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Research Article

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Studies on potential cellulase production by *Lysinibacillus sphaericus*

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Keywords

Polychaetes, *Lysinibacillus sphaericus*, cellulase enzymes, Blueberry corn, SDS-PAGE

Abstract

Coastal waters support vibrant ecosystems, are valuable natural resources, and have important environmental value. polychaetes are one of the most important protected areas and play an important role in aquaculture as food for crustaceans, fish larvae and many organisms. Therefore, this study showed that the bacterial strain *Lysinibacillus sphaericus* isolated from polychaetes is very potent against human bacterial pathogens. This includes isolation, amplification, partial purification, and application of cellulase enzymes. As a potential primary screening method for determining cellulase production, strains of *Lysinibacillus sphaericus* were screened using Congo Red Cellulose agar medium. The medium has been optimized for maximum improvement in cellulase production. The isolated strain showed growth and enzymatic activity in a wide range of 25-45 °C. Bacteria lived in environments with pH values between 3 and 11. The optimal salt content for bacterial growth and activity was found to be 3%. enzyme. Blueberry corn at a concentration of 3% has been shown to be a suitable substrate for maximum cellulase production. A maximal enzyme activity of 43 U/ml/min was found at 3% of the substrate concentration. Protein concentration was determined using Lowry's method, which showed a protein concentration of 0.33 U/mg during this period. SDS-PAGE was performed to determine the molecular weight of the partially purified cellulase enzyme from *Lysinibacillus sphaericus*. As a result, the molecular weight of the purified cellulase enzyme was between 22 and 35 kDa.

Introduction

Cellulose is the most abundant renewable natural product in the biosphere (Solomon *et al*., 1997 and Whitaker, 1998). It is made up of long chains of bound D-glucose molecules such as betas 1 and 4. Cellulases are cheaper and have the potential to form a variety of useful compounds from more complex substrates. It is an important cellular microbial enzyme that breaks down cellulose. They are produced by enzymatic biodegradation from inexpensive sources of cheap compounds such as sugars, proteins and other chemicals. Annual cellulose production is estimated at 1074.0 tonnes (Singh and Hayashi, 1995). Cellulose content in plant tissues varies from 20-45% by dry weight to more than 90% for cotton fibers (Stephens and Haichel, 1995). Recycled paper is an important source of cellulose (Crueger and Crueger, 1990) and is produced primarily by bacteria, fungi and protozoa. Many methods have been proposed and used to convert and use biomass, from direct chemical methods such as acid hydrolysis and pyrolysis to biological methods such as cellulase (Cooney *et al*, 1978). Cellulose production is primarily fungal, but interest in bacterial cellulose production has recently increased (Shao et al., 2015; Hungund and Gupta, 2010). Acid hydrolysis of cellulosic materials is cheaper than hydrolysis of cellulosic materials, but the former process often requires higher temperatures and pressures. It is also highly corrosive and accumulates unwanted by-products (Fennington *et al*., 1982).

Materials and Methods

Optimization for growth and cellulase production

Factors such as temperature, pH, salt, substrate concentration, and many other important factors are optimized (i.e. change over time) for cellulase production using screening techniques. Experiments were performed in 250 ml Erlenmeyer flasks containing production medium. After steam autoclaving, the vials were cooled and incubated under various experimental conditions and incubated at 37° C. for 72 hours.

Optimization of temperature for growth and cellulase production

The effect on growth and enzyme production was investigated by performing fermentation at various temperatures such as 25°C, 30°C, 35°C, 40°C, and 45°C. All experiments were performed to obtain an average value.

Optimization of ph for growth and cellulase production

The pH of the stock medium was optimized, but the pH of the aqueous solution was between 3, 5, 7, 9 and 11 between 1N HCl and 1NNaOH. All experiments were performed to obtain an average value.

Optimization of salinity for growth and cellulase production

Medium was prepared with different salt concentrations ranging from 1%, 2%, 3%, 4% and 5%. All experiments were performed and average values were obtained.

Effect of different substrate on cellulase production

The effects of different substrates on cellulose production have been studied using different substrates such as arnica, rice bran, wheat bran, cellulose and carboxymethyl cellulose. All experiments were performed and the average recorded.

