International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 1025-1030
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

OPTIMIZATION OF CARBOXYMETHYL CELLULASE PRODUCTION FROM *HALOMONAS* SP. ISOLATED FROM SALTPAN

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ABSTRACT

Cellulases are glycoside hydrolases involved in the depolymerization of cellulosic materials and have wide applications in industries, such as in the textile, food and feed industry. In addition, supplementation of these enzymes with animal feed improves the digestibility of plant materials. The important application is the production of bioethanol, as a result of search of alternate sources for energy needs. In this study, carboxy methyl cellulase (CMCase) producing Halomonas sp. was isolated from saltpan. The culture conditions like temperature, pH, carbon source and nitrogen source were optimized. The optimum conditions for CMCase production were 35 °C at pH 9.0 with glucose as carbon source and yeast extract as nitrogen source. Halomonas sp. PV1 showed good CMCase activity and may be used in various industrial applications.

KEY WORDS

Carboxy methyl cellulase, Halotolerant, Optimization, Enzymes

INTRODUCTION

Cellulases (EC 3.2.1.4) have increased importance in various areas of agro-industrial bioprocesses including the improvement of quality of dough for various baked products, nutritional quality of animal feed, for cleaning and anti-re-deposition action in detergent industry, denim finishing and cotton softening in textiles industry [1]. These enzymes could be used as eco-friendly means of lignocellulosic waste conversion to various useful bio-products. In recent years, the commercial production of cellulases is mainly from fungi such as *Aspergillus* sp. and *Trichoderma* sp. which may have attained increased yield, having undergone many strain improvements over the years [2]. However, these commercial enzymes are still limited by the narrow substrate reaction, instability under industrial process dynamics and high cost of production [1]. In that context cellulolytic bacterial strains are continuously being screened from various environments to find good alternative producers of cellulases [3]. The genus *Bacillus* is well known to play significant roles in the degradation of organic matter during composting and is dominant producers of cellulolytic enzymes [4]. The *Bacillus* species including *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, B. *mojavensis*, *B. halodurans* and B. *circulans* have been widely reported as dominant producers of cellulases [5,6]. Moreover, investigation of cellulolytic bacteria from extreme environment, including, marine environment is increasing interest mainly due to their remarkable adaptation and versatility to heterogeneous environmental conditions [7]. Because of these

environmental conditions, these organisms are highly acclimatized to the dynamics of industrial processes.

Cellulose may be hydrolyzed using cellulases to produce glucose, which can be used for the production of organic acids, ethanol, and other chemicals. Cellulases catalyze the hydrolysis of 1, 4 β-D glycosidic linkages in cellulose are produced by fungi, protozoans and bacteria and have various range of commercial and industrial and applications [8]. Endoglucanases cleave at random at internal amorphous sites in the cellulose polysaccharide chain, generating oligosaccharides of various lengths and consequently new chain ends. This is generally soluble derivatives of cellulose such as CMC active against acid-swollen amorphous cellulose, cellooligosaccharides [9]. Further, extremophiles are the potential source for potent enzymes with unique activity ranges. Some of the enzymes have potential application in the bioconversion of renewable cellulosic biomass to sugars, especially for the production of ethanol by a fermentation process in industrial processes [10]. Cellulases from extremophiles have a very broad range of industrial applications. Some of the cellulases can be used in animal feed and as laundry detergents in textile industries. Halophilic cellulases are commercially importance and very little works have been carried out [11]. Production of enzymes can be enhanced by optimization of the physic-chemical and nutritional conditions including initial pH of culture media, fermentation temperature and agitation speed. Hence evaluating the optimal factors is of utmost importance for increased production of the cellulase [12]. In this study, the optimal conditions for enhanced production of cellulases by *Halomonas* strain, isolated from salt pan in India.

MATERIALS AND METHODS

Screening of cellulase producing bacterial strain

The cellulolytic enzyme producing *Halomonas* sp. was isolated from the salt pan and identified. This organism previously identified by 16S rDNA sequencing and used for the production of halotolerant proteases [13]. In this study, this potent halotolerant organism was subjected for the production of cellulase. Cellulase producing ability of the bacterial isolate was screened using the medium composed of (g/L): carboxymethylcellulose, 10.0; Na_2HPO_4, 4.0; tryptone, 2.0; KH_2PO_4, 4.0; $MgSO_4.7H_2O$, 0.20; $CaCl_2.2H_2O$, 0.001; $FeSO_4.7H_2O$, 0.004; agar, 15 and pH was adjusted to 8.0 using 1 N NaOH. These plates were incubated for 48 h at 37 ˚C. The plates were flooded with Gram's Iodine to visualize the cellulolytic activity of the bacterial isolate.

