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Research Paper

Comparison of Enzyme Prodution Rate of Extracted Native Bacteria from Saline and Alkaline Soil

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Abstract

12 stain of alkalophilic bacteria extracted from saline and alkaline soils of Azerbaijan province in milk agar environment due to evaluate alkaline protease enzyme production feasibility. Amongst isolated bacteria, Bacillus genus members revealed higher product ability. Studying temperature, incubation time, carbon source, nitrogen and pH demonstrated that the best production condition is on 40 oC for 20 hours using 1.5% glucose (weight to volume), 1% Ammonium sulfate (weight to volume) and pH=9.5.

Keywords: Alkaline Protease; Bacillus Species; Alkalophile

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1. Introduction

Enzymes have broad applications in industry and household consumption. Between them, microbial protease is one of the important types of real enzymes group and comprise nearly 60% trade of total enzyme in markets. Microbial protease included Acidic, neutral and alkaline time based on their activity in special pH. Alkaline protease has extensive application in house detergent, food processing, pharmacological industry, Paper-making and X-ray films [1,2]. Bacillus species considered the best Commercial source of alkaline protease production [3] and its usage was reported numerously to produce protease enzyme [4].

Clearly, ex-situ production of protease by microorganisms related to culture medium component, especially the type of carbon and nitrogen source; and other environmental factors such as temperature, pH, and incubation time and inoculation extent [5]. As nearly 30 to 40 percent of industrial production of enzyme belongs to the growth medium, optimizing environmental composition is an important and critical issue [6]. Then, increasing microbial protease production is just possible by optimizing the environment. There is not any constant formula for all producing bacteria. Every strain had a special demand to produce the highest enzyme production [7].

Environment effects on ex-situ production of protolithic enzymes were inevitable to induce or inhibit enzyme production [8]. Protease production requires to reach carbon and nitrogen sources of the environment to play as a regulator for enzyme synthesis [9].

The aim of this study was isolating bacteria producing alkaline protease enzyme from alkaline soil, evaluating production condition and optimizing this situation and introducing the best environment for enzyme production.

2. Materials and methods

Isolating and screening microorganism: totally 50 samples collected from alkaline soils of Lushan, Manjil and Rudbar from Azerbaijan province to

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extract alkalophile bacteria and planted in milk Agar environment containing skim (100 g/l) and yeast extract (10 g/l) with pH=9. 5 stains showed positive reaction (Halo formation) to enzyme production and identified for the following experiments.

Identification of bacteria: gram lamella staining prepared firstly and shape arrangement, and gram reaction conclusion were evaluated. Then lamella related to spore staining were prepared. Other conformational experiments were performed, included gelatinize experiment, nitrate reduction, starch hydrolysis, Voges Proskauer and sugars fermentation of [10].

Evaluation enzyme production: these strains were re-cultured in medium included enzyme production. For this, 1ml of above cultured bacteria were incubated in 250 ml flask, included Casein (10 g/l), malt extraction (10 g/l), peptone (10 g/l) and sodium carbonate (10 g/l) with pH=9 for 24 h in 34 oC and 250 rpm (under shaker). Then cultures were centrifuged under 8000 rpm for 20 min and 4 oC and enzyme activity measured in superficial suspension without cells [11]. Among subjected bacteria, strain with the highest activity was selected for next survives.

Enzyme production optimization: evaluating incubation time effects on enzyme production, cultures of bacteria strain were isolated from above in 34 ^{o}C and pH=9 from 5 to 48 hours. Then sampling performed in special intervals and enzyme activity were evaluated next.

In the next step to measure the effect of temperature on optimizing enzyme production, experiments were designed in 30-45oC in an enzyme production environment and pH=9. Subsequent test to optimize pH from 8-12 in enzyme production environment performed in 40 oC; then the influence of using carbon sources means sucrose, glucose, fructose and lactose (1.5 % weight to volume) and yeast extraction (1% weight to volume) with peptone (0.5 % weight to volume), nitrate ammonium and casein sources (1% weight to volume) were evaluated in optimum condition [12-14].

2.1 Enzyme Performance Assessment:

Protolithic enzyme activity enhanced by casein as substrate [15]. Casein was dissolved in 1 M Tris-HCl buffer with 1.5 % concentration and pH=9. Measuring enzyme activity performed using 450 µl substrate and 50 µl of upper suspension resulted from centrifuging in pH=9. Reaction mixture was incubated in 45oC for 20 min and finished with adding 500 µl TCA 10%. Then sample was centrifuge for 10 min in 5000 rpm and sediment removal of supernatant used for further evaluations.

Determining protease enzyme activity was based on tyrosine released from the supernatant [15]. Each unit of enzyme activity defined as enzyme extent which can release 1µg tyrosine per minute in 45oC.

Only in 5 strain of 22 alkalophile bacteria strain surrounding milk agar colony environment, transparent halo was observed. This strains were identified and considering to the membership of Bacillus genera, one isolated were selected to subsequent studies.

The effect of time: however, production observed in whole fermentation process from 5-48 hours; the highest production designated in the late stage of logarithmic phase (nearly 20 h of beginning) (figure 1). The effect of pH and temperature: results showed that bacteria produced the highest enzyme in pH=9.5 and 40 oC (figure 2). The effect of carbon source: while different members of the Bacillus genus can use diverse carbon sources; the best sources are glucose and then fructose and Mannitol (figure 1). About the effect of mineral and organic source of nitrogen, between mineral ammonium sulfate and organic sources, setoff yeast extraction and peptone showed the best results (Table 1).

