Optimization of Conditions and Production of Carboxy Methyl Cellulase by Bacteria Isolated from Higher Termite Soil

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Abstract

The current work deals with the studies of isolation and preliminary characterization of the bacteria isolated from higher termite soil. Termites play an important role in the turnover and mineralization of complex biopolymers, such as wood and other cellulose and hemicelluloses containing materials. Lignocellulose is the most predominant component of the woody and dead plant materials, as well as it is the most abundant biomass on earth, especially in terrestrial ecosystems. Thus the work focuses on using lingo cellulose waste efficiently for production of Carboxy Methyl Cellulase (CMC). As CMC has higher hydrolyzing capacity than the other two cellulases, cellobiase and cellobiohydrolases, it has wide applications in industries such as food, baking industry, laundry etc. Thus the work focuses on identification of efficient bacterial species in this work 6 bacterial species were isolated among which I4 produced higher CMC enzyme. After the morphological, biochemical and identification test, the isolate was found to be Bacillus sp.

Keywords: Carboxy methyl cellulase (CMC); Cellulase

Introduction

Termites are a ubiquitous feature of tropical and subtropical soils, where their number exceeds 6000 m⁻² and their biomass densities (>50 g m⁻²) often surpass those of grazing mammalian herbivores (0.0-7.5 g m⁻²). Both higher and lower termites have microbes and enzymes in their hindgut, and this is therefore where the most symbiosis occurs. Soil on the other hand is a highly heterogeneous environment [1] that contains a high diversity of microorganisms [2].

These microorganisms influence above ground ecosystems by contributing to soil structure and fertility among other roles [3]. Soil microorganisms are a valuable source of natural products providing important antibiotics for pharmaceuticals, enzymes and bioactive compounds for industries [4]. Since soil being a good habitat for the growth of many number of microorganisms, majorly observed microorganisms are bacteria: Bacillus sp., Klebsiella sp., Pseudomonas sp, Serratia sp, Xanthomonas sp etc. Many fungal species are also obtained from higher termite soil, which include Aspergillus sp., Phoma sp., Neurospora sp, Trichoderma sp, Penicillium sp. The major actinomycetes species observed are Streptomyces sp, Geosmin sp, Nocardia sp. Cellulase consists of three different types of enzymes named as endoglucanases, exoglucanases and cellobiases. Several novel enzymes capable of degrading cellulose into sugars have insights from this discovery to create a high performance enzyme cocktail for processing plant biomass into biofuel.

Two cellulases, endoglucanase (CMCase), exoglucanases or β-1,4 glucan hydrolases enzymes were first reported to exist in termite mycetes conidiophores of the “fungus garden” . Bacterial cellulases have proved to be a better candidate than other microbial cellulases, with their secreted free cellulose complexes comprising all three components of cellulose. Plant cell walls are the most abundant renewable sources of fermentable sugars on the earth [5] and are the major reservoir of fixed carbon in nature. The major components of plant cell walls are cellulose, hemicellulose and lignin, with cellulose being the most abundant component. But plant woods commercially used for the production of paper. So an alternative substrate is required for production of cellulase enzyme. Rice straw, wheat straw, rice bran, wheat bran, when used as substrate there are some bacterial species mainly considered pathogen and degrades these substrates easily.

Several studies were carried out to produce cellulolytic enzymes from bio waste degradation process by many microorganisms including fungi such as Trichoderma, Penicillium, Aspergillus spp. etc. by Mandels M et al., Hoffman RM et al., Brown JA et al., Lakshmi Kant et al. [6-9] etc. Similarly cellulolytic property of bacterial species like Pseudomonas, Cellulomonas, Bacillus, Micrococcus and Cellovibrio sp were also reported. The specific cellulolytic activity shown by the bacterial species is found to be depending on the source of occurrence [10]. Some features of natural cellulose materials are known to inhabit their degradation or bioconversion [11,12]. These are degree of crystallinity, lignification and the capillary structure of cellulose to cellulolytic enzymes and other hydrolytic agents. However, many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulose materials have been reported [12,13]. Since the production of cellulase enzyme is a major process and economically viable, much work has been done on the production of cellulase from lignocellulosics and major attention has been given to use bagasse as substrate [12,13]. The bioconversion of various complex cellulose waste materials such as bagasse [13], corn cob [14]; saw dust [12] have been reported (Figure 1).