Production of cellulases in submerged fermentation

Production medium contained (g/L) peptone 0.75, glucose 0.5, $FeSO_4$ 0.01, $MgSO_4$ 0.5 and KH_2PO_4 0.5 gm. About 100 ml culture medium was taken in a 250 mL conical flask. The flask was sterilized at 121°C for 15 min, and after cooling, it was inoculated with overnight grown bacterial culture. Then, the inoculated medium was incubated at 37 °C in shaker incubator for 48 h. Further, the culture medium was centrifuged at 10000 rpm for 10 min to obtain the crude extract, which served as the source of enzyme.

Cellulase assay

Cellulase activity was measured following by standard method. Briefly, a reaction mixture composed of 0.1 ml of crude sample and 1.0 ml of 0.5% CMC in 0.05 M sodium phosphate buffer (pH 7.5). It was incubated at 37 ˚C and 3 ml DNS reagent was added. The colour was then developed by boiling the reaction mixture for 10 min and OD of the samples was measured at 540 nm against reagent blank.

Optimization of cellulase production by traditional method

Cellulase production by *Halomonas* sp. was optimized by varying the physical factors and nutrient sources.

Effect of temperature on enzyme production

The culture medium was prepared at pH 7.5 was inoculated with overnight grown *Halomonas* sp. The culture was incubated at various temperatures (35, 40, 45, 50, 55, and 60°C) for 48 h. After 48 h of incubation, the culture was centrifuged at 10,000 rpm for 10 min and cell-free culture filtrate was obtained.

Effect of pH on enzyme production

To evaluate the effect of pH on cellulase production, the pH of the culture medium was adjusted to 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0 in various Erlenmeyer flasks using 1 N HCl or 1 N NaOH and autoclaved. The culture was inoculated and incubated at 37 °C. After 48 h of incubation, the cell-free culture filtrate was obtained and used as crude enzyme.

Effect of carbon sources on cellulase production

Effect of carbon sources on cellulase production was studied by supplementing various carbon sources such as glucose, starch, maltose, lactose, sucrose and xylose at 1% (w/v) level each in the basal medium inoculated with 100 µl of culture strain. Culture medium without

any of the above carbon sources was considered as control. After 48 h of incubation, the culture was centrifuged and cellulase activity was assayed in the culture supernatant.

Effect of nitrogen sources on cellulase production

Effect of nitrogen sources on cellulase production was studied by supplementing various nitrogen sources such as ammonium sulphate, yeast extract, beef extract, peptone and oat meal at 0.5% (w/v) level each in the basal medium inoculated with 100 µl of culture strain. Culture medium without any of the above nitrogen sources was considered as control. After 48 h of incubation, the culture was centrifuged and cellulase activity was assayed in the culture supernatant.

RESULT AND DISCUSSION

The bacterial isolate was screened from the salt pan using CMC-agar plate. Initially the selected organism was screened for the production of protease and further subjected for the production of cellulases. It showed 5 mm zone on CMC agar plates (Fig. 1). Cellulase producing bacteria were isolated from various environments. In general, aerobic bacteria produce low levels of filter paperase, Avicelase, and β-glucosidase. Rastogi *et al*. [14] found that, *Geobacillus* sp. DUSELR7 and *Brevibacillus* sp. DUSELG12 were produced maximum filterpaperase activity. In a study, Soares *et al*. [15] found that only 9.1% of bacterial isolates were able to degrade Avicel on agar plates. Bacteria belonging to the genera *Cellulomonas*, *Clostridium*, *Thermomonospora*, *Cellulosimicrobium*, *Bacillus*, *Ruminococcus*, *Streptomyces*, and *Paenibacillus* have been observed to produce various kinds of cellulase when incubated under anaerobic or aerobic conditions [16]. Many studies have been carried out to elucidate the CMCase activity of aerobic bacteria. For example, a maximum CMCase activity of *Acinetobacter anitratus* was observed in the late logarithm phase [17].

