Isolation of Thermostable Bacillus Specie AHM1, Producing Industrially Important Extracellular Cellulase Enzyme

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ABSTRACT

Cellulose is the most abundant and plentiful polymer on earth. It is an essential constituent of plant cell wall as it gives rigidity and strength to plant tissue. Complex structure of cellulose can be degraded in to glucose submits by the action of cellulase enzyme. Bacterial cellulases possess huge applications in different industrial processes. The aim of current study was to isolate themostable cellulase producing bacteria from sea water and to assess an effect of temperature and carbon substrate on cellulase activity. Isolated and purified potent bacterial strain was identified as *Bacillus* specie AHM1 on the basis cellular, colonial morphology and biochemical characterization. It was found that the strain grew well and produced enzyme optimally at 50°C. The enzyme was stable over wide range of temperatures i.e. 30-60°C. Furthermore, it was noticed that utilization of various cellulosic biomass such as filter paper, cotton and grass along with carboxymethylcellulose showed significant enzymatic activity.

Keywords: Agriculture substrates, Bacillus, cellulose, cellulase, physicochemical parameters, thermostable

INTRODUCTION

Agricultural substrates are essential source of lignocellulosic biomass which is renewable, unexploited and cheap. Agricultural residues mainly includes leaves, stems, and stalks, corn fiber, rice straw, rice hulls, woody products, corn stover, sugarcane bagasses, and forest biomass. Cellulases belong to class hydrolases that act on complex structure of cellulose and release monosaccharides like beta glucose or oligomers. Cellulases hydrolyze the 1,4-beta-D-glycosidic bonds in cellulose, lignin and hemicellulose. Cellulases are widely dispersed in microbial domain such as bacteria, fungi, algae etc. However, in industrial biotechnology bacterial source is mostly preferred over other sources due to their rapid generation time, easy cultivation and enzyme purification strategies.

Cellulases possess broad range of commercial applications. They are utilized in textile, detergent, paper and pulp, bioethanol and pharmaceutical industries (Sethi *et al.*, 2013; Kushwaha *et al.*, 2013; Patagundi *et al.*, 2014). Cellulases from extremophilic microbes are attractive candidates as they are active and stable in harsh environmentalconditions like extreme temperature, pH, salinity etc., hence they are suitable for several harsh industrial processes (Anbu *et al.*, 2017).

Current study is based on the isolation of cellulase producing bacteria from sea water and evaluation of influence of substrate concentration and temperature on cellulase activity.

OBJECTIVES

Isolation of thermostable bacteria for cellulase production from sea water.

Evaluation of effect of temperature and carbon source on bacterial growth.

Assessment of influence of temperature and different substrate concentrations on cellulase activity.

METHODOLOGY

Sample collection and strain isolation

Water sample was collected aseptically from Seaside, Karachi, Pakistan. The bacterial strains were isolated using serial dilution and pour plate method.

Qualitative analysis of cellulose hydrolysis

To screen the potential of cellulase production from isolated strains, the bacterial cultures were grown on nutrient agar plate containing 1% cellulose. After 24 hours incubation at 37°C, the plates were flooded with iodine solution in order to visualize the zone of cellulose hydrolysis(Qazi *et al.*, 2017;Kasana *et al.*, 2008).

Morphological and biochemical characterization of isolated bacteria

Strain with maximum diameter of zone of hydrolysis was then selected and subjected to taxonomical and biochemical studies recommended in Bergey's Manual of Determinative Bacteriology.

Measurement of temperature optima for bacterial growth

Isolated culture was inoculated in nutrient broth containing 1% cellulose in 4 flasks and incubated at 25°C, 37°C, 50°C and 60°C for 48 hrs. Effect of temperature on growth of bacteria was determined by measuring absorbance of cell growth at 546nm in UV-Vis. spectrophotometer after 24 and 48 hrs of incubation.

Effect of different cellulosic substrates on bacterial growth

The selected isolate were grown in 1% CMC, filter paper, grass, cotton and cellulose separately at 37°C. Effect of cellulosic substrate on growth was determined by measuring absorbance of cell growth at 546nm in UV-Vis Spectrophotometer after 24 and 48 hrs of incubation.

Batch cultivation of cellulase enzyme and separation of cell free fluid (CFF)

The selected isolate was grown in nutrient broth containing 1% cellulose for 5 days at 37°C in shaking incubator. The extracellular enzyme solution was collected by centrifugation at 6000rpm for 10min in cold centrifuge. The CFF was used as an enzyme source in an enzyme assay.

Qualitative evaluation of effect of various temperatures on enzyme activity

Aliquots of 300µl CFF was placed at 25°C, 37°C, 50°C, 60°C, 70°C, 80°C and 90°C. 50µl CFF from each aliquot was used in enzyme assay after 30min, 1 hr, 1.5hrs, 2 hrs and 2.5hrs by using 1% cellulose in plate assay. Zone of hydrolysis was visualized by flooding iodine 24 hrs of incubation.

Qualitative evaluation of effect of different substrates concentration on enzyme activity

The selected isolate was grown in different concentrations 0.5%, 1%, 1.5% 2% and 2.5% of various substrates like CMC, filter paper, grass, cotton and cellulose at 37°C in an incubator for 48 hrs. After incubation, the CFF was separated. 300 µl CFF was loaded in well made in cellulose agar plates. Zone of hydrolysis was visualized by flooding iodine solution after 24 hours of incubation.

RESULTS

Characterization of isolated bacterial strain

Bacillus was isolated from sea water which is capable of producing cellulase.The colony appear to be light yellow, smooth, raised and translucent, on gram staining gram positive, rod shaped and scattered, and in

biochemical test TSI positive, MR test negative, Voges Proskauer positive, urease positive, catalase positive and fermentation of lactose, sucrose and glucose is indicative of Bacillus bacteria.

Effect of temperature on bacterial cell growth

Incubation temperature plays an important role in the metabolic activities of a micro-organism. In the present study, optimum temperature for culture growth was recorded at 50°C. When culture grows at 25°C to 50°C, after 24 hrs, the cell growth increased After 48hrs the cell growth decreases.

Effect of different substrates on cell growth

The effects of different substrates were studied on cell growth. The maximum cell growth was achieved with pure cellulose, when absorbance was measured in UV-Vis spectrophotometer at 546nm. While minimum bacterial growth was achieved in filter paper.

Effect of temperature on enzyme activity

CFF was placed at in a sterile eppendorf at 25°C, 37°C, 50°C and 80°C for 24hrs, to determine the optimum temperature for enzyme activity. After incubation, those incubated CFF were used in plate assay. The highest zones were obtained at 50°C while no zones appeared at 80°C, indicating that enzyme becomes inactive at 80°C.

Effect of different conc. of different substrates on cellulase activity

The máximum cellulase activity was recorded with 1.5% concentration of cellulose after 24 hours of incubation. Grass (2.5%) induced significant enzymatic activity. While 0.5% CMC gave least cellulase activity

CONCLUSION

Current study is useful for indutrial employment of bacterial cellulases in different industrial processes. It allows the use of agriculture waste for eco friendly production of cellulase through microbial fermentation.

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