Effect of different substrate concentration on cellulase production

The effect of different substrate concentrations on cellulase production was studied for maximal enzymatic activity of 1-5%. All experiments were performed to obtain an average value. The optimal substrate concentration obtained in this step was determined in subsequent experiments.

Mass culture of cellulase producing from of different parameters *Lysinibacillus sphaericus*

Based on the optimization results, a large-scale cellulase culture of *Lysinibacillus sphaericus*

was performed. 5% inoculum was inoculated into 500 ml of double production medium. Fermentation was performed in 1000 ml Erlenmeyer flasks on a rotary shaker (300 rpm). Biomass and enzyme activity were tested every 6 hours. After 36 hours, the fields were harvested to recover the cellulase enzyme.

Enzyme activity

Cellulase activity was measured by reducing sugar released by the DNA method (Krootdilaganandh, 2000). After incubating 0.5 ml of CMC solution, 0.5 ml of coenzyme and 0.5 ml of 0.05 M citric acid solution at pH 4.8, 2 ml of DNS solution was added for 30 min at 50^{o} C. The treated samples were boiled for 15 minutes. Let the colour changes on in cold water before letting it cool. The optical density at 540 nm was read with a spectrophotometer for a white detector (Thermo Spectronic, USA).

Extraction and partial purification of cellulase enzyme

Unless otherwise specified, several enzymatic purification steps were followed and performed at 4 °C. In the first purification step, the supernatant containing the extracellular enzymes of various saturation (40%, 60% and 80% saturation) was treated with solid ammonium sulfate with stirring overnight. continued (Wang et al., 2006). The precipitated enzyme was collected by centrifugation (10,000 rpm, 15 min) and dissolved in 0.1 mM phosphate buffer (pH 5.0). The enzyme solution was applied to the dialysis membrane No.1 (HIMEDIA) I tested the same pad for 48 hours, but some optional pads were replaced. Partially purified enzymes were tested in dry powder form and cellulosic activity was tested.

Determination of molecular weight by electrophoresis (SDS-PAGE)

Electrophoresis of protein samples on polyacrylamide gels is an essential analysis and in some cases the starting point for protein scientists. Electrophoresis can be used to separate and compare complex protein mixtures, to assess protein purity during separation, and to provide estimates of physical properties such as subunit composition, electronegativity, volume, and charge. All types of electrophoresis can be performed in a variety of gel formats, from microgels to gels slightly larger than stencils, to gels larger than these plates. As in many other cases, dissociation of SDS-based gels is very robust, but not all proteins are readily soluble in SDS solutions. Most proteins bind to a certain amount of SDS per microgram of protein, resulting in a uniform charge density per unit mass, resulting in a separation based on polypeptide chains. After electrophoresis, proteins are usually detected using more common protein staining methods such as Coomassie blue or silver staining. These processes immobilize the proteins in the gel matrix either through chemical bonding or by changing their color to prevent further replication of the protein bands. Electrophoresis is used to isolate complex protein mixtures, determine subunit structures, determine the uniformity of protein samples, and purify proteins for use in other polyacrylamide gel electrophoresis applications. (PAGE) In response to an electric field, proteins pass through pores in the gel. Protein migration is determined by the pore size of the matrix gel, the weight, shape, and size of the protein. Sodium polyacrylamide gel electrophoresis. The sample was dissolved in the reduced sample buffer, the same amount of protein was loaded on a 12% SDS polyacrylamide gel, and constant current (30 mA) electrophoresis was performed.

Results

Lysinibacillus sphaericus strain showed a 12 mm clearence in cellulose agar medium.

Fig.1 Zone of clearance in the Cellulose Agar medium

Optimization of growth and cellulase production

pH

The present investigating various pH values, an optimal pH value for growth and enzymatic activity was found at pH 7. The increase was 3.15 OD and the enzyme activity was about 40 U/ml/min.

