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Studies on Amylase from Pro biotic Lactic Acid Bacteria

[1]Anteneh Tamirat Bogale & [2]S.G. Prapulla

[1]Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia

[21]Microbiology and Fermentation Technology Division Central Food Technological Research Institute (CFTRI), Mysore, Karnataka, India

Corresponding author E-mail: antenehtamirat@ibc.gov.et

Abstract: The objective of the study was to investigate a-amylase enzyme production by selected probiotic lactic acid bacteria (LAB). Qualitative screening of LAB for production of a-amylase enzyme on MRS agar plate showed that 3 *Lactobacillus plantarum* strains (K_0A_1, K_7, and T_{51}) and *Lactobacillus fermentum* were found to show halos (clear zones around bacterial growth) which was taken as confirmation of amylase enzyme production and *Lactobacillus plantarum* K_0A_1 was the best amylase producing isolate with measured clear zone diameter of 2.13mm. Optimum incubation period, incubation temperature and pH for amylase production were found to be 37°C, 21h and 7.0 respectively. At 37°C and, 21h incubation period and pH 7.0, spectrometeric determination of amylase enzyme activity at a wavelength of 600nm was found to be 1.37 mg/ml of reducing sugar (maltose) and the best salt concentration for precipitation of amylase enzyme was 60% w/v.

Key Words: *Spectrometeric determination, Amylase anzyme, Probiotic, Lactic Acid Bacteria*

1. Introduction

Lactic acid bacteria (LAB) are commonly considered as powerful probiotics that exert many beneficial effects in gastrointestinal (GI) tract and enhance health as mentioned by Nowrooz*iet al.*,[6]. According to Ray and Panda [7], the probiotic *lactobacillus*spp include *Lactobacillus acidophilus*, *L. plantarum*, *L. casei*, *L. gasseri* and *L. rhamnosus*. Lactobacilli have been periodically associated with anticarcinogenic, antimutagenic and antitumourigenic activities; therefore, the consumption of LAB fermented foods may elicit the above effects. Furthermore, Steinkraus [10] noted that probiotic lactobacilliare of considerable significance in fermentation-based industries for production of variety of fermented foods ranging from fermented vegetables such as cabbage (*sauerkraut* and *kimchi*) and cucumbers, curd and yoghurt, lacto-pickles, kefir and fermented milk and according to Vishnu *et al.*, [12], to the production of lactic acid (LA) as food additive. Panda *et al.*, [8] also added that some species of lactobacilli such as *L. acidophilus* and *L. plantarum* are commonly used as ‘’starter cultures’’ in vegetable and fruit fermentation.

According to Vishnu *et al.*, [12] some LAB possess amylase activity. *Lactobacillus* amylase exhibits significant role in GI tract of chicken and mammals like pig, rabbit, horse and human beings including infants as indicated by Nowrooz*iet al.*,[6] and they degrade the starch present in food to LA and fermentable monosaccharides that can be easily assimilated in the body, thus improving utilization of dietary starch and enhancing digestion. Rincker*et al.*, [8] pointed out that LA produced in GI tract lowers the pH of the environment, thus inhibiting the growth of pathogenic bacteria like *Salmonella*, *Staphylococcus* and *Escherichiacoli*. And Nguyen *et al.*, [5] added that amylolytic LAB are now implicated in preparing high energy density cereal-based foods for improving utilization of dietary starch in infants and children. Collington*et al.*, [2] reported inclusion of probiotic (a mixture of multiple strains of *Lactobacillus* spp. and *Streptococcus faecium*) resulting in significantly higher carbohydrate enzyme activities in small intestine of piglets. Similar results were also found by Rincker *et al.*, [8] that in case of rats, broiler, chickens and rabbits and due to low pancreatic α-amylase content in young pigs, *Lactobacillus* 123 was evaluated as a source of amylase for weanling in pigs to improve digestibility of starch in small intestine. In line with the above information, the present study was carried out to investigate amylase enzyme production by some selected LAB.

2. Materials and methods

2.1. Source of microorganisms, medium and culture conditions

LAB isolates (*Enterococcus faecium*, *Lactobacillus fermentum*, *Lactobacillus plantarum* strains K_0A_1, K_7, K_{23}, and T_{51}) analyzed in this study were obtained from Microbiology and Fermentation Technology Division, Central Food Technological Research Institute (CFTRI), Mysore, India. The bacterial stock cultures were

stored and maintained on Mann Rogassa Sharpe (MRS) agar slants at 4°C as recommended by Sharpe and Elisabeth Pyer[9].

