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Cloning of cellulase gene from *Ruminococcus albus* using a shuttle vector

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Ruminococcus albus, R flavefaciens and *Fibrobacter succinogenes* are some of the rumen bacteria with cellulolytic activities (Hungate 1966, Ohmiya *et al.* 1988, Ozcan *et al.* 1996). *R albus* is reported to be one of the most active cellulolytic cocci responsible for 20% of the rumen cellulolytic activity. Although *R. albus* has got powerful cellulase enzymes, their role in the rumen is limited since it is not a predominant rumen bacteria (Mackie and Kistner 1985). *R. albus* cellulase gene (*R.albus* F-40, endoglucanase EgI) has been expressed in *B. fibrisolvens* (Kobayashi *et al.* 2003). Shuttle vectors are generally used for interspecies cloning and pBS42 is one such vector. Hence in the present study considerable effort has been put on cloning of cellulase gene from *R.albus* using a shuttle vector and studying its expression in *E.coli.*

Bacterial strains: Ruminococcus albus, isolated from bovine rumen and available in Department of Animal Biotechnology, was used for isolating cellulase gene. *Escherichia coli* DH5α was also procured from the same department and used as the host bacteria for carrying recombinant plasmids. Both *Ruminococcus albus* and *Escherichia coli* were maintained as freeze-dried cultures.

Shuttle vector: Shuttle vector PBS42 was kindly provided by Dr White Head (National Centre for Agricultural Utilization Research, Ilinois, USA) and used for cloning cellulase gene. pBS42 is a 4789bp plasmid with a chloramphenicol resistant phenotype.

Cloning of cellulase gene: Genomic DNA libraries of *R. albus* were constructed in the *Hind* III site of plasmid pBS42. The chromosomal DNA and pBS42 DNA were digested with *Hind* III enzyme (Ware *et al.* 1989). One µg of pBS42 DNA was mixed with 3 µg of partially digested *R. albus* chromosomal DNA and ligated using 10 units of T_4 DNA ligase at 15°C for 16 h. The ligation mixture was used to transform *E.coli* DH5α cells and recombinant colonies were selected on LB agar plates containing chloramphenicol (20 mg/ml).

Screening of transformants: Chloramphenicol resistant colonies were screened for cellualse activity by Congo red assay (Teather and Wood 1982). Colonies with zone of hydrolysis in congo red assay were picked up from the original plates and checked for the presence of recombinant plasmids by isolating DNA and agarose gel electrophoresis.

Assay for cellulase activity: Cellulase activity of the recombinant bacteria was determined by viscosity reduction of carboxy methyl cellulose (CMC) containing reaction mixture at 37°C and monitoring for 5 min by using viscometer. For this, Congo red assay positive recombinant cells were ground in a mortar with aluminum oxide powder and the enzyme was extracted with 50 mM phosphate buffered saline (pH 6.8). The reaction mixture contained CMC solution (1%) in 50 mM potassium phosphate buffer (5 ml pH 6.8) with 1 ml of enzyme solution. One unit of enzyme activity was defined as the amount of enzyme required to reduce the viscosity of CMC in 1 min. Cellulase activity of the recombinant bacteria was also determined by measuring the reducing sugar or total sugar released as per Somogyi-Nelson (Wood and Bhat 1988). Substrate activity of the recombinant plasmid was also assessed as per Takano *et al.* (1992). The OD at 660 nm was measured in a spectrophotometer.

Choramphenicol resistant recombinant colonies (65) were obtained following cloning in pBS42 shuttle vector. In the initial screening by congo red assay, 12/65 colonies (clones) showed cellulase activity, indicated by the presence of well marked clearing zones around the wells. These colonies had recombinant plasmids of 9.3 kb, 9.14 kb, 8.98 kb and 6.93 kb size. Among these recombinant plasmids, the 8.98 kb plasmid revealed maximum cellulose digesting ability as evidenced by congo red assay. Restriction enzyme analysis of this plasmid resulted in the release of 3.7 kb insert encoding for cellulase activity. This resembled the endo-1,4-ß-glucanase gene cloned from *R.albus* by Karita *et al.* (1993).