Fig. 1 Effect of pH on cellulase production

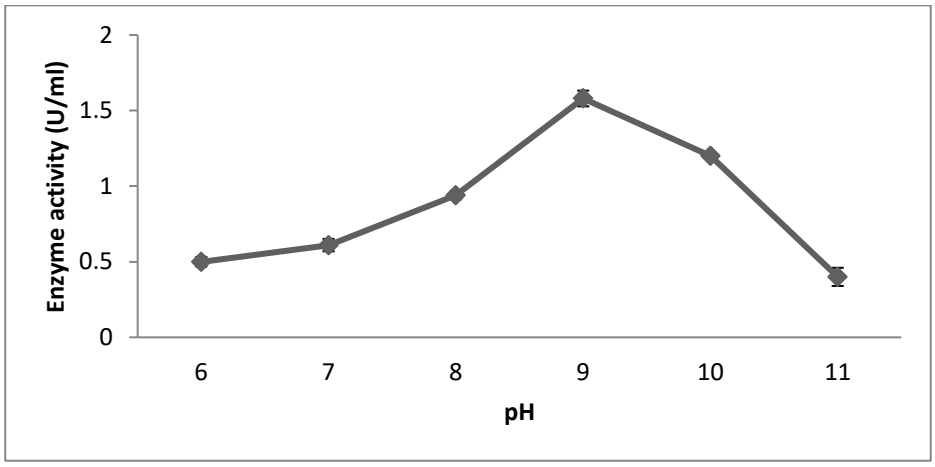


Fig. 2. Effect of temperature on cellulase production

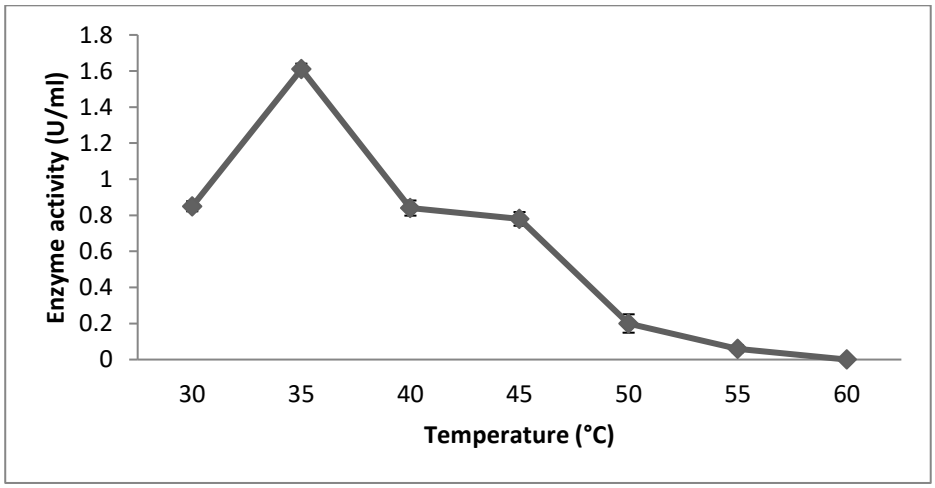
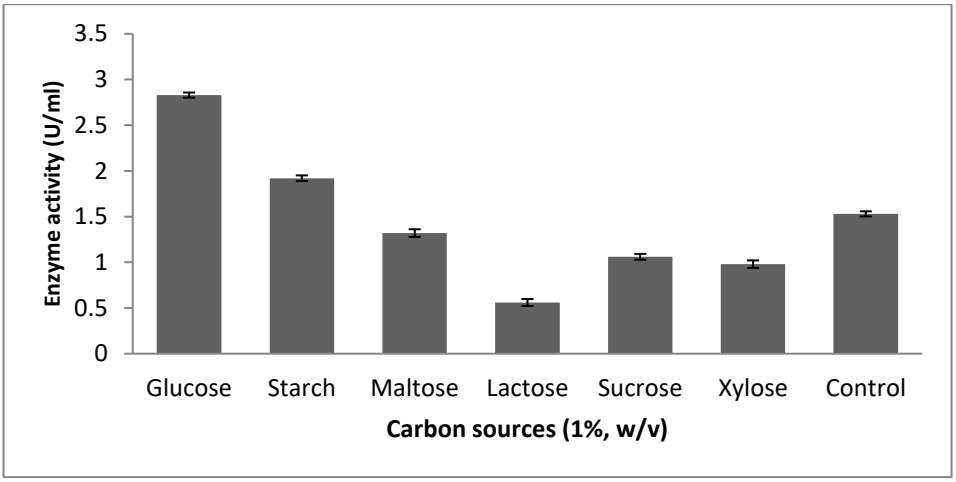


Fig. 3. Effect of carbon sources on cellulase production



Effect of pH

In the present study cellulase production was found to be maximum at pH 9.0. The selected bacterial isolate was allowed to grow in media of various pH values ranging from 8.0 to 10.0. This result was in accordance with the finding of other workers for various bacterial strains [18, 19]. An optimum pH is required to maintain the three-dimensional shape of the active site of enzyme and the change in pH value results in loss of functional shape of enzyme due to alteration in the ionic bonding of enzyme.

Effect of temperature on cellulase activity

Enzyme activity recorded at various temperatures showed that *Halomonas* yielded maximum cellulase production at 35 °C (Fig. 2). The temperature is one of the critical factors and was found to influence extracellular enzyme secretion, possibly by changing the physical properties of the cell membrane. These results are similar those of Bakare *et al*. [20] who found that the cellulase enzyme produced by *Pseudomonas fluorescence* was high at 35 °C. The optimum temperature for the production of cellulase by *Bacillus subtilis* 115 and *Bacillus subtilis* was found to be 40 °C [21]. Ray *et al*. [22] reported that maximum yield was obtained at 40 °C and 45°C by *Bacillus subtilis* and *Bacillus circulans*, respectively.