2.2. Screening of LAB that produce dietary amylase enzyme

LAB isolates were checked according to Maragkoudakis *et al.*, [4]using a medium that consisted of polypeptide (0.5%), beef extract (0.5%), yeast extract (0.2%), NaCl (0.2%), corn starch (2%), and agar (1.5%). Lab isolates were point plated (inoculated at the center) and after aerobic incubation for 48h., colonies were removed from agar medium, and then 0.2% Congo red reagent was added to the agar medium. After 30 min. the medium was decolorized with 1M NaCl in order to identify the number of colonies that had a surrounding halo zone. The experiment was repeated three times and average clear zone diameter measurementsin mm were taken into consideration. The isolate showing maximum average diameter of clear zone (enzyme activity) was selected for further analysis and propagated in broth supplemented with 2% (w/v) corn starch medium at incubator shaker at 150 rpm at 37^{0}C for 24hrs.

2.3. Starch fermentation and determination of growth (incubation period)

Lactobacillus plantarum K_0A_1 which showed the highest amylolytic halo was selected for further analysis. Fermentation was performed in duplicate in 250ml Erlenmeyer flasks at 37°C. MRS broth containing 20g/l corn starch as carbon source was used and inoculated with 10% (v/v) overnight broth culture propagated in the same medium used for the fermentation. After incubation time resultant broth was centrifuged at 10000 rpm for 10 min. Optical density at 600 nm (OD 600) was measured using a *Spectronic 401* spectrometer every 3 hours for 24 h and growth of the bacteria was determined as described by Calderon *et al*., [1].

2.4. Effect of incubation temperature and medium pH on enzyme activity

The influence of initial medium pH on amylase production was assessed by the method of Heikkila& Saris [3]by cultivating the isolate in MRS broth of pH ranging from 3.0 to 11.0. The optimal pH for enzyme activity was determined by changing the assay reaction mixture pH using the following buffers (0.1 M): sodium acetate (pH 5.0), sodium phosphate (pH 6.0–7.0), Tris–HCl (pH 8), glycine–NaOH buffer (pH 9–10) and 2% corn starch as substrate. The effect of temperature was also studied by the method of Heikkila& Saris [3]by performing the fermentation at different temperatures, 30, 37, 40, 45 and 50 °C. The optimum temperature for the enzyme activity was evaluated by measuring the amylase activity at the specified temperatures 30–50°C in 0.1 M sodium phosphate buffer (pH 7.0) and 2% corn starch

2.5. Determination of amylase activity

Production of reducing sugars from corn starch was used as a criterion of amylase activity. One ml of cell extract was mixed with 3 ml corn starch solution (20g/l) buffered to pH 7.0 with 0.1 mol/l phosphate buffer. After incubation at 37 ºC for 90 min, 2 ml of the reaction mixture were added to 3 ml of 3, 5-dinitro salicylic reagent (DNS) according to Heikkila& Saris [3]in a stoppered glass tube. The tube was heated in a boiling water bath for 15 min, cooled and diluted with 15 ml of distilled water. The color (absorbance) was measured in a spectrometer at a wavelength of 600nm. Activity was calculated and expressed as reducing capacity equal to 1 mg/ml maltose during 90 min.

2.6. Enzyme Purification

Purification of enzyme was carried out by the methods of Tahar*et al.*,[11]and VipulVerma*et al*., [13] by precipitating the enzymes present in the crude sample. A range of Ammonium Sulphate (NH4)2SO4 salt was used from 30%-85% to precipitate the enzyme.

3. Results and Discussion

Extracellular amylase enzyme producing ability of LAB isolates was determined qualitatively by observing the formation of halos (clear zones) around the colonies on MRS agar medium plates. Only four LAB isolates displayed varying level of amylase producing ability. Maximum amylase

Isolates	Trial.1 diameter	Trial.2 diameter	Trial.3 diameter	Average diameter
L. fermentum	1.5	1.7	1.4	1.53
Lactobacillus plantarum K_7	1.7	1.5	1.7	1.63
Lactobacillus plantarum K_0A_1	1.8	2.1	2.5	2.13
Lactobacillus plantarum T_{51}	1.1	0.9	0.8	0.93
Lactobacillus plantarum K_{23}	-	-	-	-
E faecium	-	-	-	-

Table.1. Isolates showing halos (clear zone)

production was shown by *Lactobacillus plantarum* K_0A_1 (clear zone size 2.13 mm), followed by *Lactobacillus plantarum* K_7 (clear zone size 1.63 mm), *L. fermentum* and *L. fermentum* (clear zone size 1.53 mm) and *Lactobacillus plantarum* T_{51} (clear zone size 0.93 mm) as shown in the above Table.1 .

