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Research Article

ANALYSIS OF GUT FLORA FROM DAMP WOOD TERMITES (*TRINERVITERMES* SPP.) AND EXTRACTION, CHARACTERIZATION OF CELLULASE FROM THE ISOLATE

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ABSTRACT

Objective: The objective of the study was to isolate, characterize, identify, and compare the potentials of cellulolytic strains isolated from the gut of damp wood termites (*Trinervitermes* species) collected from Chennai, Tamil Nadu, India.

Methods: Termites were collected and used as hoard of cellulase producers and screened for the cellulase producers using carboxymethyl cellulose as the sole source of carbon and were authenticated using Congo red plate screening method. The isolates showing a significant zone of clearance were further confirmed by biochemically characterized.

Results: Nine effective isolates were characterized and three strained were used for analysis. The organisms were subjected with substrate, temperature (25°C, 37°C, and 45°C), and pH to optimize the cultural condition. The enzyme activity was estimated using endoglucanase, FPase after incubating at appropriate conditions. Five isolates showing a significant zone of clearance were selected, out of which three belonged to *Bacillus* and one each to *Staphylococcus*. Optimization of media and genetic modification of the strains can further convalesce their competence. All the isolates have ensuring application in view of use in future.

Conclusion: An effective strain of bacteria was isolated from the gut of termites can be used as a potential candidate for the production of cellulase in industries.

Keywords: Trinervitermes, Cellulase, Lignocellulose material.

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INTRODUCTION

Termites are the most copious terrestrial insects in many damp and dry areas of tropical and subtropical ecosystems [1-3]. They are called as "white ants;" however, they differ from ants in morphological and phylogenetical characters [4]. Termites are polymorphic insects, living in large groups of some hundred to several million individuals, comprised reproductive (Winged) forms together with numerous sterile soldiers and workers [5,6]. All termites live in colonies within the confines of excavations within wood above-ground, or in subterranean and epigeal nest systems [7]. Termites portray an immense role in terrestrial ecosystem by recycling biomass containing the mixture of cellulose, lignin, and hemicellulose [8-10]. Termites are soil insects that efficiently decays lignocellulose with the aid of the gut-associated microbes to simplify carbohydrate that later fermented to ethanol using fungi.

They are a key functional group of animals in the tropics and can achieve high dense population. Their numerous colonies have great influence in ecosystems.

METHODS

Collection of termites

Damp wood termites were collected and identified as *Trinervitermes* spp. based on their shape and parts. The termites were surface washed with distilled water further dried on a filter paper. The surface sterilization was performed with 70% ethanol. The head was trimmed off, and the entire gut was removed with sterile forceps and mixed in a mortar and pestle [11].

Isolation of microbes from termite gut

The guts were homogenized in 1 ml of sterile distilled water, centrifuged at 10,000 rpm for 5 minutes to remove large gut debris. The supernatant was serially diluted and spread plate method was performed on nutrient agar, agar and plate count agar. Colonies were picked up from the agar plates after incubating at 37°C overnight and inoculated into respective broths [12].

Isolation and generic level identification

From the resultant Petri plate of enumeration study of crushed mixture of termite, nine types of bacterial colonies were obtained. These colonies were isolated and cultured in individual Petri plates [13].

For proper characterization, the colonies were labeled as NSP_1, NSP_2,.... NSP_9. Standard procedures of bacterial identification such as gram staining, spore staining, capsule staining, motility, IMViC, and other essential tests were carried out to identify bacteria at least up to the generic level [14].

Screening of bacteria for cellulase production

Enzyme production

The isolated organisms were inoculated in 150 ml Erlenmeyer flask which contains 30 g of cellulose initially the moisture content of the substrate is determined. The minimal salt medium was prepared with the following content (g/L), and the pH of the mineral salt medium (MSM) is adjusted before sterilization [15].

Ammonium sulfate - 10 g, potassium phosphate - 3 g, magnesium sulfate - 0.5 g, calcium chloride - 0.5 g, yeast extract - 7 g, glucose (dextrose) - 15 g. The MSM was prepared and sterilized [16].

Congo red method
The different bacterial species were inoculated in the minimal medium and were kept in the shaker for 5-7 days. The medium was then filtered and centrifuged at 10,000 rpm for 15 minutes. The agar was prepared with the following composition and autoclaved at 121°C for 30 minutes. The medium was poured in the sterile Petri plates and left for 15 minutes. 20µl of the supernatant of centrifuged bacterial medium was added to the wells made in plates using micro pipette. These were incubated at 25-30°C for 72 hrs. The Petri plates were logged with 1% of Congo red and left for 15 minutes. The detaining of the plates was done with 1 M NaCl solution for 15 minutes. The zone of clearance was observed for the cellulose hydrolysis surrounding the colonies [17].

