

## Biosynthesis of Alkaline Phosphatase by *Escherichia coli* Efrl 13 in Submerged Fermentation

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**Abstract:** In the present study cultural conditions for alkaline phosphatase production from *E.coli* EFRL 13 using molasses in batch wise submerged fermentation was investigated. The effects of time period (2-48Hours), carbon sources (glucose, fructose, galactose, starch, molasses and date syrup) nitrogen sources (peptone, tryptone, yeast extract, ammonium chloride, sodium nitrates and potassium nitrate) were checked on enzyme production. The highest level of alkaline phosphatase was achieved using mineral medium containing 2.0% molasses and 2% sodium nitrate as carbon and nitrogen source, respectively after 24 hours of incubation at 40°C initial pH was adjusted to 9.0. In this study cost effective substrate is utilized for alkaline phosphatase production.

**Key words:** *Escherichia coli* EFRL 13 • Molasses • Submerged fermentation • Alkaline Phosphatase

### INTRODUCTION

Alkaline phosphatase (orthophosphate monoester phosphohydrolases E. C. 3.1.3.1.) are metalloenzymes, nonspecific, phosphomonoesterases [1], which exists in various organisms from bacteria to mammals [3-5]. The phosphatases are grouped according to optimum pH as alkaline phosphatases (EC 3.1.3.1, optimum pH 8.0) and acid phosphatases (EC 3.1.3.2 optimum pH 6.0). Alkaline phosphatase hydrolyzes a wide variety of phosphate esters and is classified as alkaline phosphatase according to its optimum pH ranging from 7.5 to 11.0 [6, 7]. Phosphatases are one of the most crucial enzymes for survival of organism that hydrolyze phosphate esters and provide inorganic phosphate (Pi). The fact that phosphate can not be synthesized by microbes makes alkaline phosphatases crucial for their survival and the cell obtains Pi from nucleic acids, phosphorylated sugars, proteins, etc [8, 9]. Phosphatases show great structural and functional diversity with respect to subunit size, metal ion requirements and substrate specificities [8]. Many APases are monomeric with sizes as small as 15.5 kDa to as large as 160KDa [10, 11], while others have multiple subunits such as the trimeric 143KDa protein from *Thermus aquaticus* [8]. The importance of alkaline phosphatase in clinical medicine and molecular biology has made it a popular subject for scientific study and commercial utility [12, 13]. In practical, the alkaline

phosphatase has wide range of applications in the diagnostics, immunology and molecular biology which serve as biochemical markers in quantitative measurements of disease [12-20]. The work described in this article deals with optimization of cultural conditions for alkaline phosphatase production by *E. coli*.

### MATERIAL AND METHODS

**Organism:** The *Escherichia coli* were isolated from the soil sample of garden, Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro, Pakistan. The microorganism was identified in Enzyme and Fermentation Biotechnology Research Laboratory, IBGE. The organism was maintained on nutrient agar medium containing meat extract 3.0g; peptone 3.0g; Sodium Chloride 3.0g and Agar 15.0g/ liter of distilled water and organisms was re-cultured with one week interval.

**Fermentation medium:** The fermentation medium contain glucose (1%) peptone (0.5%), CaCl<sub>2</sub> (0.1%), NaCl (0.1%) and sodium dihydrogen phosphate (0.3%). The *E. coli* was grown in 50ml of fermentation medium in 250ml conical flask and the initial pH of the medium was maintained at 7.0. Flasks were cotton plugged and autoclaved at 1.5 kg /cm<sup>2</sup> for 20 minutes. The Sterilized media cooled at room temperature and inoculated with

0.5 ml of *E.coli* culture. The flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at  $37\pm 2^\circ\text{C}$ . The bacterial biomass was separated from culture broth after an interval of 2 hours incubation period through centrifugation and further analysis was carried out from culture broth.

**Effect of Time of Incubation:** The alkaline phosphatase production was determined from culture broth with two hour interval for 48 hours using 1.0% glucose as a carbon source through batch wise submerged fermentation.

