Indian Journal of Agricultural Sciences **85** (7): 970–2, July 2015/Article

Isolation and identification of cellulose demoting symbionts from gut of subterranean termite, *Odontotermes obesus*

D R BAJYA[1], DEEPIKA ARYA[2], M RANJITH[3], M C LAKHARAN[4] and S K RAZA[5]

Institute of Pesticides Formulation Technology, Sector 20, Udyog Vihar, Gurgaon, Haryana 122 016

Received: 28 November 2014; Accepted: 5 May 2015

ABSTRACT

The study was carried out to isolate and identify the symbionts, viz. cellulose demoting bacteria and fungus in termite gut. The experiment was conducted during (February 2013- July 2013) at biotechnology laboratory, Institute of Pesticide Formulation Technology, Gurgaon (Haryana). Termites are wood eating insects and are among the most important ligno cellulose- digesting insects and possess a variety of symbiotic microorganisms in their gut. Nutrient agar, potato dextrose and Carboxy Methyl Cellulose (CMC) were used to isolate the dry bacterial strain and fungus. The cellulose is demoted in termite gut by the production of cellulase enzyme which is detected by Congo red stain. Colony morphology and staining technique such as Gram's staining, Congo red staining and oxidase test for bacterial strain gave an idea for the presence of genera Citrobacter and Enterobacter. *Aspergillus nidulans* has been isolated and identified at division of plant pathology, IARI, New Delhi. These bacteria and fungus were able to assimilate CMC which aid in digestion of cellulose in subterranean termite *Odontotermes obesus* (Rambur) and this study abetted to understand more about the symbionts associated with digestive mechanism of termites.

Key words: Bacteria, Cellulose, Fungi, Identification, Isolation, *Odontotermes obesus*

Symbiotic digestion of cellulose is a complex series of events involving both the host and gut symbionts (Ulrich *et al.* 2004). Enzymes such as endogenous cellulases (endo-b-1, 4-glucanase and b-glucosidase) are excreted from salivary glands or mid-gut, have been identified and characterized in both lower and higher termites (Ohkuma 2003). Host digestive processes mainly take part in foregut and midgut; whereas symbionts digestive processes are focused on hindgut. In higher termites, the digestive activity is distributed on foregut, midgut and hindgut; whereas the digestive activity is more concentrated in the hindgut of lower termites. There are many types of bacteria found in termite gut with different functions and significance. They were hemi-cellulose degrading bacteria (Schafer *et al*. 1996), lignolytic bacteria (Borji *et al*. 2003), cellulolytic bacteria (Wenzel *et al.* 2002), aromatic degrading bacteria (Harazono *et al*. 2003) and nitrogen-fixing bacteria (Frohlich *et al*. 2007). *Staphylococcus* and *Bacillus* sp. are the most abundant bacteria in termite's gut (Breznak 1982 and Konig 2006). Other different bacteria found in Formosan termite *Coptotermes formosanus* are *Enterobacter* and *Serratia marcescenes* (Adams and Boopathy 2005) and *Enterobacter* have been used to decolorize azo dyes (Moutaoukki *et al.* 2004). *Desulfovibrio intestinalis* was isolated from the hindgut of the lower termite *Mastotermes darwiniensis* (Jurgen Frohlich *et al*. 1999) and hemi cellulose degrading bacteria and yeast have also been found (Schafer *et al*. 1996). Phenol oxidizing laccases have been found from the gut of termite *Reticulitermes flavipes (*Coy *et al.* 2010*)*. Bacterial isolates from lower as well as higher termites have been described as aerobes and facultative or strict anaerobes and were predominantly strains of *Streptococcus*, *Staphylococcus*, *Bacteroides, Enterobacteria* and *Bacillus*.

MATERIALS AND METHODS

Laboratory studies were carried out to isolate the cellulose demoting bacteria and fungi from termite gut, to identify and screen the cellulose degrading bacteria and to study the morphological and biochemical characteristics of isolated bacterial strains.

Termites were collected from the fields of wheat crop at IPFT research farm Gurgaon,during *rabi* season 2012-13. Boxes used to trap them were covered with net. Termites were identified as workers of *Odontotermes obesus* (Rambur).