Materials and Methods

Sample collection

The higher termite soil sample was collected from three different regions of Palakkad district (Nelliyampati, Kallepully, Chittur). The samples were collected, serially diluted and was spread plated.
Isolation of bacteria from higher termite soil

The soil samples were isolated and were spread plated from the three soil samples. The six Colonies with visibly distinguishable morphologies were selected and restreaked on Nutrient agar to obtain pure cultures isolates were labeled as I1, I2, I3, I4, I5, I51, I52 (Tables 1 and 2).

Identification of bacteria

Bacteria were identified and classified based on their physical and biochemical characteristics. Various biochemical test such as IMViC, TSI, Carbohydrate fermentation, Catalase, oxidase test, starch hydrolysis test were performed to identify the bacterial species (Tables 3-5).

Screening

The six isolates were inoculated by spot inoculation and incubated at 24-48 hrs. 1% of Congo red solution was added to the spot inoculated plates and then excess stains were removed by using 1M NaCl and zone of clearance were observed as in Figure 1.

Optimization of physiological condition

Cellulases have versatile applications in textile, laundry, pulp and paper, fruit juice extraction, and animal feed additives [15]. In addition, they find use in saccharification of lignocellulosic agro residues to paper, fruit juice extraction, and animal feed additives [15]. In addition, there were reports that the cellulase production by Aspergillus niger and bacterial strains such as Cellulomonas sp was observed over a wide range of temperatures between 30 to 50°C [19,20]. In the current research since the I4 isolate utilized the Carboxy methyl cellulose during the screening test. The isolates was further analysed for the optimization: Temperature and pH.

Effect of pH

For estimation of optimum pH, the enzyme activity was carried out at five different pH (4, 5, 6, 7, 8). In order to find the effect of pH also 0.5 ml of Carboxymethyl cellulose is used as substrate.

Effect of temperature

For estimation of optimum temperature, the enzyme activity was carried out at five different temperatures (20°C, 30°C, 40°C, 50°C and 60°C) using 0.5 ml of Carboxymethyl cellulose substrate.

Results and Discussion

The three samples were collected from higher termite soil. Serially diluted higher termite soil sample showed distinct colonies at 10^4 dilution. The selected colonies were labeled as I1, I2, I3, I4, I5, I51, I52, and was subjected to physical and biochemical characterization, the results were tabulated in Tables 1 and 2.

The Figure 1 shows the specific screening and CMCase assays. After the preliminary screening the I4 isolate was found to be more efficient for the CMCase activity. Optimization of physiological conditions (pH and temperature) were analyzed as in Figures 2-5, thus I4 isolate at temperature 40°C at pH 6 was more efficient as in Figures 6-9. In addition there were reports that the cellulase production by Aspergillus niger and bacterial strains such as Cellulomonas sp was observed over a wide range of temperatures between 30 to 50°C [19,20]. In the current research since the I4 isolate utilized the Carboxy methyl cellulose during the screening test. The isolates was further analysed for the optimization: Temperature and pH.

Table 1: Enumeration of bacterial colonies.

| Serial dilution | Colonies obtained (one quadrant) | Colonies obtained (four quadrant) |
|-----------------|---------------------------------|----------------------------------|
| 10-1            | 525                             | 2100                             |
| 10-2            | 326                             | 1304                             |
| 10-3            | 245                             | 980                              |
| 10-4            | 200                             | 800                              |
| 10-5            | 163                             | 652                              |
| 10-6            | 113                             | 452                              |
| 10-7            | 88                              | 392                              |

Figure 2: For estimation of optimum temperature, the enzyme activity was carried out at five different temperatures (20°C, 30°C, 40°C, 50°C and 60°C) using 0.5 ml of Carboxymethyl cellulose substrate for 5 days.