**Effect of carbon source:** The effect of different carbon sources (glucose, fructose, galactose, starch, molasses and date syrup) were incorporated in the fermentation medium to analyze their effect on alkaline phosphatase production.

**Effect of nitrogen sources:** The effect of different nitrogen sources (peptone, tryptone, yeast extract, sodium nitrate, potassium nitrate and ammonium chloride) were incorporated in fermentation medium.

**Effect of initial pH:** The effect of pH on alkaline phosphatase production was observed by adjusting initial pH of fermentation medium in the range (4-11) using 0.1N HCl or NaOH.

**Effect of temperature:** The effect of temperature of incubation was checked on the yield of alkaline phosphatase by *E.coli* ranging from  $30^\circ\text{C}$  to  $60^\circ\text{C}$ .

**Assay of enzyme activity:** Alkaline phosphatase activity was measured spectrophotometrically by monitoring the release of para-nitrophenol from para-nitrophenyl phosphate (PNPP) at 400 nm as reported by De Prada *et al.* [8]. The culture broth was centrifuged at 6,000 rpm for 15 minutes at  $4^\circ\text{C}$ . 1ml of cell free supernatant was mixed with 1ml substrate. The mixture was incubated at  $30^\circ\text{C}$  for 10 min. At the end of the incubation 0.05ml of 4 M NaOH was added to stop the reaction. The colour intensity was measured at 400nm against a blank.

One unit of enzyme defined as the amount of alkaline phosphatase required to release one milligram of orthophosphoric acid under assay conditions.

## RESULTS AND DISCUSSION

Ten bacterial strains were isolated from the fertile soil of institute of biotechnology and genetic engineering and screened for alkaline phosphatase activity in the culture medium. The primary selection criteria were: 1) maximal secretion of alkaline phosphatase into the culture medium and 2) production of alkaline phosphatase using cost effective substrate. Four of isolated strains showed alkaline phosphatase secretion, but one was selected, which produced maximum for further optimization of cultural conditions for alkaline phosphatase production. The strain was identified by morphological and biochemical tests carried out according to Bergey's Manual of determinative Bacteriology as *E.Coli*, [21]. *E.coli* strain showed best growth after 24 hours in

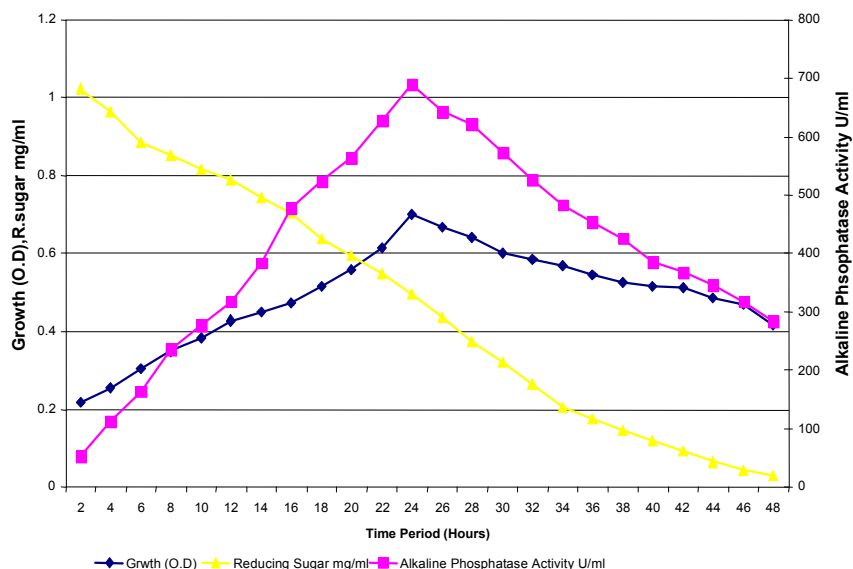


Fig. 1: Effect of Time of Incubation on Biosynthesis of alkaline Phosphatase by *Escherichia coli* when incubated at  $37\pm 2^\circ\text{C}$  and pH was adjusted to 7.0.