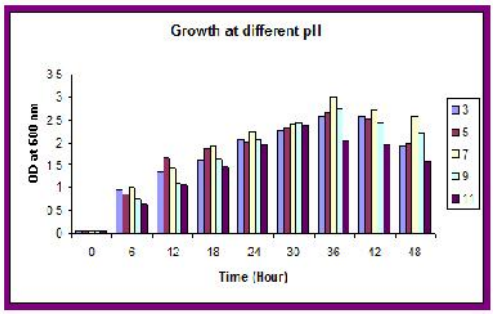


Fig. 2 Showing Bacterial growth at different pH

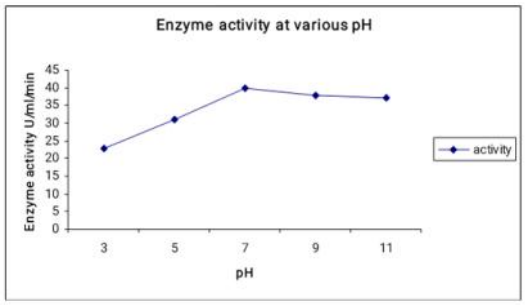


Fig. 3 Showing Enzyme activity at various pH

Optimum temperature for growth and cellulase production

Among the various temperatures studied, the optimal temperature for growth was found to be 35 °C. The increase at 600 nm was an OD of 3.42, which was observed after 36 hours. The enzyme activity was 35 units/ml/min.

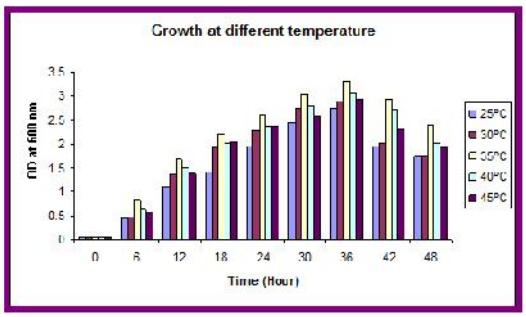


Fig. 4 Showing Bacterial growth at different temperature

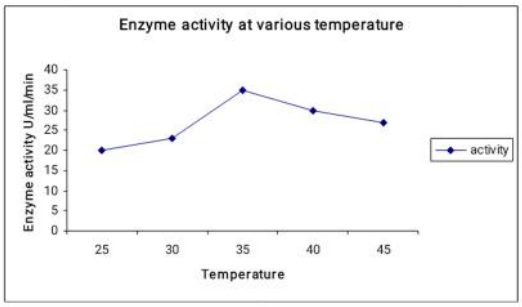


Fig. 5 Showing Enzyme activity at Various Temperature

Optimum salinity for cellulase production

Enzyme activity of up to 3% was determined in the various saline conditions tested. The enzyme activity was about 39 units/ml/min. The increase was 3.31 OD.

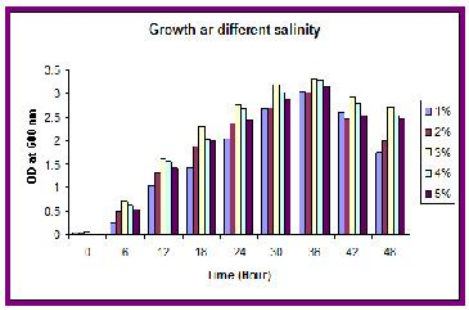


Fig. 6 Showing Bacterial growth at different salinity

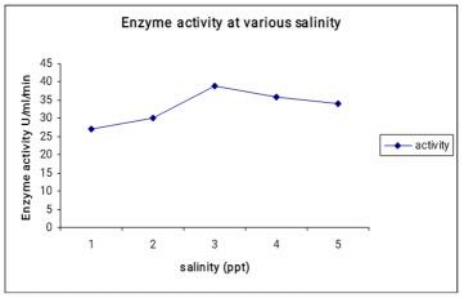


Fig. 7 Showing Enzyme activity at various Salinity

Optimum substrate for cellulase production

Among the various substrates used, maize was observed to have the highest growth and activity. The enzyme activity was about 40 units/ml/min. The maximum increase in OD was set to 3.63.