Figure 3: For estimation of optimum temperature, the enzyme activity was carried out at five different temperatures (20°C, 30°C, 40°C, 50°C and 60°C) using 0.5 ml of Carboxymethyl cellulose substrate for 10 days.

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Table 2: Physical Characterization.

| S No | Isolate | Size | Shape | Colour | Margin | Surface | Elevation | Transparency | Viscosity |
|------|---------|------|-------|--------|--------|---------|-----------|-------------|-----------|
| 1.   | I1      | Large| Undulate| Yellow | Undulate| Smooth | Flat      | Opaque      | Moist     |
| 2.   | I2      | Medium| Circular| Light yellow| Entire | Smooth | Flat in growing | Transparent | Moist     |
| 3.   | I3      | Small| Circular| Light white| Entire | Smooth | Low convex | Translucent | Dry       |
| 4.   | I4      | Large| Circular| Creamy| Entire | Smooth | Flat in growing | Opaque      | Moist     |
| 5.   | I5₁    | Small| Circular| White | Diffuse | Finely granular | Flat | Opaque | Ropy      |
| 6.   | I5₂    | Small| Irregular| White | Diffuse | Glossy | Flat | Opaque | Mucoidal  |

Table 4: Carbohydrate Fermentation Test.

| S No | Bacterial isolates | Sucrose | Glucose | Mannitol | Inositol |
|------|-------------------|---------|---------|----------|----------|
| 1.   | Isolate 1         | Ap/NG   | Map/NG  | Map/NG   | Map/NG   |
| 2.   | Isolate 2         | Ap/NG   | Map/NG  | Map/NG   | Ap       |
| 3.   | Isolate 3         | Ap/NG   | Map/NG  | Map/NG   | Map/NG   |
| 4.   | Isolate 4         | Ap/NG   | Nc      | Ap/NG    | Map/NG   |
| 5.   | Isolate 5₁        | Map/NG  | Map/NG  | Map/NG   | Map/NG   |
| 6.   | Isolate 5₂        | Ap/NG   | Map/NG  | Map/NG   | Map/NG   |

Abbreviations: Ap/NG: Acid production/No gas; NG: No gas; Map: Minor acid production; Ap: Acid production; Nc: No change

Table 5: Tripe sugar iron test.

| S No | Name | Size | Shape | Colour | Margin | Surface | Elevation | Transparency | Viscosity |
|------|------|------|-------|--------|--------|---------|-----------|-------------|-----------|
| 1.   | I1   | Large| Undulate| Yellow | Undulate| Smooth | Flat      | Opaque      | Moist     |
| 2.   | I2   | Medium| Circular| Light yellow| Entire | Smooth | Flat in growing | Transparent | Moist     |
| 3.   | I3   | Small| Circular| Light white| Entire | Smooth | Low convex | Translucent | Dry       |
| 4.   | I4   | Large| Circular| Creamy| Entire | Smooth | Flat in growing | Opaque      | Moist     |
| 5.   | I5₁  | Small| Circular| White | Diffuse | Finely granular | Flat | Opaque | Ropy      |
| 6.   | I5₂  | Small| Irregular| White | Diffuse | Glossy | Flat | Opaque | Mucoidal  |

Figure 4: For estimation of optimum temperature, the enzyme activity was carried out at five different temperatures (20°C, 30°C, 40°C, 50°C and 60°C) using 0.5 ml of Carboxymethyl cellulose substrate for 15 days.

Figure 5: For estimation of optimum temperature, the enzyme activity was carried out at five different temperatures (20°C, 30°C, 40°C, 50°C and 60°C) using 0.5 ml of Carboxymethyl cellulose substrate for 20 days.

Figure 6: The best pH for enzyme activity was determined by enzyme assay at different pH levels (4, 5, 6, 7, 8). In order to find the effect of pH also 0.5 ml of carboxymethyl cellulose is used as substrate for 5 days.
suggestion that Bacillus sp at specific pH and temperature were found to be more potential.

References

1. Daniel R (2004) The soil metagenome—a rich resource for the discovery of novel natural products. Curr Opin Biotechnol 15: 199-204.

