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Formation of proteases from newly isolated strain isolated from Saudi Arabia

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ABSTRACT

Comparative study was carried out on the production of protease using isolate from moraine. The isolates were screened on gelatin plates for gelatin hydrolyses by the different isolates. Among the all tested isolate No. 7 EHN produced the highest activity, while under the fermentation conditions after 3 days the maximum yield of protease was 277 U/ml. The protease production from marine isolate could be commercially used for applications purpose.

Keywords: Protease, screening, isolations. Extracellular, Identification, gelatin ager, production.

INTRODUCTION

proteases account for approximately 40% of the total enzyme sales in various industrial market sectors, such as detergent, food, pharmaceutical, leather, diagnostics, waste management and silver recovery. This dominance of proteases in the industrial market is expected to increase further by the year 2005 (Godfrey and West, 1996). Proteases are capable of cleaving proteins into peptides and amino acids, they are characterized by their optimal pH (acid, neutral or alkaline), their temperature, their ability to hydrolyze specific proteins (collagenase, keratinase, etc...), their homology to well characterized enzymes as chymosine, chymotrypsin, pepsin and trypsin (trypsin-like, pepsin-like, etc.), and their stability. The major uses of proteases are in the biotechnological production of detergents (Bailey *et al.*, 1977), dairy industries as milk-clotting agents (calf rennet composed mainly of (Fox, 1982) and as an agent for meat tenderization (Bernholdt, 1975). Proteases have also clinical and medical application in reduction of tissue inflammation (Nout *et al.*, 1990). Microorganisms elaborate a large array of proteases, which are intracellular and/or extracellular. Intracellular proteases are important for various cellular and metabolic processes, such as sporulation and differentiation, protein turnover, maturation of enzymes and hormones and maintenance of the cellular protein pool. Extracellular proteases are important for the hydrolysis of proteins in cell-free environments and enable the cell to absorb and utilize hydrolytic products (Kalisz, 1988). At the same time, these extracellular proteases have also been commercially exploited to assist protein degradation in various industrial processes (Kumar and Takagi 1999; Outtrup and Boyce 1990).

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Proteases of microbial origin have long been used in industry (Pavlukova *et al.*, 1998), easily extracted and separated (Phadatare *et al.*, 1993).

Microbial proteases are among the most important hydrolytic enzymes and have been studied extensively since the advent of enzymology. There is renewed interest in the study of proteolytic enzymes, mainly due to the recognition that these enzymes not only play an important role in the cellular metabolic processes but have also gained considerable attention in the industrial community. The objectives of this investigation, therefore isolation a novel isolate which capable of producing higher amount of extracellular proteases and to determinate the values of their proteolytic activities for applications purpose.

MATERIALS AND METHODS

Source of samples

Samples of sea water and sediment (30-m depth) were collected in sterile containers along the Red Sea coast from Jeddah, Saudi Arabia.

Isolation of bacteria

Bacteria were isolated for protease enzyme using a serial dilution method described by Sjudahl *et al.* (2002). Samples were inoculated on skim milk agar plates containing peptone (0.1%), NaCl (0.5%) and skim milk (10%) medium prepared using sea water, then incubated at $28 \pm 2^\circ\text{C}$ for three days (Uyar *et al.* 2011). Bacterial isolates were primarily purified on nutrient agar medium and routinely maintained at 4°C on culture purity was determined from colony morphology.

Screening for best strain produced protease

The isolates were Screening for best strain produced protease by plate assay using protease specific medium containing (g/l) K_2HPO_4 2.0, glucose 1.0, peptone 5.0, gelatin 15.0, and agars 15. The clear zone diameters were measured after 24h of incubation at 28°C by flooded the plates with mercuric chloride solution, this method was referred as gelatin clear zone method (Abdel Galil 1992).

Quantities estimation of protease

The isolates strains were inoculated in 50 ml of protease specific medium broth containing (g/L) glucose, 5.0; peptone, 7.5; ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5.0; KH_2PO_4 , 5.0; and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1, pH-7.0 and were culture in a rotary shaker (180 rpm) at 28°C for 3 days. After the completion of fermentation, the whole fermentation broth was centrifuged at 10,000 rpm at 4°C , and the clear supernatant was recovered. The crude enzyme supernatant was subjected to further studies (Josephine *et al.*, 2012).

Measurement of enzymatic activity

Protease activity in the culture supernatant was determined according to the method of Tsuchida *et al.* (1986) by using casein as a substrate. A mixture of 500 μl of 1% (w/v) of casein in 50 mM phosphate buffer, pH 7 and 200 μl crude enzyme

extract were incubated in a water bath at 40°C for 20 minutes. After 20 minutes, the enzyme reaction was terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid (TCA) and was kept at room temperature for 15 minutes. Then, the reaction mixture was centrifuged to separate the unreacted casein at 10,000 rpm for 5 minutes. The supernatant mixed with 2.5 ml of 0.4M Na_2CO_3 . 1 ml of 3-fold diluted Follin Ciocalteus phenol reagent, was adding. The resulting solution was incubated at room temperature in the dark for 30 minutes and absorbance of the blue color developed was measured at 660 nm against a reagent blank using a tyrosine standard (Lowry *et al.* 1951). One unit of protease is defined as the amount of enzyme that releases 1 μg of tyrosine per ml per minute under the standard conditions of supernatant solution.

