EXPRESSION OF ENDOGLUCANASE GENE FROM CELLULOLYTIC BACTERIA OF TERMITE GUT

N.V. ARUNA

I. D. No. MVM 10001 (ABT)

Thesis submitted in partial fulfillment of the requirement for the degree of

MASTER OF VETERINARY SCIENCE

IN

ANIMAL BIOTECHNOLOGY

to the

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

CHENNAI – 600 051

DEPARTMENT OF ANIMAL BIOTECHNOLOGY

MADRAS VETERINARY COLLEGE

TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

CHENNAI – 600 007

2012

CERTIFICATE

This is to certify that the thesis entitled **"EXPRESSION OF ENDOGLUCANASE GENE FROM CELLULOLYTIC BACTERIA OF TERMTE GUT"** submitted in partial fulfillment for the requirement of the degree of **MASTER OF VETERINARY SCIENCE** in **ANIMAL BIOTECHNOLOGY** to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, is a record of bonafide research work carried out by **N.V. ARUNA** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or the other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

K. Vijayarani

Place : Chennai - 7
Date : 29.06.2012

Dr. K. VIJAYARANI
Chairman

Date : TIRUPATI
Place : 30-7-2012

RECOMMENDED

EXTERNAL EXAMINER

APPROVED

K. Vijayarani 14.8.12

Chairman : **Dr. K. VIJAYARANI**

Members : - Absent -
1. Dr. M. PARTHIBAN

Krishnamohan

2. Dr. Y. KRISHNAMOHAN REDDY

ABSTRACT

Title	:	**Expression of endoglucanase gene from cellulolytic bacteria of termite gut.**
Name of the student	:	**N.V. ARUNA**
Degree for which submitted	:	**M.V.Sc in ANIMAL BIOTECHNOLOGY**
Name of the chairman	:	**Dr. K. VIJAYARANI, Ph.D.,** Professor , Department of Animal Biotechnology Madras Veterinary College Chennai-600 007.
University	:	**Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051.**
Year	:	**2012**

The present study was undertaken to amplify, clone and express endoglucanase gene from cellulolytic bacteria isolated from termite gut and to characterize it by SDS-PAGE and Western blotting.

Cellulolytic bacteria was isolated from the gut of fungus growing termite, *Odontotermes formosanus*, by plating homogenised gut microbes on nutrient agar containing 1% cellobiose and by repeated subculturing. The isolate was identified as *Salmonella sp.* based on its morphology, Gram's staining characters, motility and its growth characteristics in selective media like Brilliant Green agar, MacConkey agar and Selenite F broth. The isolated bacteria was further characterized as *Salmonella enterica* based on its biochemical tests and sugar fermentation tests. Cellulolytic property of the isolate was determined by Congo red assay. Genomic DNA was isolated from the overnight grown culture and 16S rRNA gene was amplified using genomic DNA as template. The 16S rRNA gene PCR amplicon was sequenced and BLAST analysis done for confirmation of the isolated bacteria.

Endoglucanase gene was amplified from the genomic DNA isolated from *Salmonella enterica* using gene specific primers with CACC overhang in the forward primer to facilitate its directional cloning. Endoglucanase gene PCR amplicon of 1.72 Kb was gel purified and cloned into pET100 prokaryotic expression vector. The recombinant clone was confirmed by restriction digestion, PCR and sequencing. The recombinant pET100 plasmid was transformed into *E.coli* (BL21 DE3) cells.

The recombinant pET100 colonies were induced with 1mM IPTG to analyse the expression of the endoglucanase enzyme in *E.coli* (BL21 DE3). The expressed protein was analyzed using 12% SDS-PAGE. The expressed protein with the molecular weight of 65 kDa was obtained with the 5 hr induced cultures, which further reacted with Anti His (G-term)-HRP antibody on immunoblotting. The specific activity of the enzyme was measured using Di-nitro salicylic acid (DNS) assay and the activity was found to be 54 Units/ml.

Key words: *Termite - Salmonella enterica - Endoglucanase gene – Cloning - Expression in pET 100 - SDS-PAGE - Western blotting – DNS assay.*