Assay of cellulase
Endoglucanase assay was performed by incubating 1 ml of crude enzyme with 1 ml of 1% carboxy methyl cellulose (CMC) in 50 mM sodium citrate buffer (pH 4.8) at 50°C for 30 minutes. At different growth period, the matrix was washed with phosphate-buffered saline, and the cell-free extract was used for analysis. The bacterial crude extract was prepared by centrifuging 10 mL of cell-free extract at 5000 rpm for 15 minutes. The activity of cellulase was studied, using dinitrosalicylic (DNS) assay method. The assay was carried out as follows. Culture filtrate 0.2 mL was mixed with 1% CMC in a test tube and incubated at 40°C for 30 minutes. The reaction was terminated by adding 3 mL of DNS reagent. The tube was then incubated at 100°C for 15 minutes followed by the addition of 1 mL of salt solution. The optical density was taken at 575 nm against blank. One unit of cellulase activity is defined as the amount of enzyme required to liberate 1 µmol of reducing sugars per minute under the assay conditions [18].

FPase assay was performed using 30 mg strip of Whatman filter paper No. 1 under the same conditions with incubation time of 1 hr [19].

The amount of protein in culture supernatant was determined using the method reported by Lowry *et al.* [20].

Optimization of nutritional parameters for cellulase production
Bacillus strain that had the ability to utilize various additional carbon sources. It was observed that CMC gave maximum cellulase activity at 1.8% concentration for enzyme production when compared to other carbon sources [21].

Effect of pH on enzyme production
Optimization was carried out using minimal medium of 2, 4, 6, 8 and 10. The pH was adjusted by using 1 N HCL or 1 N NaOH. Then, minimal salt medium was enriched with CMC powder. Autoclaved and then different bacterial (NSP_1, NSP_4, NSP_2, and NSP_5) batch cultures were maintained [22].

Effect of temperature on enzyme production
Optimization was carried out by placing the substrates containing minimal salt medium and the bacterial (NSP_1, NSP_4, NSP_2, and NSP_5) at different temperatures of 25°C, 30°C, 35°C, 40°C, 45°C, 50°C and 55°C [23].

RESULTS

Identification study:

The organisms isolated were tabulated in Table 1.

Screening of cellulase from isolated organism
Congo red method was used to identify the potential strain from collection of bacterial isolates identified from the gut of termites (Fig. 1).

Enzyme activity
The enzyme activity was estimated using CMC as a substrate by DNS method. Table -2 lists the FPase and Endoglucanase activity of different microorganism isolated.

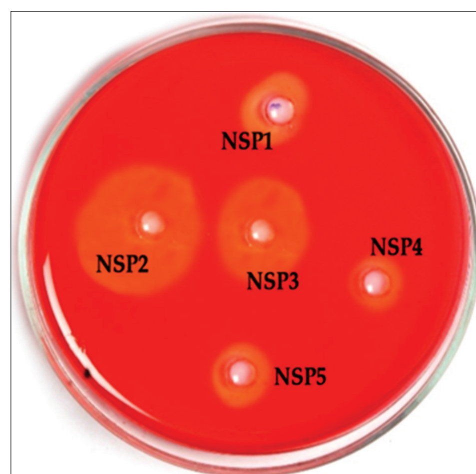

Fig. 1: Screening of cellulase producing organism

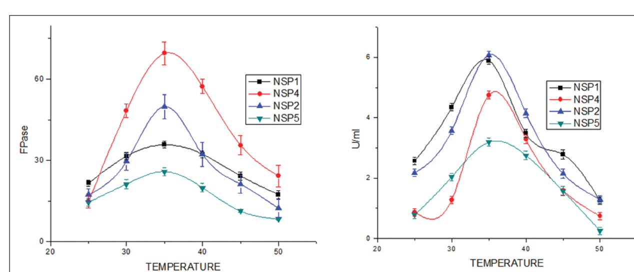

Fig. 2: Effect of temperature on enzyme production

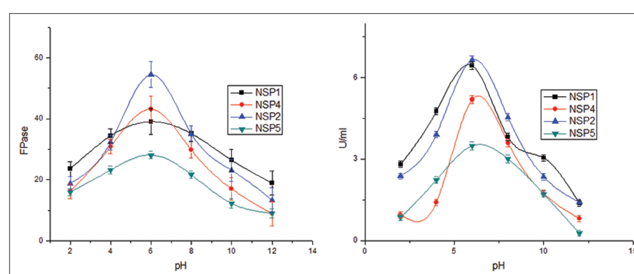

Fig. 3: Effect of pH on enzyme production

Statistical tests
The results were statistically analyzed using IBM, Statistical Package for the Social Sciences software and analysis of variance with $p<0.05$.