2. Liesack W, Janssen PH, Rainey FA, Ward-Rainey NL, Stackebrandt E (1997) Microbial diversity in soil: The need for a combined approach using molecular and cultivation techniques. In: Modern Soil Microbiology. Elsas JD, Trevers JT, Wellington EMH (Eds.). Marcel Dekker, New York, USA, pp: 375-439.

3. O’Donnell AG, Seasman M, Macne AN, Davies T (2001) Plants and fertilisers as drivers of change in microbial community structure and function in soils. Plant and Soil 232: 135-145.

4. Strohl WR (2000) The role of natural products in a modern drug discovery program. Drug Discov Today 5: 39-41.

5. Himmel ME, Ruth MF, Wyman CE (1999) Cellulase for commodity products from cellulolytic biomass Curr Opin Biotechnol 10: 358-364.

6. Mandels M, Reese ET (1999) Fungal cellulases and the microbial decomposition of cellulosic fabric. J Ind Microbiol Biotechnol 22: 225-240.

7. Hoffman RM, Wood TM (1985) Isolation and partial characterization of a mutant of Penicillium funiculosum for the saccharification of straw. Biotechnol Bioeng 27: 81-85.

8. Brown JA, Collin SA, Wood TM (1987) Development of a medium for high cellulase, xylanase and β-glucosidase production by a mutant strain (NTG II/6) of the cellulolytic fungus Penicillium pinophilum. Enzyme Microbial Technol 9: 355-360.

9. LakshmiKant, Kamal, Mathur SN (1990) Cellulolytic activities of Chaetomium globosum on different cellulolytic substrates. World J Microbiol Biotechnol 6: 23-26.

10. Saxena S, Bahadur J, Varma A (1993) Cellulose and hemicellulose degradation bacteria from termite gut and mound soils of India. Int J Microbiol 33: 55-60.

11. Solomon BO, Layokun SK, Nwesigwe PK, Oluwole PO (1990) Hydrolysis of saw dust by cellulase enzyme derived from Aspergillus flavus Linn. Isolates NSPR 101 beyond the initial fast rate period. JNISCHE 9: 1-2.

12. Solomon BO, Amigun B, Betiku TV, Ojumu T, Layokun SK (1999) Optimization of cellulase production by Aspergillus flavus Linn. isolates NSPR 101 grown on bagasse. Journal of Nigerian Society of Chemical Engineers 16: 61-68.

13. Kanosh AL, Essant SA, Zeitn AM (1999) Biodegradation and utilization of bagasse with Trichoderma reesi. Polyon Degrad Stab 62: 273-276.

14. Ojumu T, Solomon V, Bamidele O, Betiku E, Layokun SK, et al. (2003) Cellulase production by Aspergillus flavus Linn Isolate NSPR 101 fermented in sawdust, bagasse and corn cob. African J Biotechnol 2: 150-152.

15. Bhat MK (2000) Cellulases and related enzymes in biotechnology. Biotechnol Adv 18: 355-383.

16. Sánchez OJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. Biosourc Technol 99: 5270-5295.

17. Jo KD, Lee YJ, Kim BK, Lee BH, Chung CH, et al. (2008) Pilot-scale production of carboxymethylcellulase from rice hull by Bacillus amylolequakens DL-3. Biotechnol Bioprocess Eng 13: 182-188.

18. Chipeta ZA, du Preez JC, Christopher L (2008) Effect of cultivation pH and agitation rate on growth and xylanase production by Aspergillus oryzae in spent sulphite liquor. J Ind Microbiol Biotechnol 35: 587-594.

19. Jaradat Z, Dawarneh A, Jabbaneh Q, Aalasoun I (2008) Influence of culture conditions on cellulase production by Streptomyces sp. (Strain 2J). Jordan J Biol Sci 1: 141-146.

20. Milala MA, Shugaba A, Gidado A, Ene AC, Wafar JA (2005) Studies on the use of agricultural wastes for cellulase enzyme production by Aspergillus niger. Res J Agric Biol Sci 1: 325-328.