Termites were surface sterilized with 70% ethanol and then washed in sterile distilled water. Under sterile conditions, the heads were removed and the bodies were crushed with the help of glass rods. The paste obtained

[1] Officer Incharage (e mail: deva.bajya@gmail.com), [3] JRF (Ph D Scholar), [4] JRF (Ph D Scholar), Division of Bioscience, [5] Director, [2] M Sc Biotechnology Scholar, Department of Biotechnology, Maharishi Markandeshwar University, Mullana, Haryana

Table 1 Gram staining of the strains

Name of the strain	Observation	Result
N1	Pink, rod	Gram negative
N2	Pink, rod	Gram negative
N3	Pink, cocci	Gram negative
N4	Pink, rod	Gram negative
N5	Pink, rod	Gram negative
N6	Pink, rod	Gram negative
N7	Pink, rod	Gram negative
N8	Pink, cocci	Gram negative
N9	Pink, rod	Gram negative
N10	Pink, rod	Gram negative
N11	Pink, rod	Gram negative
N12	Pink, cocci	Gram negative
P1	Pink, rod	Gram negative
P2	Pink, rod	Gram negative
P3	Pink, rod	Gram negative
P4	Pink, rod	Gram negative
P5	Pink, rod	Gram negative
P6	Pink, rod	Gram negative
P7	Pink, rod	Gram negative
P8	Pink, rod	Gram negative

Table 2 Congo red staining

Name of the strain	Diameter of zone of inhibition (in cm)				
	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
N1	1.3	1.5	2	1.7	1.5
N2	1.2	2	0.6	0.8	
N3	2.3	2	2.5	2.7	2.6
N4	1	1.2	1.2	1.6	2
N5	3	2.7	2.9	3.4	3.1
N6	1.3	1.4	1	1.6	1.5
N7	1.5	1.6	1.7	1	0.8
N8	0.7	0.6	0.9	0.5	
N9	2.5	2	2.6	1.9	2.3
N10	1	1.7	1.4	1.6	1.8
N11	3.2	2.8	3	3.5	3.4
P1	0.5	0.8	0.9	0.7	0.6
P2	1.6	1.8	1.6	1.9	1.5
P3	2.6	2.4	2	2.5	
P4	0.5	0.7	1	1.2	0.8
P5	0.8	0.7	0.6	0.6	
P6	2.3	2.7	1.8	1.5	2
P7	0.9	0.7	0.6	0.5	
P8	1	1	1.4	1.6	1.8
P9	0.4	0.6	0.5	1	0.3

was used for the isolation of the bacteria and fungus with the help of inoculating loop.

Three different media were used for inoculation of the bacteria, e.g. nutrient agar, potato dextrose agar, carboxy methyl cellulose (CMC).

Each media was supplemented with carboxy methyl cellulose (CMC) as cellulose source. Nutrient agar was used for the isolation of general bacteria. Potato dextrose agar was used for the isolation of fungus. CMC, which measures endo- β -1, 4-glucanase activity, is one of the most popular artificial substrates for measuring cellulase activity because of its high solubility in water and so CMC was used for the identification of cellulolytic activity of bacteria. Sub culturing was done several times to get the pure culture of bacteria and fungus.

The agar plates with 1% of CMC were prepared. Strains were streaked and incubated at 37 ^{o}C for 48 hours. Petri plates were then flooded with 0.1% Congo red reagent and then left for 20 minutes. Then plates were washed with 1M NaCl. Clearance zone known as halo zones were seen indicating the positive result.

Identification of bacteria was done from colony morphology and staining techniques such as Gram staining and Congo red reagent test. Fungus isolated was identified at Indian Type Culture Collection Identification centre, Division of Plant Pathology, IARI Pusa, New Delhi.

RESULTS AND DISCUSSION

Gram's staining of all cultures were done for all the strains to identify the gram negative and gram positive bacteria. Of the twenty strains, eighteen strains were observed to be pink and rod shaped gram negative bacteria. Only two strains N8 and N12 were pink and cocci, both belonging to gram negative (Table 1). The results of biochemical tests for bacterial strains gave an idea of the isolated bacterial strains to be of genera *Citrobacter* and *Enterobacter* as all strains were gram negative and oxidase positive which falls in line with the findings of (Wenzel *et al*. 2002).

The Congo-red assay done to check the cellulose demoting activity of the isolated strains resulted in strains that digest cellulose as CMC in the media gave zone of inhibition when treated with Congo-red dye. The diameter of zones of inhibition were measured which corresponded to the cellulose digesting ability of the respective strains. The difference between the diameters of zone of clearance gave the basis of comparison between the highest cellulolytic activity and the lowest cellulolytic activity showing colonies. The measurements of zone were taken in centimetres (cm) (Table 2).

