

Partial Purification and Characterisation of Carboxymethyl Cellulase from *Bacillus sp.* Isolated from Sugar Cane Baggase (SCB) Dump Site

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Abstract

Carboxymethylcellulase (CMCase) was extracted from *Bacillus sp.* isolated from sugar cane baggase dump site. The enzyme was partially purified using ammonium sulphate precipitation and Sephadex G-200 gel and characterized at various pH and temperature values. The optimum pH of the purified enzyme was pH 6.5 and its temperature optimum was 50°C. The Michaelis-Menten constants, K_m and V_{max} , of the enzyme using carboxymethyl cellulose as substrate were found to be 5 and 0.89 $\mu\text{mol}/\text{min}$ respectively.

Key words: Agricultural residues; carbon sources, nitrogen sources; pH activity; temperature activity

Introduction

Lignocellulosic (agro-industrial waste) waste forms a large proportion of solid waste in our cities, thus constituting an environmental problem. Agro-industrial wastes and byproducts are renewable form of resources generated round the year all over the world. Wheat, rice bran, sugar cane bagasse, corn cobs, citrus and mango peel are some of the important wastes of food industries and are accumulated in enormous amounts and could serve as source of environmental pollution (GOP, 2001).

Studies have shown that conventional waste treatment such as chemical hydrolytic strategies have failed to ameliorate this problem (Jaafaru, 2013). Chemical hydrolysis of lignocellulose is accompanied with the formation of toxic components that are harmful to the environment (Ninawe and Kuhad, 2005). The use of microbial enzymes in lignocellulosic waste treatment has been shown to be an alternative that is efficient and cost effective (Jaafaru, 2013). Conversion of agricultural wastes to useful products is also an attractive option as a remedy for air pollution, energy production and other environmental concerns (Doran *et al.*, 1994).

Another significant application of agro-industrial wastes such as sugar cane bagasse and wheat bran is the biotechnological production of enzymes such as cellulases, especially carboxymethyl cellulase, for their food applications. There is an increasing tendency among the people to use chemical free foods. The use of enzymes (cellulases) in food processing could meet such public demands (Pandey *et al.*, 2002).

Cellulose, the major structural polysaccharide of plants, is a hydrophilic linear glucose polymer with the units bonded by β -1, 4-glucosidic linkage. The depolymerization of cellulose into monomeric sugars either by strong acids or by commercial enzymes remains a major economic problem, which has stimulated investigations into the structure and function of microbial cellulases and development of improved strains with increased cellulase

production (Jamil *et al.*, 2003). Cellulose biodegradation refers to the breakdown of cellulose into its component glucose units through the action of enzymes (Jamil *et al.*, 2003).

The most studied are the extra cellular cellulase systems in bacteria that have three components: Endo-glucanases (EC 3.2.1.4), cellobio-hydrolases (3.2.1.9.1) and β -glucosidases (EC 3.2.1.2.1) (Coughlan and Ljungdahl, 1988). Endo-glucanases, also referred to as CM-cellulases, attack an amorphous cellulose by random cleavage of β -glycosidic linkages.

Carboxymethylcellulase (endo-glucanase) converts the polymeric form of cellulose into oligosaccharide form, then cellobiohydrolase (Exoglucanase) separates cellulose by acting on non-reducing end. CMCases (Endo- β glucanase) have great potential for its utilization in the food industry. It can be used in bread production to increase loaf volume and maintain freshness, brewery and wine biotechnology, extraction and clarification of juices, production of fruit nectars and purees to improve the hedonic response (Harada *et al.*, 2005). Presently little efforts are being made to prepare the hydrolytic enzymes like carboxymethyl cellulase by fermenting the agricultural wastes/byproducts through microorganisms. For cost effectiveness, the best possible solution may be the utilization of the indigenous, cheaper and underutilized substances (SCB) as substrate for the production of this valuable enzyme and then its further application in various food preparations. This communication is aimed at purification and characterization of CMCase from *Bacillus sp.*, using sugarcane baggase as carbon source.

Materials and Methods

Soil sample (20 g) was collected from a sugar cane baggase (SCB) dump site in Nsukka, Enugu State, Nigeria. Ten bacterial cultures were screened for carboxymethylcellulase (CMCase) activity. The isolates were identified based on the basis of morphological, cultural and biochemical properties according to the method of Sneath (1994).

The culture was maintained as described by Kamble and Jadhav (2012). The bacterial isolate was maintained in solid medium in basal salt solution (BSS) containing 1% carboxymethylcellulose (CMC) at pH 7.5 and stored at 4°C.

