Production of Alkaline Protease from Alkalophilic Thermophilic Bacteria and its Application in Bological Detergents

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Abstract

Eleven alkalophilic thermophilic bacterial isolates producing alkaline protease(s) were selectively isolated from soil and compost samples, using solid medium amended with 1% wheat meal at pH 10.5.

Proteolytic activity was qualitatively investigated, measuring the ratio of clear zone diameter to colony diameter. Isolate no. 3T, 4T and 9T were respected as the relatively most active organisms, which were subsecuently identified and designated as Bacillus sp.

Qualitative assessment of alkaline protease activity was done on solid medium using agar diffusion method, by which isolate 4T showed the relatively highest enzyme activity.

Optimal growth conditions influencing enzyme production were determined. Results showed that 930 unit / ml of alkaline protease were produced after (3) days of incubation at 50 °C and pH 10.

A commonly used detergent powder supplemented with 1% crude enzyme preparation showed better effect in removing blood smear from cotton cloths than control powder.

Introduction

Alkaline proteases are mainly produced by alkalophilic microorganisms. Thermophilic microorganisms have already made a large impact in biotechnological applications (1). The advantage of alkaline proteases from thermophilic microorganisms is their stability to a wide range of high temperature and ph values, besides to high reaction rates and activity at high temperature(2, 3).

Various taxonomic groups including bacteria, actinomycetes and fungi are producing alkaline heat stable protease enzymes (4).During the last two decades, most of the work has been done on alkaline proteases produced by genus Thermus, Bacillus and Streptomyces, as those microorganism are easy to maintain and grow well in both small and large scale culture (5). At least six different species of genus Bacillus, as being thermophilic, are producing alkaline protease (2), the most notable among them are Bacillus thermoproteolyticus, B. licheniformis and B. subtilis (6).

Today, the most promising thermophilic bioproducts are thermostable enzymes, not only because of their enhanced thermostability, but also due to their high resistance against denaturing agents and tolerance to high detergent concentrations (3, 4).

The biological detergents are one of the numerous products in biotechnological industry, which containing enzymes that have been obtained from alkalophilic bacteria (1, 2). Their commercial success can be measured by the fact that the market share for detergents containing enzymes , is about 75% in Europe , 55% in Japan and 45% in USA(7).

This paper describes the isolation and cultivation of alkalophilic thermophilic Bacillus sp. producing alkaline protease, and assaying its application in biological detergents.

Materials and Methods

Selective isolation of bacteria. Bacteria were isolated from soil and compost samples. A 1 g of sample was suspended in sterile distilled water , vortexed, and few drops were spread plated on solid medium containing (w/v) : wheat meal 1% , glucose, 0.5% ; KH2PO4 0.1% ; Na2CO3 1% ; and agar, 1.5 % which dissolved in tap water , pH adjusted at 10.5 . Inoculated plates were incubated at 50 C for (2-3) days. Colonies that form clear zone on solid medium were picked and subcultured on the same medium (8).

Qualitative assessment for the detection of alkaline protease was done by culturing bacterial isolates on LC-agar (L-agar plus 1% casein), pH 10.5 (9). Morphological and physiological characteristics for three

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isolates which were designated as (3T, 4T and 9T) showed a higher

ability to produce alkaline protease were done following (10). Enzyme

activity was qualitatively estimated using agar diffusion method on casein digest agar diffusion method on casein digest agar as described by (9).

Determination of growth conditions influencing maximal production of alkaline protease: Experiments were carried out by growing the selected isolated Bacillus sp. 4T in the liquid basal

medium which contains (w/v): Soy bean meal, 1%; glucose 2%; polypeptone 1%; KH2PO4 0.1% and Na2CO3 1%; pH was adjusted to 10 (unless otherwise stated) and autoclaved. 100 ml aliquots of basal medium in 500 ml conical flasks were inoculated (0.63 ± 0.2 × 10 3 cell/ ml) incubated at 50 C (unless otherwise stated) , with shaking at 150 rpm , for different time intervals (8) .Amount of produced enzyme was estimated according to (11).