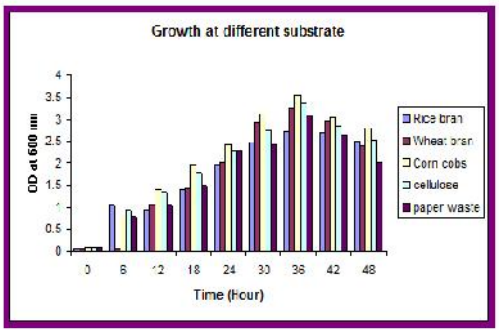


Fig. 8 Showing Bacterial growth at different substrates

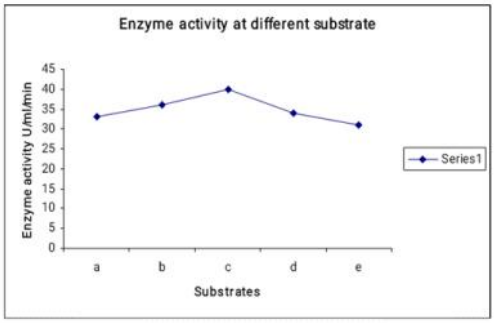


Fig. 9 Showing Enzyme activity at different substrates

Optimum substrate concentration for cellulase production.

An increased maximal enzyme activity (3.75 OD) at various substrate concentrations used was found to be 3%. Enzyme activity was found to be 43 units/ml/min.

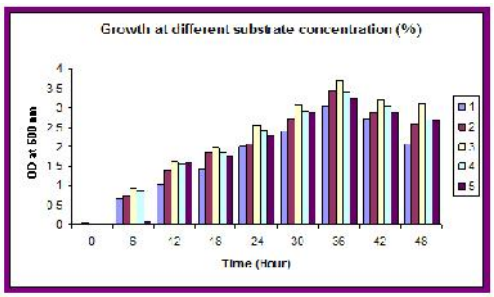


Fig. 10 Showing Bacterial growth at different corn cobs concentration (%) as substrate

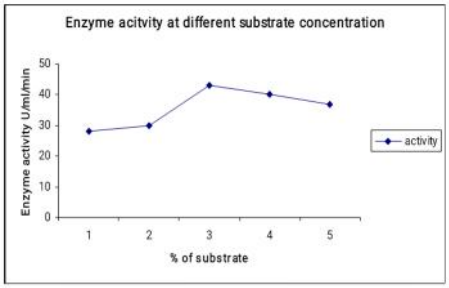


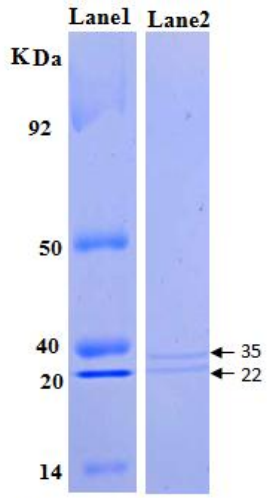
Fig. 11 Showing Enzyme activity at Different substrate concentration

Ammonium sulphate precipitation for the partial purification of extracellular cellulase enzyme

Perhaps the *Lysinibacillus sphaericus* strain was chosen for mass culture under optimal conditions. 500 ml of cell-free supernatant was obtained. Maximum protein deposition (0.84 g) at cellulosic enzymes, additional amounts of ammonium sulfate (30, 40, 50, 60 and 70) and 50% ammonium sulfate, 40 (0.56 g) and 30% (0.17 g). Washing was initiated by dialysis in seamless cellulose tubes reconstituted with phosphate buffer for 24-48 hours. Dialysis membranes are partially permeable and have a molecular weight between 12,000 and 14,000.

Molecular weight determination by SDS-PAGE analysis

Polyacrylamide gel analysis was used to determine the molecular weight of the resulting enzyme. Assays were performed under uncertain conditions to determine cellulase.



Lane 1: Molecular weight markers
Lane 2: purified cellulase.

Fig. 12 SDS-PAGE of purified cellulose

The results exhibited that the purified cellulase enzyme has a molecular weight range from 22 to 35 kDa.