The selected strain was tested for its ability to produce extracellular CMCase under solid state fermentation (SSF). Sugar cane baggase (SCB) was used as the substrate. The strain was cultured in an Erlenmeyer flask (500ml) containing 10g of SCB. After 72hr, the solid substrate was removed and suspended in 100mM sodium phosphate buffer (pH 7.5). The content was then centrifuged at 4000rpm for 60min. the cell-free supernatant was filtered through Whatman No. 1 and the filtrate used as crude enzyme.

Carboxymethylcellulase (CMCase) Assay: The activity of CMCase was measured according to Ghosh (1987). An assay medium containing 900 μ l of 1% CMC solution was added with 100 μ l enzyme in a test tube. DNS reagent (1.5ml) was added and incubated at 50°C for 5min in an automated water bath. The absorbance was measured at 540nm. One unit of CMCase activity was defined as the amount of enzyme that liberates 1 μ mol of glucose equivalents per minute under the assay conditions.

Protein content estimation: Total protein content was measured according to Bradford (1976). Bovine serum albumin was used as standard protein.

Partial Purification of CMCase: Ammonium sulphate crystals was added to the supernatant and incubated for 12 hr, precipitate was centrifuged at 4000 rpm for 60 min and resuspended in 100mM sodium phosphate buffer pH 7.5.

Sephadex G-200 Gel Filtration: The precipitated solution was loaded on Sephadex G-200 column (25cm x 1.7cm).

Characterization of Partially Purified Enzyme: The optimum temperature of the partially purified enzyme was determined at different temperatures ranging from 30 - 100. Also, the optimum pH of the enzyme was studied at 100 mM sodium acetate (3.5 – 5.5), Tris-HCl buffer (8 – 10.5) and sodium phosphate buffer (6 – 7.5).

Substrate Concentration Study: The effect of substrate concentration on the activity of CMCase was determined using 1% CMC as substrate. The V_{max} and K_m values of the enzyme were determined using Lineweaver-Burk plot.

Results and Discussion

Twelve bacterial strains which showed maximum growth and enzyme production on SCB agar plates isolated from soil collected at SCB dump site were

picked for further studies. The identification of these bacterial isolates was based on cell morphology, colony morphology and other physiological and biochemical tests.

After CMCase incubation with ammonium sulphate, 60% saturation gave the highest CMCase activity as represented in Fig. 1. Fractions of 35ml were collected. This result is in agreement with the works of Zhang *et al.* (2009) and Minghal *et al.* (2012).

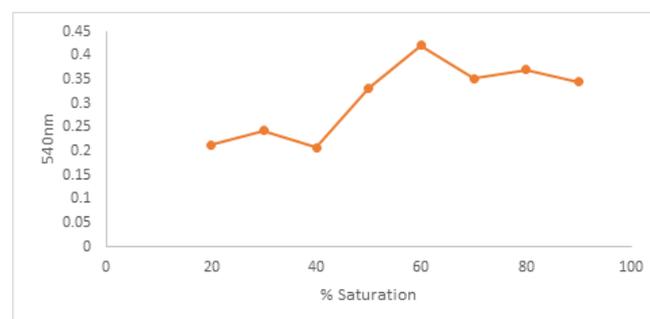


Fig. 1: Ammonium sulphate profile

The optimum pH for the activity of CMCase cultured on SCB was pH 6.5 as shown in Fig. 2. At less pH values, gradual increase in the enzyme activity was noticed until its peak of 6.5. This was preceded by a sharp decline in the enzyme activity at higher pH values. This could be attributed to denaturation caused by the very alkaline solution. Odeniyi *et al.* (2009), obtained similar result from *Bacillus coagulans* cultured on palm fruit industrial residues, and Dhillon *et al.* (1985) obtained a similar result from *Bacillus licheniformis*-1. This suggests that *Bacillus sp* acts best at near neutral pH.

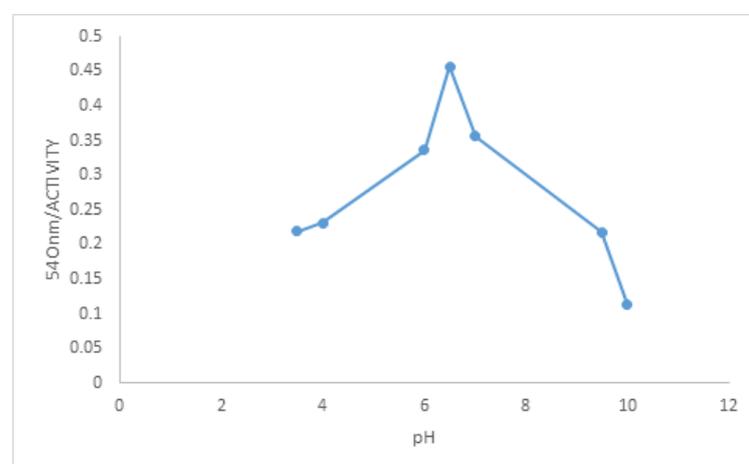


Fig. 2: Effect of pH on CMCase activity

The effect of temperature on CMCase was studied. Optimum temperature was observed at 50°C as shown in Fig. 3. Subsequent increase in temperature produced a gradual decrease. This

decrease could be as a result of enzyme denaturation caused by high temperature. The report of Vijayaraghavan and Vincent (2012) on their study of *Bacillus sp* from a paddy field has 50°C as its optimum temperature. Mawadza *et al.* (2000), obtained a similar result from *Bacillus* CH43 and HR68.