Enzyme assay : Activity of alkaline protease was assayed following (12).A one unit of enzymatic activity could be defined as the amount of enzyme required to produce an increase in absorbance at 280 nm equal to 0.001 per min . Under the assay conditions.

Qualitative assessment of alkaline protease activity in combination with detergent; the method described by (4) was modified as follows: Two cotton fabric pieces contaminated with 5 drops of blood, smeared and left to dry for 1 h., were soaked separately, the first in test solution which is; 1 ml of crude enzyme added to 1% (w/v) detergent powder dissolved in 100 ml tap water, ph adjusted to 10; the second in control solution which is: 1% detergent solution minus enzyme. Both preparations were incubated at 50 C for 20 min. with shaking. Both fabric pieces were washed by tap water for comparison by direct visual examination.

Results and Discussion

Alkalophilic thermophilic microorganisms can be isolated from natural environments, such as soil and compost (1).

Isolation of proteolytic bacteria:

Alkaline proteases of microbial origin have a number of specific properties; the most remarkable one is the optimum of their action which lies in alkaline range of ph values between 8-12 (6).

Eleven isolates of alkalophilic bacteria were isolated from soil and compost samples, selectively cultivated at 50 °C and ph 10.5 among them, three isolates which were designated as 3T, 4T and 9T showed better ability for producing alkaline protease. Isolate 4T was able to exhibit relatively the highest enzymatic activity.

Characterization of the bacterial isolates:

The three isolates (3T,4T and 9T) were aerobic , rod – shaped , gram positive , spore forming bacilli, motile , catalase positive , maximum temperature for growth was 70 °C , which could tentatively characterized as genus Bacillus.

Growth conditions affecting the production of alkaline:

Temperature and pH are probably the most important factors affecting the synthesis of alkaline protease (13). Fig 1. shows that, maximal enzyme was at 50 °C, when initial ph of the growth medium was adjusted to 10. Fig. 2 shows that enzymatic was at its most referring to the amount of produced enzyme (pH 10). As bacterial cells grown to the adequate period of the time, this would give the relatively highest amount of produced enzyme (which was after 3 days of incubation) as shown in Fig. 3.

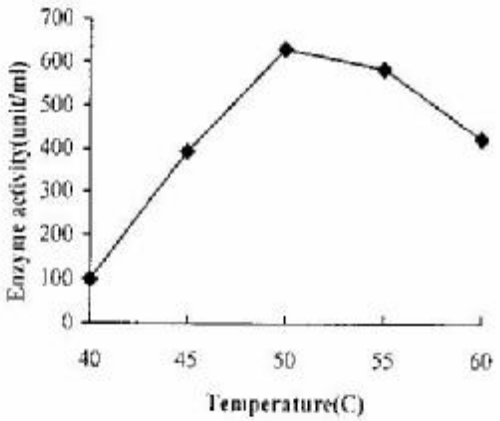

Figure (1): Effect of growth temperature on the production of alkaline protease by Bacillus sp. isolate 4T, grown in liquid basal medium for (2) days at pH (10).

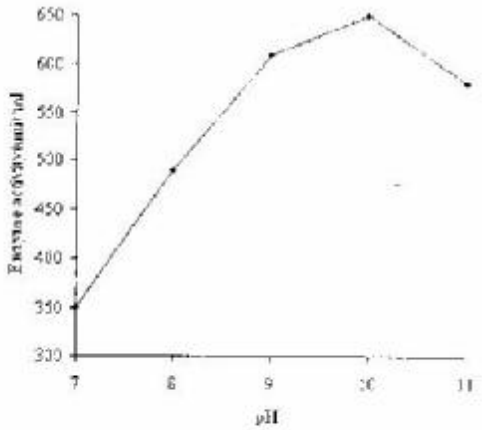

Fig. (2): Effect of pH on the production of alkaline protease by Bacillus sp. isolate 4T, grown in liquid basal medium for (2) days at 50°C.