Identification of isolated bacteria

The isolated bacteria with strong productivity for protease were purified according to the procedure described by Peculytė (2007). Finally, the isolated and purified bacterial strain producer was identified by means of morphological examination, cultural studies and biochemical characterization according to the methods of Buchanon and Gibbons (1974).

Results and dissection

A total of 7 isotates bacterial strains were isolated from marine in Jeddah. Screening and quantitative analyses for extracellular protease was done then the selected isolate was identified of protease producing bacteria.

Isolation and screening of microorganisms producing Protease

Fig. 1 shows the isolate bacterium producing protease enzyme a clear zone around the colony as the protein near the colony is utilized. The all isolates were screened for producing protease using gelatin agar media. The isolate No. 7 produced a larger clear zone than the others, indicating higher proteases activity Fig (1). Depending upon the zone of clearance, the isolate No.7 EHN was selected for further experimental studies.



Fig. 1: The zone of hydrolysis by isolate No.7 on gelatin agar plates.

Gupta *et al.*, (2005) performed isolation of bacterial strains from environmental samples and screened their capability of protease production using skim agar and reported that the *Streptomyces sp.* CD3 was the maximum producer of protease among the isolated strains. On the hand seventy fungal isolates were screened by Chekireb *et al* (2009) for their abilities to produce extracellular protease by means of formation of clearing zones around the fungal growth in gelatin agar plates.

Quantitative analyses for extracellular protease

The isolates bacterial were screened for their abilities to produce extracellular protease during their growth on production medium for protease (Folasade and Joshua 2005). The inoculated bacterial strains which produced high amount of extracellular protease were selected. The results of this screening were depicted in Fig 2. It was observed that all the 7 isolates exhibited protease producing ability. Its ability to produce protease was further confirmed by the formation of halo zone and hydrolysis in gluten agar plates. But the formation of isolate No. 7 was the best producers of extracellular protease (277 U/ml). The Production of extracellular protease from Isolate No.7 showed the maximum enzyme production was observed at 72 hours.

This result was in concordance with similar screening work in which a total of 118 bacterial strains were isolated from soil and *Bacillus sp.* predominant was used for protease production with activity (380U/ml) Mohsin *et al* (2011). Other investors, reported that both *Bacillus anthracis*, S-44 and *Bacillus cereus* S-98 exhibited their maximum ability to biosynthesize proteases within 60 h incubation period since the productivity reached up to 126.09 units/ml-1 for *Bacillus anthracis*, S-44 corresponding to 240.45 units/ml-1 for *Bacillus cereus*, S-98 respectively (Johnvesly *et al* 2012). Moreover, Soundra *et al.* (2012) found that a high level of extracellular thermo stable protease activity was observed after 24 h incubation.

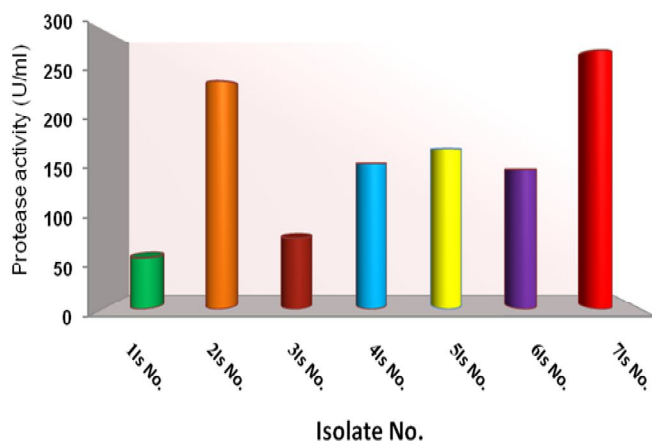


Fig. 2: Screening For Protease Producers Under Production Medium.

Identification protease producing bacterium

Bacterial strain producing protease enzymes were isolated from marine. Among them, isolate No.7 showed the highest proteolytic enzymes productivity. The potent bacteria was

identified based on morphological characterizations. The morphological characteristics are presented in Fig (3). The results showed that the strain is spherical shape, motile, Gram-negative, cocci-shaped, nonspore-forming bacterium it identified to be a strain of *streptococci* (Kim *et al* 1998) and named it *streptococci sp* 7EHN.

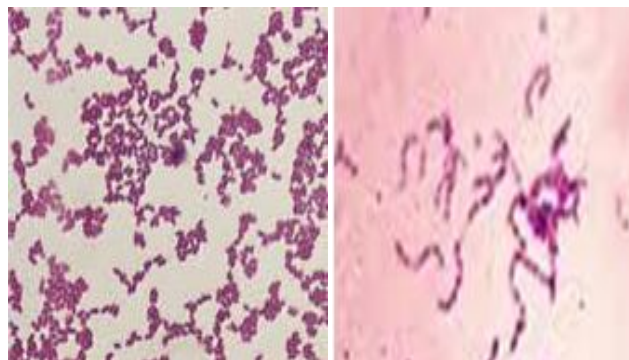


Fig. 3: Gram-negative spherical shaped cocci strain.

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