Effect of temperature on cellulase activity (filter paper and endoglucanase assay)
Cellulolytic enzyme production is also temperature dependent. In the experiments, three different strains of *Bacillus* and one strain of *Staphylococcus* subjected at different temperature to study the production of enzyme at different temperature. Temperature is also an essential role in the influences the cellulase production from the organisms. The effect of temperature on cellulase production using high yield strain (NSP_1, NSP_4, NSP_2, and NSP_5) was studied by varying the temperature range from 25 to 55°C (Fig 2).

The maximum production of cellulase was obtained at 35°C for CMC enriched medium that was optimized. The rate of enzyme catalyzed reactions increased with temperature up to a certain limit. Enzyme activity decreases with increase in temperature because of enzyme stability. There are previous reports [24,25] correlating with the results.

Table 1: Characterization of the different bacterial sample

Sample	Staining			Shape	Motility	Biochemical test								Organisms identified
	Gram	Spore	Capsule			I	MR	VP	Ci	Co	U	N	H_2S	
NSP_1	+	+	-	Bacilli	-	-	-	+	+	-	-	+	-	*Bacillus* sp.
NSP_2	+	+	-	Bacilli	-	-	-	+	+	-	-	+	-	*Bacillus* sp.
NSP_3	-	-	-	Bacilli	+	+	+	-	-	-	-	+	-	*Escherichia coli*
NSP_4	+	+	-	Cocci	-	-	-	-	+	+	-	-	-	*Staphylococcus aureus*
NSP_5	+	+	-	Bacilli	-	-	-	+	+	-	-	+	-	*Bacillus* sp.
NSP_6	-	+	-	Bacilli	+	-	-	+	+	-	-	+	-	*Serratia* sp.
NSP_7	+	-	-	Bacilli	-	-	-	+	+	-	-	+	-	*Bacillus* sp.
NSP_8	-	-	-	Bacilli	+	-	+	-	+	-	-	-	+	*Salmonella typhi*
NSP_9	-	-	-	Bacilli	+	+	-	+	-	-	+	-	+	*Proteus vulgaris*

I: Indole, MR: Methyl red, VP: Voges prokauer, Ci: Citrate, Co: Coagulase, U: Urease, N: Nitrate, H_2S: Hydrogen sulfate

Table 2: FPase and endoglucanase assay results for the isolated strains of bacteria

Sample	Organisms identified	Cellulase activity	
		FPase	U/ml
NSP_1	*Bacillus* sp.	36.72±0.78*	4.02±0.24*
NSP_2	*Bacillus* sp.	49.45±0.91	5.65±0.21
NSP_3	*Escherichia coli*	17.97±1.21	2.16±0.18
NSP_4	*Staphylococcus aureus*	38.61±1.04	4.45±0.23
NSP_5	*Bacillus* sp.	27.19±1.54	3.17±0.17
NSP_6	*Serratia* sp.	11.78±1.25	1.31±0.14
NSP_7	*Bacillus* sp.	8.53±0.92	1.02±0.11
NSP_8	*Salmonella typhi*	11.29±0.85	1.26±0.94
NSP_9	*Proteus vulgaris*	9.25±0.24	1.11±0.87

*Mean square error

Effect of pH on cellulase activity (filter paper and endoglucanase assay)

The optimal pH for endoglucanase activity is found to be at pH 6 for bacterial cellulase. Increasing or decreasing the pH beyond this resulted in decline in enzyme activity. Cellulase yield by NSP_4 appeared to depend on pH value. Results illustrated by Fig. 3 clearly show that the optimum pH for maximum production of cellulase. It was observed that the activity decreased with more increase in pH which indicates reduction in the activity [26,27].

CONCLUSION

The strong relationship between the microbes and the termites was well established. The organisms present in the midgut of termites are responsible for the degradation of lignocellulose material (Wood). By exploiting this phenomenon, the organisms were isolated from the termites and isolated, identified was used for the production of cellulase enzyme. From these isolated, the potential producers were identified and characterized at different temperature and pH. These organisms could be considered as effective candidates for the production of cellulase in large scale.

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