Oxidase test done to demonstrate the ability of a bacterium to produce the enzyme cytochrome-c oxidase, capable of reducing oxygen, yielded positive results as there was a colour change to blue, then to dark purple or black, within 10 to 30 seconds, which distinguished aerobic and anaerobic metabolism.

A fungus of phylum Ascomycota, genus *Aspergillus* and species *Aspergillus nidulans* has been isolated and identified by Indian type culture collection identification, IARI Pusa- New Delhi. *Aspergillus nidulans* was also found to be associated with the digestion of cellulose as it was grown on PD supplemented with CMC.

The study proved to be useful as two bacterial strains and one fungal strain was isolated from the gut of *O. obesus* and the role of these symbionts in cellulolytic activity of

O. obesus was studied which gave a better understanding on the digestive mechanism of termites.

REFERENCES

Abe T, Bignell D E and Higashi M. 2000. *Termites: Evolution, Sociality, Symbioses, and Ecology*, pp 209–27, 307–32. Kluwer Academic Publishers, Dordrecht.

Adams L and Boopathy R. 2005. Isolation and characterization of enteric bacteria from the hindgut of Formosan termite. *Journal of Bioresources Technology* **96**: 1 592–8.

Borji M, Rahimi S, Ghorbani G, Vand Yoosefi J and Fazaeli H. 2003. Isolation and identification of some bacteria from termites gut capable in degrading straw lignin and polysaccharides. *Journal of Faculty of Veterinary Medicine University of Tehran* **58**: 249–56.

Breznak J A. 1982. Biochemical aspects of symbiosis between termites and their intestinal microbiota. (*In*) *Invertebrate-Microbial Interactions*, pp 173–203. Anderson J M, Rayner ADM and DWH Walton (Eds.). Cambridge University Press, Cambridge, UK.

Coy M R, Salem T Z, Denton J S, Kovaleva E S, Liu Z, Barber D S, Campbell J H, Davis D C, Buchman G W, Boucias D G and Scharf M E. 2010. Phenol oxidizing laccases from the termite gut. *Insect Biochemistry and Molecular Biology* **40**(10): 723–32.

Frohlich J, Koustiane C, Kampfer P, Roselle-Mora R and Valens M. 2007. Occurrence of rhizobia in the gut of the higher termite *Nasutitermes nigriceps*. *Journal of Systematic and Applied Microbiology* **30**: 68–74.

Harazono K, Yamashita N, Shinzato N, Watanabe H, Fukatsu T and Kurane R. 2003. Isolation and characterization of aromatics degrading microorganisms from the gut of the lower *Coptotermes formasanus*. *Journal of Biosciences Biotechnology and Biochemistry* **67**: 889–92.

Jürgen Fröhlich, Henrik Sass, Hans-Dietrich Babenzien, Thomas Kuhnigk, Ajit Varma, Shailendra Saxena, Christine Nalepa, Peter Pfeiffer, Helmut König. 1999. Isolation of *Desulfovibrio intestinalis* sp. nov. from the hindgut of the lower termite *Mastotermes darwiniensis*. *Canadian Journal of Microbiology* **45**(2): 145–52.

Konig H. 2006. Bacillus species in the intestine of termites and other soil invertebrates. *Journal of Applied Microbiology* **101**: 620–7.

Moutaouakki A, Zeroual Y, Dzayri F Z, Talbi M, Lee K and Blaghen M. 2004. Decolorization of azo dyes with Enterobacter agglomerans immobilized in different supports by using fluidized bed bioreactor. *Current Microbiology* **48**(2): 124–9.

Ohkuma M. 2003. Termite symbiotic systems: efficient bio-recycling of cellulose. *Applied Microbiology and Biotechnology* **61**: 1–9.

Schafer A, Konrad R, Kuhnigk T, Kampfer P, Hertel H and Konig H. 1996. Hemicellulose-degradating bacteria and yeasts from the termite gut. *Journal of Applied microbiol*ogy **80**: 471–8.

Ulrich Radek R, Yang H and Brune A. 2004. "Endomicrobia" cytoplasmic symbionts of termite gut protozoa form a separate phylum of Prokaryotes. *Applied and Environmental Microbiology* **71:** 1 473–9.

Wenzel M, Schonig I, Berchtold M, Kampfer P and Konig H. 2002. Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis*. *Journal of Applied Microbiology* **92**: 32–40.