which produces alpha amylase. This alpha amylase has potential to digest starch.

In the present study,***Lactobacillus acidophilus*** was isolated from the curd. The growth profile was studied. The alpha amylase production from ***Lactobacillus acidophilus*** was also observed. Optimum conditions were also observed during the present research.

Lactobacillus acidophilusis fast and easy growing bacteria even in simple and cheaper media and so they secreted enzymes and proteins directly into the growth agar medium. This reduces the cost of production and purification. So in the present work we have selected this bacterium for their growth study and alpha amylase enzyme production study. To avoid sporulation, batch culture system was used for the present research work.

Modern biotechnology approaches that ***Lactobacillus acidophilus***can be used for the alpha amylase production study directly in the growth medium. This enzyme can be useful to hydrolyze the starch into maltose at lower cost. And can be beneficial to old aged people who feel difficulty in digesting starch.

In this research work, to study the enzyme activity, two assays that are Iodine assay and DNSA assay techniques are used.

MATERIALS AND METHODS

Microorganisms and Culturing

Lactobacillus acidophilus bacteria were isolated form the curd and taken for study; grown separately on nutrient agar plates and slants at 37°C and sub cultured at 24h, 48h, 72h and 96hours.

Inoculum and Growth –alpha amylase production media

The inoculum was prepared by the addition of bacteria from freshly grown agar slant or plate into sterile distilled water. From this 0.5ml of suspension was inoculated in to 3ml of sterilized nutrient broth and incubated at 37°C for 24hours. From this 1 ml culture was inoculated into 9ml of nutrient broth to make 10 ml cultures and incubated at 37°C for 24hours. From this 3 ml culture was inoculated into 27ml of nutrient broth to make 30 ml cultures and incubated at 37°C for 24hours. From this 10 ml culture was inoculated into 90ml of nutrient broth to make 100 ml cultures and incubated at 37°C for 24hours.

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 production of enzyme.

Growth absorbance

Growth absorbancewas determined by measuring the absorbance of cultures at 600 nm

using a Spectrophotometer Model DIGISPEC-

200Gl. The appropriate range of optical density

was observed in spectrophotometer (between 0.3 –

0.7 absorbance) and sample was diluted by the appropriate dilutions in the distilled water. The

growth absorbance was observed using distilled

water for adjusting 0% Absorbance/100 %

transmission. The control, un-inoculated broth, was used as a control, against broth with growth.

Enzyme Assay

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

Optimization of process parameters for maximal amylase production from *Lactobacillus acidophilus*

Temperature

The optimum temperature for ***Lactobacillus acidophilus*** was studied.

pH

In the present study for the research work, the optimum pH was selected 7.0 on which whole research work was carried out.

Fermentation duration

During the journey up to the optimum duration, the enzyme production increases with the time. But if incubation time is more than the optimum duration, enzyme activity begins to decline. This could be due to the either depletion of the mediumnutrient or release of the toxic substances in the medium nutrient. ***Lactobacillus acidophilus*** is an important source for alpha amylase production.

The α-Amylase Enzyme Extraction

After incubation, the culture broth was centrifuged at 8000 rpm for 20 minute in cooling centrifuge. The supernatant collected and supernatant volume 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, enzyme solution was warmed at 40°C in a water bath; aliquots of the substrate stock solution are mixed with the enzyme solution. The reaction mixture was incubated at room temperature and reaction was carried out for 10 minutes. After reaction by the substrate, reaction was terminated. Reaction mixture was taken and added to Iodine reagent. Finally, after dilution of this, absorbance was read at 620nm.

The DNSA method is to detect the saccharifying action of α-amylase enzyme. This method was proposed by Bernfield in 1955. In this method, the dinitrosalicylic was used. The aliquots of the substrate stock solution are mixed with the enzyme. It is followed by 0 minute, 3 minutes, 6 minutes, 9 minutes, 10 minutes, 12 minutes, 15 minutes and 40 minutes of 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 extent of saccharification of starch by α-amylase. This method is also known as dinitrosalicylic acid assay.

RESULTS AND DISCUSSION

Isolation of Bacteria

The bacterial species ***Lactobacillus acidophilus*** were isolated by culturing on nutrient agar plates. ***Lactobacillus acidophilus*** were observed with 1-3 mm in diameter of one colony size, surface colonies show different size and shape, round to irregular shape with some margins around the colony, rough surface and white shiny color colonies were observed.Fifteen to twenty colonies appeared on the plate which was further streaked on nutrient slants, separately to get pure culture. All the bacteria isolated were further processed for enzyme activity.