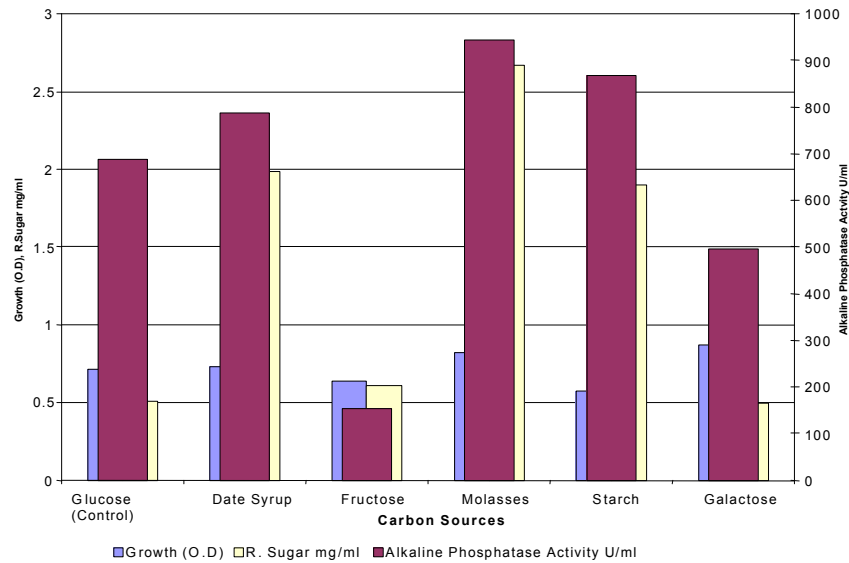


Fig. 2: Effect of carbon source on production of alkaline Phosphatase by *Escherichia coli* after 24 hours of incubation at  $37\pm 2^\circ\text{C}$  and pH was adjusted to 7.0.

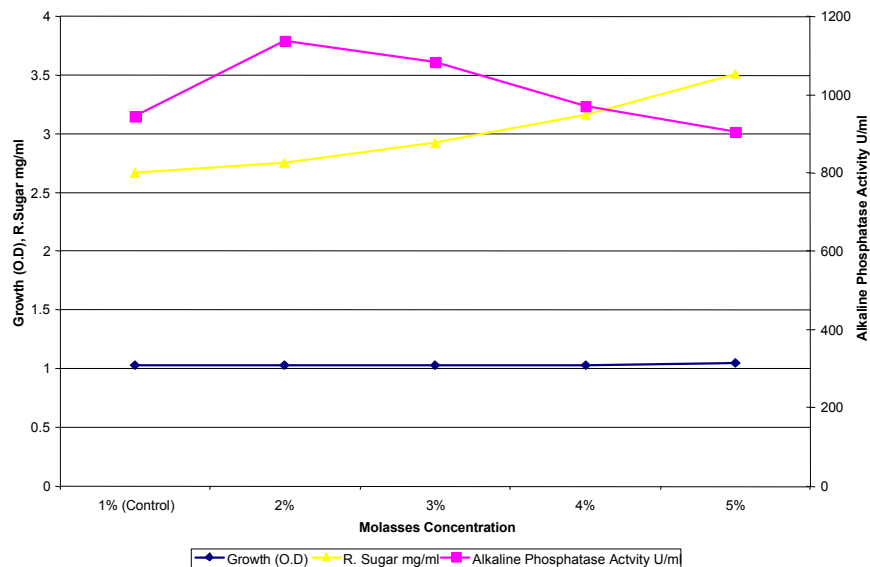


Fig. 3: Effect of different concentration of molasses on biosynthesis of alkaline Phosphatase by *Escherichia coli* after 24 hours of incubation at  $37\pm 2^\circ\text{C}$  and pH was adjusted to 7.0.

nutrient broth medium at  $37^\circ\text{C}$ . The strain was capable of growing in the pH range of 4-11 and  $30-60^\circ\text{C}$  temperature. Effect of time of incubation on synthesis of alkaline phosphatase by *Escherichia coli* is shown in Figure 1.