CMCase activity of the recombinant cells determined by CMC-viscosity reduction method is shown in Fig.1. Cellulase activity increased with the growth of culture. The viscosity of CMC reduced after the addition of the recombinant cells to CMC solution. When 24 h grown culture was tested, CMC

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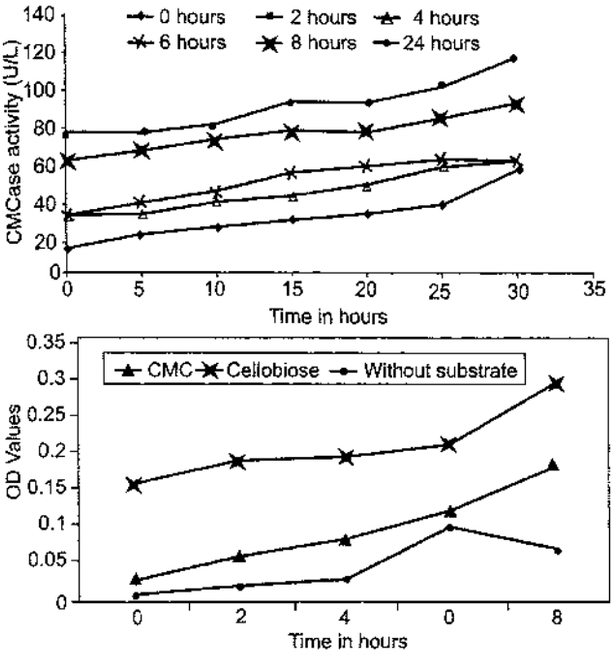

Figs 1–2. **1.** CMCase actity of recombinaut bacteria at different time intervals (viscosity reduction method) **2.** Growth curve OD values of the recombinant bacteria using different substrates.

digesting ability was increased from an initial ability of 53 µl (30 min after the addition of culture of CMC solution) to 116 µl at 24h. Carboxy methyl cellulase (CMCase) or endo-1, 4-ß-glucanase is a fibre digesting enzyme which catalyses the hydrolysis of cellulose, releasing glucose by breaking the ß-1,4 linkages. Cellulase activity of the recombinants was tested using the above property also by measuring the amount of reducing sugar released (Wood and Bhat 1988). One millilitre of the recombinant cells liberated 792 µg of glucose, indicating cellulase activity. Cellulase activity of the recombinant cells was also checked by their ability to utilize various substrates. The growth curve studies indicated that cellobiose was utilized (Fig. 2) more efficiently than CMC which again confirmed the celluloytic activity of the recombinants.

It is concluded that the 3.7 kb cellulase encoding gene cloned into the shuttle vector pBS42 had celluloytic activity and resembled endo-1,4-ß-glucanase enzyme.Further attempts are being made to transfer this gene, expressed in *E.coli,* into dominant anaerobic rumen bacteria by transconjugation.

SUMMARY

The present study describes the cloning of a cellulase gene isolated from the rumen cellulolytic bacteria *Rumincoccus albus* into the shuttle vector pBS42. 8.98 kb recombinant plasmid carrying an insert of 3.7 kb was found to have cellulolytic activity as assessed by Congo red assay. The cellulolytic activity of the recombinant cells was assessed by carboxy methyl cellulose - viscosity reduction methods, ability to release reducing sugar and ability to utilize substrates like cellobiose and carboxy methyl cellulose. Cellulase activity increased with the growth of the culture and also when cellobiose was used as a substrate.

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REFERENCES

Hungate R E. 1966. *The Rumen and its Microbes.* Academic Press, New York, USA.

Mackie R I and Kistner A. 1985. Some frontiers of research in basic ruminant nutrition. *South African Journal of Animal Sicences* **15**: 72–76.

Ozcan N , Cunningham C and Harris W J. 1996. Cloning of the cellulase gene from rumen anaerobe *Fibrobacter succinogenes* SD35 and partial characterization of the gene product. *Letters in Applied Microbiology* **22**: 85–89.

Karita S, Morioka K, Kajino T, Sakka K, Shimada K and Ohmiya K. 1993. Cloning and sequencing of novel endol,4-ß-glucanase gene from *R. albus. Journal of fermentaion and bioengineering* **73**: 79–88.

Kobayashi Y, Taguchi H, Goto T N, Koike S and Ohmiya K. 2003. Expression and export of a *Ruminococcus albus* cellulase in *Butyrivibrio fibrisolns* through the use of an alternative gene promoter and signal sequence *Canadian Journal of Microbiology* **49**: 375–82.

Ohmiya K, Nagashima, Kajino T, Hoto E, Tsukada K and Shimizu S. 1988. Cloning of the cellulase gene from *Ruminococcus albus* and its expression in *E.coli. Applied Environmental Microbiology* **54**: 1511–15.

Takano M, Mariyama R and Ohmiya K. 1992. Structure of a ß-glucosidase gene from *R.albus* and properties of the translated product. *Journal of Fermentation and Bioengineering* **73**: 79–88.

Teather R M and Wood P J. 1982. Use of congo red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Applied Environmental Microbiology* **43**: 777–80.

Ware C E, Bauchop T and Gregg K. 1989. The isolation and comparison of cellulase genes from two strains of *Ruminococcus albus.* Journal of General Microbiology **135**: 921–30.

Wood T M and Bhat K M. 1988. Methods for measuring cellulase activities. *Methods in Enzymology* **160**: 87–112.