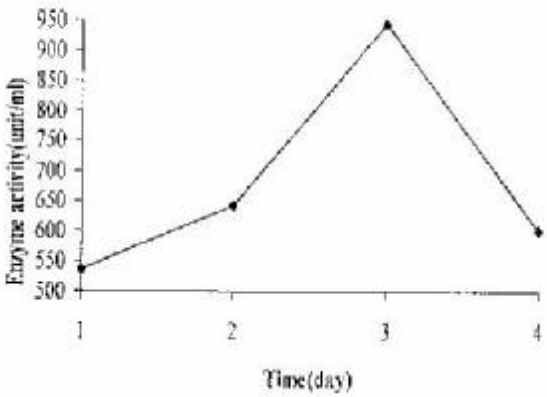

Fig.(3): Effect of incubation time on the production of alkaline protease by Bacillus sp. Isolate 4T,growth in liquid basal medium at 50 °C and pH(10).

A temperature favorable for growth of a microorganism is usually favorable for enzyme synthesis as it has a great influence on microbial growth and enzyme production (14).

It was found that the optimum temperature for the production of alkaline protease by mesophilic and thermophilic bacteria ranges from 35-40 °C and 45-60 °C respectively (6). In Bacillus licheniform, maximal production of alkaline protease was carried out at 40 °C and pH 8.5 after 20 h of incubation (15), whereas (13) reported that maximum amount of enzyme produced by thermophilic Bacillus sp. was at 40 C and pH 9 grown for 20-24 (14) also noted that, maximal enzyme production by B. lichniformis are commonly used in industrial application as builders for biological detergents.

In the current assay , it was obvious that local detergent solution amended with crude enzyme , efficiently removed blood smear from cotton fabric after 30 min , compared by direct visual examination with control detergent solution , such result would mean that , application of such enzyme in industrial production will motivate local scientific research towards new horizons for the development of national industry .

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الخلاصة

عزلت (11) عزلة بكتيرية محبة للحرارة ومنتجة لأنزيم البروتييز القاعدي Alkaline protease(s) من نماذج الترب والمياه الملوثة بالنفط باستخدام الوسط ... رقم الهيدروجيني (8.5) والحاوي على 1% حليب منزوع الدسم . استخدم وسط LC-agar (L-agar with 1% casein) لاختبار قدرة العزلات على انتاج الأنزيم من خلال قياس قطر المنطقة الشفافة الى قطر المستعمرة كدليل لاختيار افضل العزلات المنتجة للأنزيم . شخصت العزلات على انها تعود لجنس Bacillus sp. قيست فعالية انزيم البروتييز المنتج من العزلات الثلاث باستخدام طريقة الانتشار في الاكار Agar diffusion method وتبين ان العزلة Bacillus 4T اعطت اعلى فعالية . درست الظروف المثلى (درجة الحرارة ، الرقم الهيدروجيني وفترة الحضانة) للعزلة Bacillus 4T لانتاج انزيم البروتييز القاعدي في الوسط السائل ، ووجد ان اقصى انتاجية للأنزيم (950 وحدة / مل) كانت بعد (3) ايام وبدرجة حرارة (50م) وعند الرقم الهيدروجيني (10) . استخدم الأنزيم الخام للعزلة Bacillus 4T لتحسين كفاءة مساحيق الغسيل ، واظهرت النتائج ان المنظفات المعاملة مع 1% من الأنزيم الخام ... في ازالة بقع الدم من الأنسجة القطنية مقارنة بالسيطرة على مجموعة السيطرة و انخفاضا في مستويات الشحوم الدهنية عالية الكثافة و الثلاثية الكليسيرات أيضا لدى المرضى .

من المحتمل جدا أن تستجيب خلايا أورام الدماغ ... من الشحوم وأن هؤلاء المرضى يحتاجون الأدوية المخفضة للشحوم الدم .