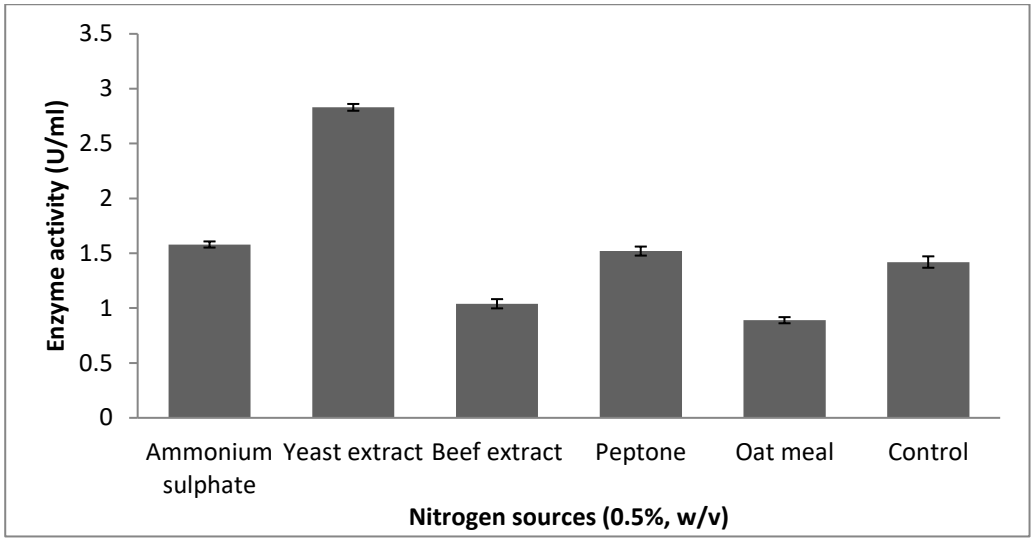
Effect of carbon source

Various carbon sources such as glucose, starch, maltose, lactose, sucrose and xylose were used. In the present study, glucose significantly enhanced the production of cellulase after 48 h of incubation at 35 °C. Starch and maltose also showed high cellulase production at 48 h of incubation. Ishihara *et al*. [23] studied the utilization of D-xylose as carbon source for the production of cellulase. Ramana *et al*. [24] stated that glucose, sucrose, and mannitol were suitable for optimum levels of cellulase production. Wei *et al*. [25] studied on cellulase production by *Bacillus* sp. from terrestrial sources and found cellulose as the good carbon source, while xylan proved to be the suitable carbon source for cellulase production from *Bacillus cereus* MRK1 [26].

Effect of nitrogen source

Production of cellulase has been shown to be sensitive to repression by different nitrogen and carbohydrate sources. Among the nitrogen sources yeast extract significantly enhanced the production of cellulase (Fig. 4). This result is in accordance with a report on cellulase production by other bacterial species. Kumar *et al*. [26] suggested that yeast extract is the best nitrogen source for cellulase production. In another study, peptone was reported as the best nitrogen source for cellulase production [27]. Likewise, another report suggested yeast extract as nitrogen source for cellulase production from fungal isolate, *T. viride* [28]. Casein and peptone were reported as good nitrogen sources for cellulase production from the marine bacterium *Marinobacter* sp. MSI032 [29]. These findings suggest that yeast extract, ammonium sulphate, and peptone may be preferred source to enhance the production of cellulase.

Fig. 4. Effect of nitrogen sources on cellulase production



CONCLUSION

Cellulases find great application in various industrial sectors. It is mainly employed in detergent industry, textile industry, animal feed and fuel production from biomass. With increase in demand, novel activity, its higher yield, and economical production are highly desirable. This study reports optimized production of carboxy methyl cellulase from the salt pan isolate, *Halomonas* sp. Enzyme production was found to be maximum, when this organism was grown at 35 °C, pH 9.0, glucose and yeast extract. This enzyme can be used for various purposes in detergent industries, pharmaceutical industries and food industries. The high enzyme activity between alkaline and neutral pH and high temperature will be of use in various biotechnological and industrial applications.

ACKNOWLEDGEMENT

Authors greatly acknowledged Smykon Biotech Pvt. LtD, Nagercoil 629 001, Kanyakumari District, Tamilnadu, India for laboratory facilities.

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Received:08.05.18, Accepted: 09.06.18, Published:01.07.2018

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