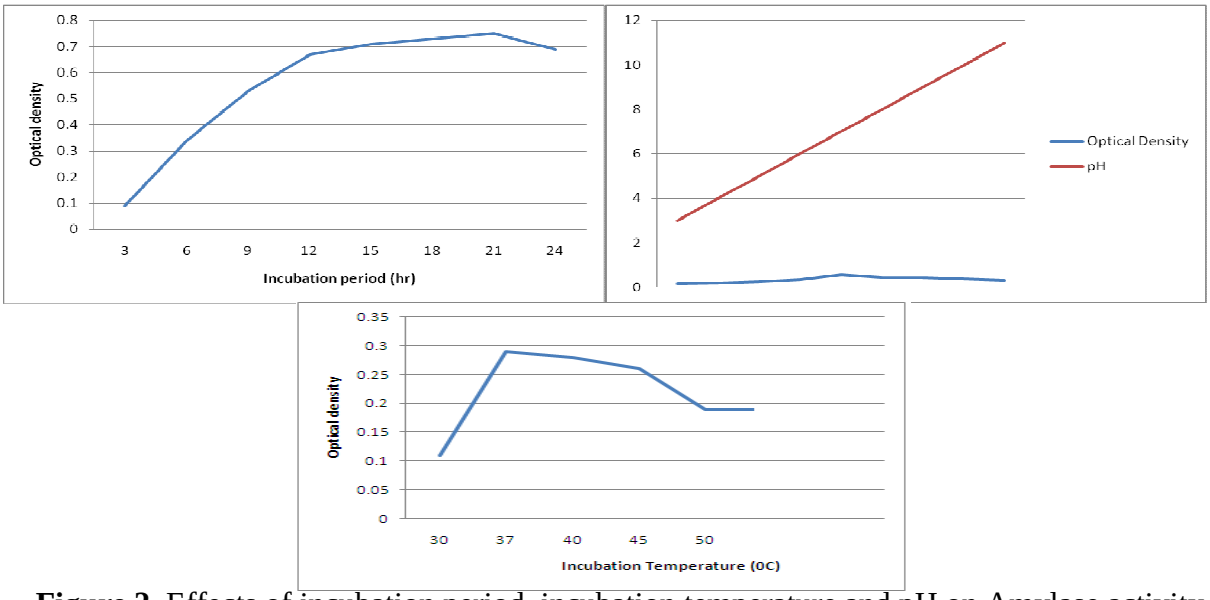

Figure 2. Effects of incubation period, incubation temperature and pH on Amylase activity

As shown in the above figure.2, effects of incubation period, incubation temperature and pH on the enzyme activity were determined and the activity of amylase produced by *Lactobacillus plantarum* K_0A_1 increased from 0 to 21h with maximum enzyme activity measured at 37°C at the 21st hour. The optimum pH for amylase activity was 7.0 and the highest amylase activity was measured at pH 7.0 and incubation temperature of 37°C and the best enzyme Activity.

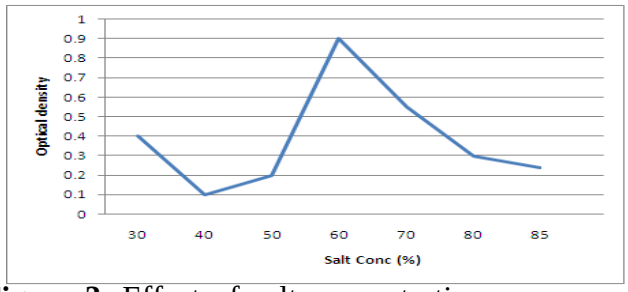

Figure 3. Effect of salt concentration on enzyme precipitation.

In this study, Ammonium sulphate was used to precipitate amylase enzyme for purification purpose and the best salt concentration was found to be 60% w/v and at other salt concentrations significant precipitation was not found (Figure 3.).

4. Conclusion & Recommendation

This study revealed that *Lactobacillus plantarum* K_0A_1 possesses probiotic properties and was the best probiotic isolate among the obtained isolates and showing production of amylase enzyme with good enzyme activity expressed by production of 1.37 mg/ml of reducing sugar (maltose) from corn starch. The active enzyme was also purified using 60% w/v salt concentration.The strain could be potential candidate for production of probiotic products for human and animal health if further characterization studies are undertaken.

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