The growth and enzyme yield increased with passage of time and the maximum enzyme secretion was found (0.688 U/ml) after 24 hours but on prolong incubation production decreased may be due to change in pH or synthesis of inhibiting metabolites. The effect of different carbon sources (glucose, galactose, fructose, starch, molasses and date syrup) was determined on the

production of enzyme. It is shown in Figure 2 that *Escherichia coli* grows better and secreted maximum alkaline phosphatase when grown on 1% molasses mineral medium at  $37\pm 2^\circ\text{C}$  after 24 hours of incubation in comparison to other carbon sources.

Agro-industrial waste causing pollution, which can be utilized as raw material for the production of enzymes and many value added products. The utilization of agro-industrial residues on one hand provides useful products and on another help in solving pollution problems [22]. Among various low cost raw materials in present study

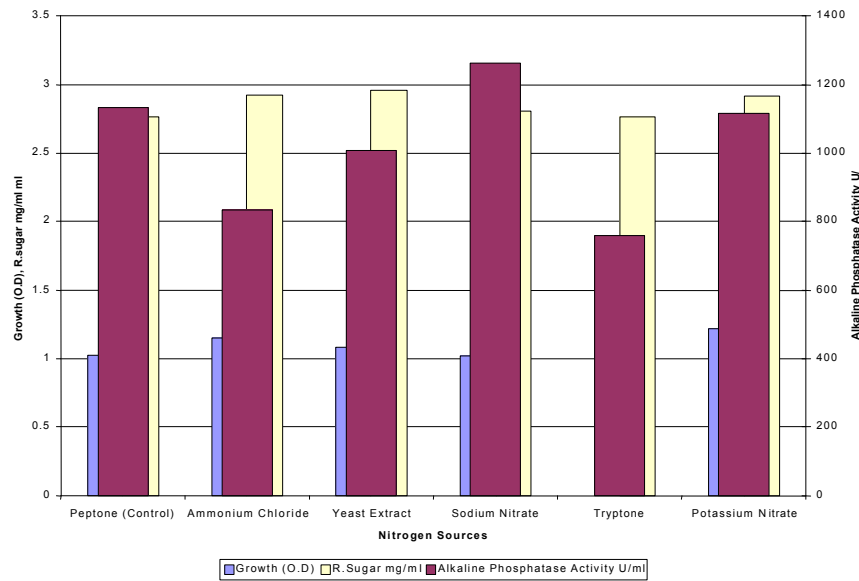


Fig. 4: Effect of nitrogen source on biosynthesis of alkaline Phosphatase by *Escherichia coli* after 24 hours of incubation at  $37\pm 2^\circ\text{C}$  and pH was adjusted to 7.0.