Discussion

In this study, the *Lysinibacillus sphaericus* strain showed a distance of 12 mm in the Congo Red test. A bacterial study in the Congo Red test was performed as a preliminary study to select cellulase producers. In another study, approximately nine bacterial strains (EB1-EB9) tested positive for the Congo Red test, with EB3 exhibiting degradation with the highest ratio of apparent area diameter to colony diameter. Cellulose excess on CMC agar plates (Woo and Park, 2003). *Salini vibrio* S. NTU-05 isolated from Szutsau salt showed a clear area around the colonies on CMC agar plates after staining with 1% Congo red solution, indicating a high amount of cellulose (Wang, 2009). Cellulase was produced by *Aspergillus niger* and Bacillus subtilis by solid-phase fermentation using pine cone shell as a substrate. *Bacillus subtilis* has the highest enzyme production compared to Niger (Sunita et al., 2006). Aerobic celluloid bacteria were found in some mossy forests and agricultural areas, and the proportion of celluloid bacteria in forest soils was higher than in agricultural soil samples (Hatami, 2007). A nitrogen-fixing strain of *Paenibacillus* was isolated from soil in a nitrogen-free environment. Three of these cellulase-positive isolates were identified as E, H and SH *Paenibacillus* strains by CM Case (Emtiazi et al., 2007). The stars used in this study generate a maximum activity of 45 units/mL per minute. Company availability is displayed. Optimal physical and chemical parameters for growth and enzyme production indicate that strains used for industrial production are optimal. This study also demonstrated that the optimal temperature for cellulase production is 35 °C. An optimum temperature for the production of the same cellulase was observed using pineapple peel as a substrate (Sunita, 2006). The effect of temperature on the activity and stability of CM Case produced by *Bacillus subtilis* was measured at 50°C (Kim, 2009). The optimum temperature for pure cellulose at 70 °C is *Salini vibrio* sp. In stock. NTU-05 (Wang, 2009). The optimal salinity was found to be 3, but in a corresponding study, *Salini vibrio* sp. The optimal NaCl concentration was found by purification and characterization of the novel cellulase halo. NTU-05 was discovered (Wang, 2009). Among the various substrates used, corn starch showed an increase in enzyme activity at the optimal substrate concentration 3, and *Bacillus subtilis* showed the maximum enzyme activity with an increase in the skin substrate concentration (10%) of the skin. Pineapple (Sunita, 2006).

The molecular weight of cellulase was determined by SDS-PAGE analysis. In this study, the molecular weight of the purified cellulase enzyme was found to be 22-35 kDa. Bacillus sp. The molecular weight range of purified CMKase is 30-65 kDa (Arifin, 2006). The molecular weight of the CH43 and HR68 strains estimated by SDS-PAGE was 40 kDa (Mawadza, 2000). Hallucinogenic cellulase with a molecular weight of 29 kDa was purified by SDS-PAGE (Wang, 2009). The *Lysinibacillus sphaericus* used in this study produced promising amounts of cellulase, allowing the extension of the cellulase-producing family into the genus Bacillus. In another study, the *Bacillus* mutant (BpCRI 6) provided several advantages, including increased activity in the presence of substrates such as CMC and cellobiose (Kotchoni, 2003). Two types of bacteria, the genera *Acinetobacter anitratus* and *Moraxella*., they will break up. *Archachina marginata* extracted from giant blood lymph nodes of giant African snails. The intracellular activity of the culture medium during bacterial growth was determined by measuring the decrease in glucose release from carboxymethylcellulose (CMC) (Ahee, 2002). Vetriselvi (2007) contains 5 types of bacteria such as *Pseudomonas, Proteus, Micrococcus, Serratia* and *Bacillus* and 5 types of fungi such as *Aspergillus niger, Trichoderma halzianam, Milotesium loridam, Culbra realnata* and *Fusarium oxysporum*. During the study period, 15 isolates were obtained from decomposed litter compost, including fast-growing bacterial isolates of Bacillus, Cellulomonas and Pseudomonas. The three types of cellulose that are degraded by *Bacillus* are *B. subtilis B. licheniformis,* and *B. polymyxa*. Reported by Majumdar (2001). Cellulase was produced by *Aspergillus niger* and *Bacillus subtilis* by solid-phase fermentation using pine cone shell as a substrate. *Bacillus subtilis* has the highest enzyme production compared to Niger (Sunitha, 2006). Aerobic celluloid bacteria were present in some northern forests and agricultural areas, and the proportion of celluloid bacteria in forest soils was greater than in agricultural soil samples (Hatami, 2007). A nitrogen-fixing strain of *Paenibacillus* was isolated from soil in a nitrogen-free environment. Three of these cellulase-positive isolates were identified as E, H and SH *Paenibacillus* strains by CMCase (Emtiazi, 2007). The stars used in this study generated activity of up to 45 U/mL/min. It showed firm availability. Optimal physicochemical parameters for growth and enzyme production indicate that strains used for industrial production are ideal.

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