Table 1. The effect of carbon and nitrogen source on Alkaline protease enzyme production with bacillus stain in pH=9.5 and 40oC in 20h incubation

Enzyme activity (U ml-1 min-1)	Nitrogen source (1% w/v)	Enzyme activity (U ml-1 min-1)	Carbon sources (1.5% W/v)
55.2	NH4NO3	42.55	sucrose
55.89	NH4Cl	59.8	Fructose
58.19	NaNO3	43.93	Mannitol
54.74	NH4H2PO4	64.63	Glucose
70.84	(NH4)2SO4	41.86	Maltose
57.04	Pepton		
56.12	Casein	42.55	lactose
65.55	Pepton+yeast extraction		

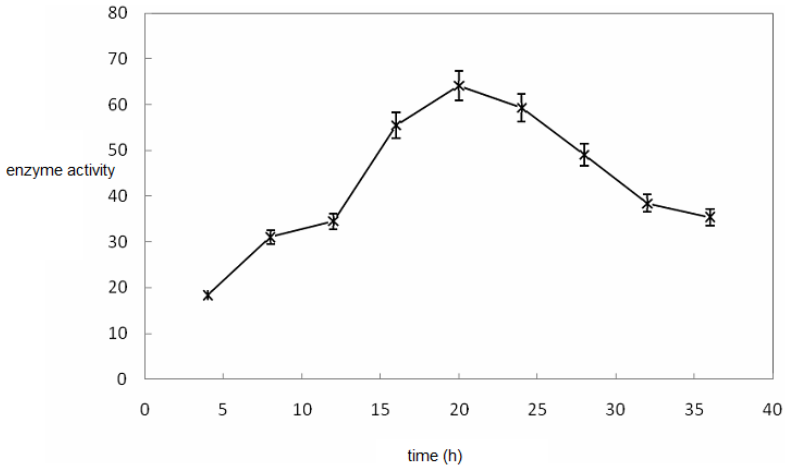

Figure 1. Culturing time on alkaline protease enzyme production base on (U ml-1 min-1) with bacillus strain

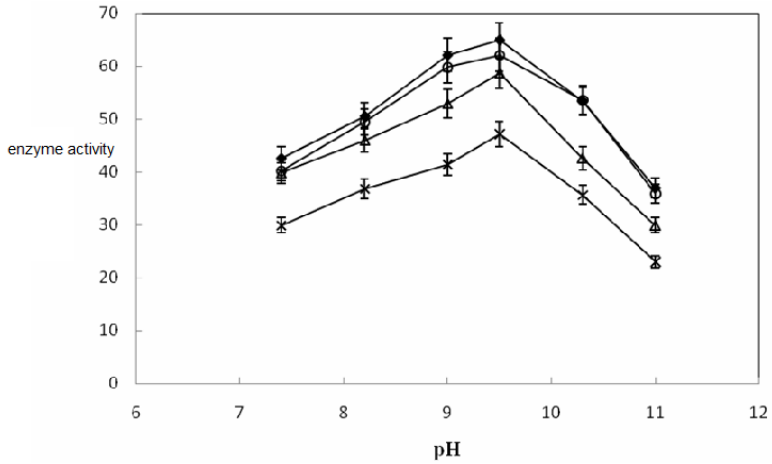

Figure 2. The effect of pH on temperature (oC) on alkaline protease enzyme production base on (U ml-1 min-1) with bacillus strain 35 (○), 40 (♦), 45 (Δ), 50 (×).

3. Discussion

One of the main determinant factors to produce the enzyme in fermentation procedure is time. In this study, the maximum enzyme production observed 20 hours after the beginning of the process. So it sounds that the highest production corresponded to logarithmic phase. In this way, other researchers reported the same results [13].

Ward et al (1986) reported the most enzyme production of different species of Bacillus genus I the late of logarithmic phase [16]. Apparently, production related to protein production changes during the sporulation process.

In temperature and pH point of view, the authors mentioned different restriction to optimal production of the enzyme. The best temperature reported 45oC and the most proper pH obtained between 9-11 [17].

Sen et al (1993) found glucose as the best carbon source to produce enzyme and others confirm the increasing role of glucose rather than other carbon sources [18]. Results also support this claim. Many researchers try to prove that this enzyme can use glucose and starch in addition to nitrogen sources such as yeast, peptone and etc. But about using carbon sources and affordable energy much research has not been done [19]. However, in some cases proved that using combination carbon source rather a separated substrate, such as glucose or lactose had higher efficiency in production point of view [20].

Using different mineral and organic sources showed the Ammonium sulfate privilege between other used mineral nitrogen sources and also yeast extraction was used by peptone through nitrogen sources. Fujiwara et al (1991) introduced nitrogen sources as the best items to enzyme production [21]. Different organic and mineral sources were introduced to enzyme production. Recently in several types of research using the mixture obtained by fish protein hydrolysis known as a nitrogen source [22]

It can be concluded that to produce alkaline protease enzyme in 40 oC and pH=9.5 during 20 h from the beginning of the process in an environment containing 1.5% carbon sources (weight to volume) glucose and 1% nitrogen as

Ammonium sulfate, given strain was the best choice. Of course, more research might be required to assess using this strain for industrial purposes.

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