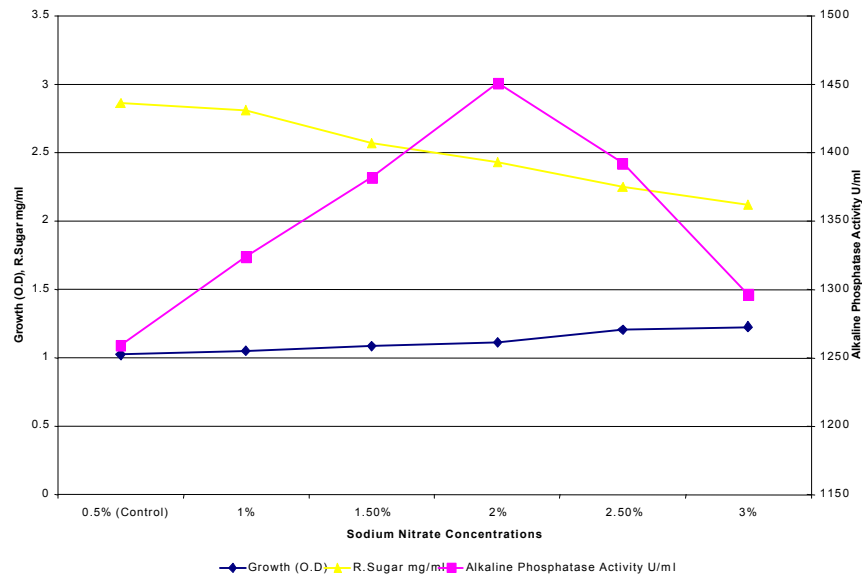


Fig. 5: Effect of different concentrations of sodium nitrate on biosynthesis of Alkaline Phosphatase by *Escherichia coli* after 24 hours of incubation at  $37\pm 2^\circ\text{C}$  and pH was adjusted to 7.0.

molasses which is the sugar industry by-product produced maximum alkaline phosphatase. The superior effect of molasses on enzyme synthesis may be due to presence of growth promoting substances in enough quantity which fulfill the requirement of bacterial growth along with synthesis of alkaline phosphatase. In the next step effect of varying molasses concentrations (1-5%) synthesis of alkaline phosphatase by *Escherichia coli* was investigated as shown in Figure 3.

Alkaline phosphatase biosynthesis increased with increasing molasses concentration up to 2.0% and then it falls because the higher concentration of molasses acts as suppressor. Figure 4 shows the effect of organic and inorganic nitrogen sources (0.5%) on alkaline phosphatase production by *E. coli*.

In this study peptone was replaced with ammonium chloride, potassium nitrate, sodium nitrate, yeast extract and tryptone. It is noted that the *Escherichia coli* produced maximum alkaline phosphatase when grown in

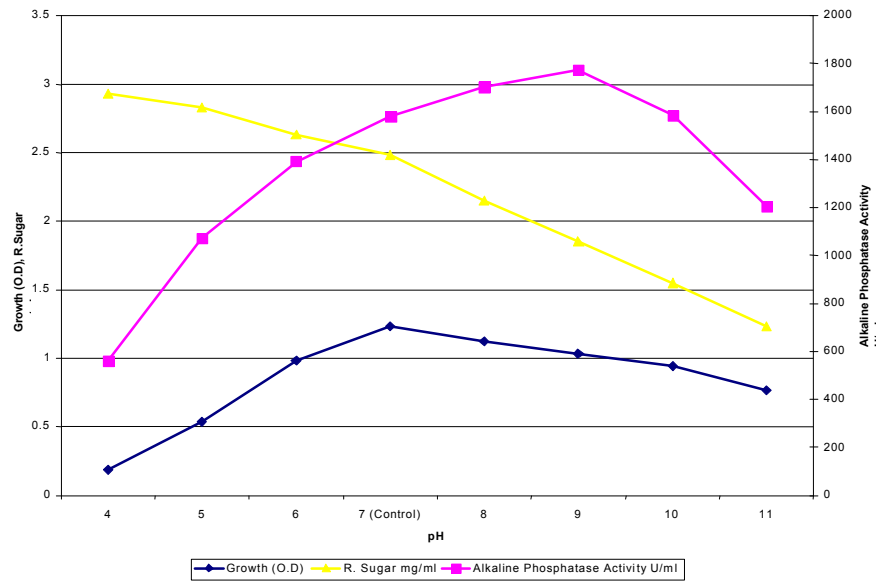


Fig. 6: Effect of initial pH on production of alkaline Phosphatase by *Escherichia coli* after 24 hours of incubation at  $37\pm 2^\circ\text{C}$ .