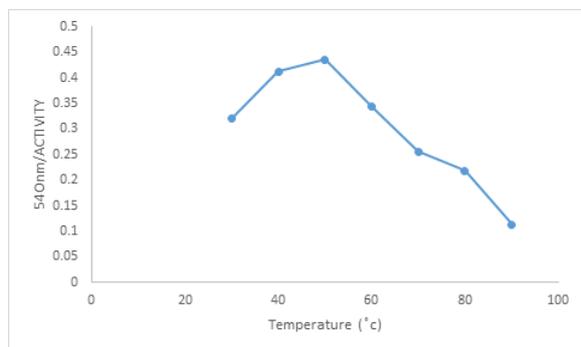


Fig. 3: Effect of temperature on CMCase activity

The substrate concentration of 0.6mg/ml has the highest CMCase activity as shown by Fig. 4. The kinetic parameters K_m and V_{max} of 5.0 μ g/min/ml and 0.89 μ mol/ml respectively were deduced from Lineweaver-Burk (Fig. 5) plot at 50°C. The findings of Bansod *et al.* (1993) and Nakamura *et al.* (1995) were in the ranges of 0.5 and 19.6 mg/ml and 260 - 350 μ mol/min/ml.

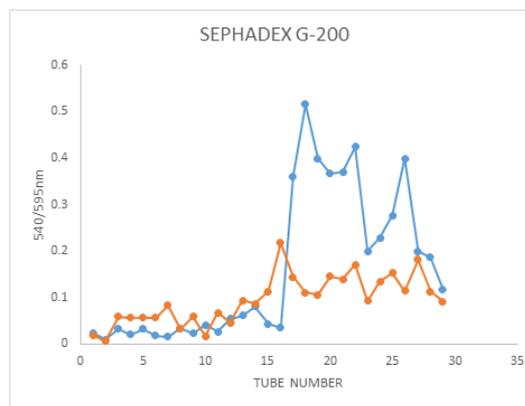


Fig. 4. Sephadex G-200 elution profile

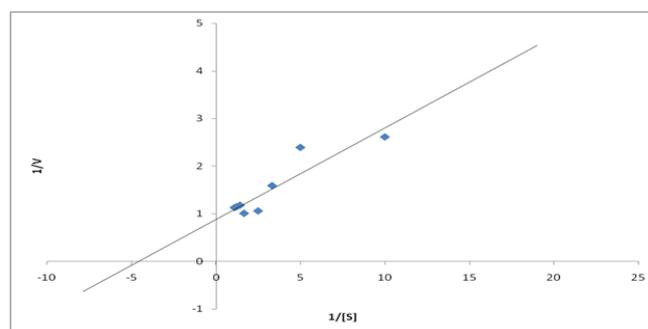


Fig. 5: Lineweaver Burk plot

Table 1: Purification table of CMCCase

Purification Steps	Protein Conc. (μ g/ml)	Activity (U/Min)	Specific Activity (U/mg)	Total Activity (U)	% Yield	Purification Fold
Crude Enzyme	106	90	0.18	45000	100	1
Ammonium Sulphate Precipitation	173	143	1.10	18590	16.36	6.11
Sephadex G200 Filtration	36	233	7.06	7689	15.58	39.22

There is reduction in total protein from the crude values of 106 to 36 μ /ml after purification, this could be due to the removal of some unwanted proteins. Also, there was an increase in the specific activities of the enzyme to 7.06U/mg (Table 1) after the purification step. This was probably because some biomolecules interfering with the enzyme activity were removed during the purification process.

Conclusion

The results of this research show that CMCCase obtained from *Bacillus sp* cultured on SCB could be effective in the hydrolyses of lignocellulose rich source such as sugar cane baggase at mild laboratory conditions of 50°C and pH 6.5. Sugar cane baggase (SCB), an agro-waste that was used in this work is a waste product that pollutes the environment. It is could be used industrially for the production of CMCCase from *Bacillus sp*. This would

help in cleaning the environment and lead to the reduction in the cost of the enzyme production.

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