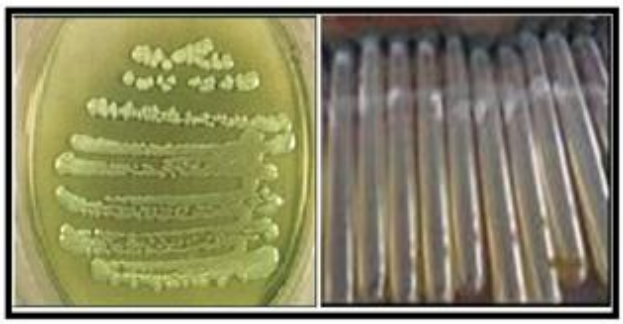

Fig:1 Growth of the *Lactobacillus acidophilus*

Optimization of process parameters for maximal amylase production from *Lactobacillus acidophilus*

Temperature

The optimal temperature for enzyme secretion is 37°C. This bacterium can survive harsh environments by turning into spore-form; when conditions are good, it will turn back into a vegetative state.

pH

In the present study for the research work, the optimum pH was selected 7.0 on which whole research work was carried out.

Fermentation Duration

It was studied for different fermentation hours and studies revealed a high yield of alpha amylase was observed after 48 hours of fermentation.

Growth studies

Bacterial divides by binary fission. This range observed shows that when we inoculated the bacterial inoculum 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 scarcity of the water.

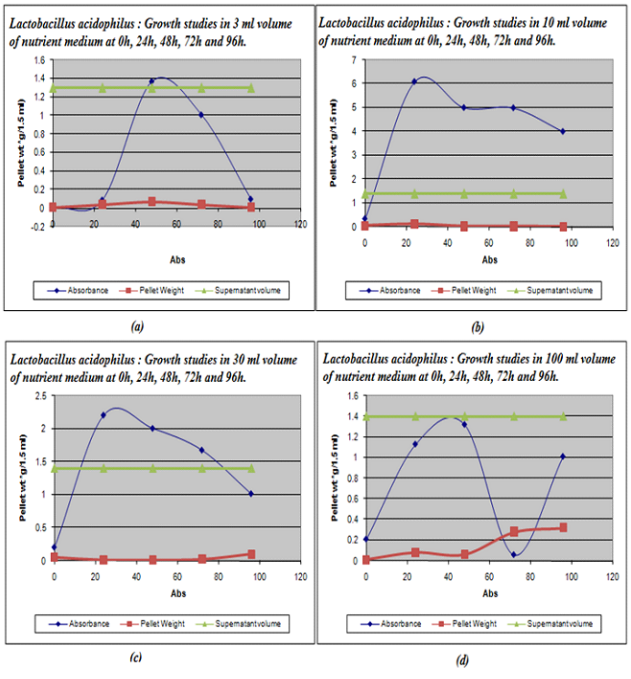

Fig:2 Growth studies of *Lactobacillus acidophilus*

Production of Enzyme

The enzyme when assayed at 24 h, 48 h, 96 h in the 30 ml and 100 ml series of ***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 from *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 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. 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 Liquefying action of α-amylase enzyme from *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.

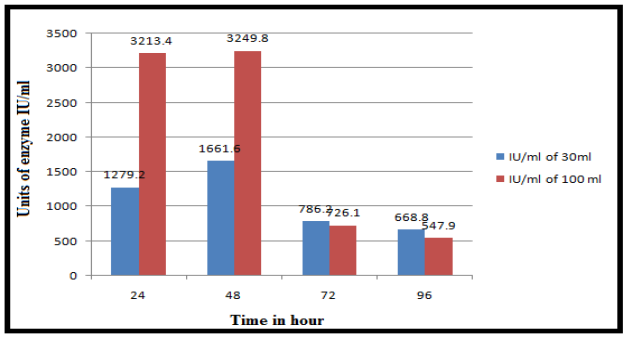

Fig:3. Enzyme α-amylase (assayed as liquefying activity) secreted during growth of *Lactobacillus acidophilus* in 30 ml and 100ml volumes of the Nutrient broth.

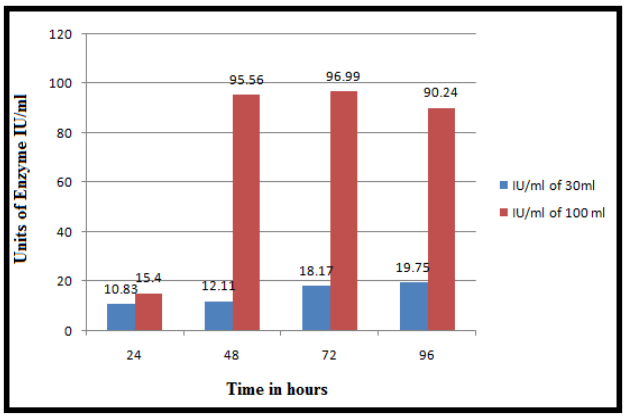

Fig:4 Enzyme α-amylase (assayed as Saccharifying activity) secreted during growth of *Lactobacillus acidophilus* in 30 ml and 100ml volumes of the Nutrient broth.

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.

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