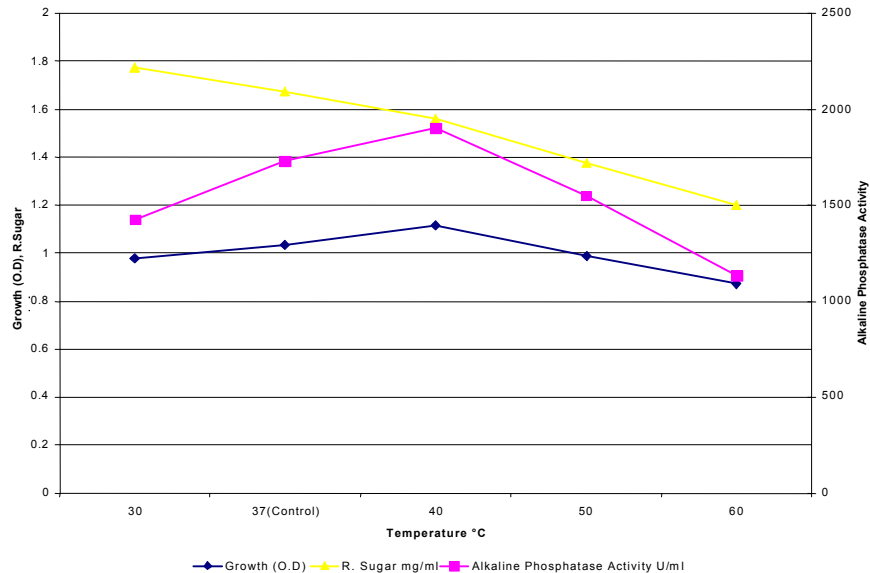


Fig. 7: Effect of temperature on biosynthesis of alkaline Phosphatase by *Escherichia coli* after 24 hours initial pH was adjusted to 9.0.

mineral medium containing 0.5% sodium nitrate and 2.0% molasses. The effect of different concentrations of sodium nitrate (0.5-3%) were also checked on the production of alkaline phosphatase the yield was increasing up to 2% sodium nitrate the results are depicted in Figure 5.

Figure 6 shows the influence of initial pH on the production of alkaline phosphatase by *Escherichia coli* using 2.0% molasses and 2.0% sodium nitrate in mineral medium when incubated at  $37^\circ\text{C}$  for 24 hours.

Production of phosphatase was observed in a wide pH range (4.0-11) production shows no correlation with growth. The optimum pH 7.0 and 9.0 were noted for growth and enzyme production respectively. The growth was extremely slow at pH 4.0 and 11.0 and reached maximum after 24 hours of incubation at pH 7.0. Though production of enzyme was maximum at pH 9.0 (1773 U/ml), beyond this production rate was low. The pH dependent enzyme production might have been due to pH control over the growth of bacteria or pH dependent control the

enzyme synthesis gene expression [23]. The results are in the accordance with the reported results of Danielle and Raymond [24] in the case of alkaline phosphatase production by *E.coli* at pH 8.3 and Dahot *et al.* [25] in the case of alkaline phosphatase production by *Penicillium expansum* at pH 9.5. Figure 7 shows the effect of incubation temperature on the synthesis of alkaline phosphatase by *Escherichia coli* (30-60°C) when grown on 2.0% molasses and 2.0% sodium nitrate mineral medium, which was incubated for 24 hours at the initial pH 9.0.

The maximum yield of alkaline phosphatase was noted at 40°C. Similar results are reported by Danielle and Raymond [24] in the case of alkaline phosphatase production by *E.coli* at 37°C after 16 hours of incubation and Dahot *et al.* [25] in the case of alkaline phosphatase production by *Penicillium expansum* at 40°C. Temperature influence all the physiological activities in a living cell and is one of the important environmental factor to control the growth, microbial activities, normal functioning of enzyme and many enzymes control the nutritional requirement of the cell and subsequently its composition [26].

## CONCLUSION

The present study is carried out for the utilization of industrial waste as carbon source for the production of alkaline phosphatase from *E.coli* EFRL 13. The molasses is economically cheap substrate for commercial alkaline phosphatase production using local isolated *E. coli* strain.

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