Journal of Microbiology and Biotechnology
Vol. 4, No. 2, 102-107, 1994

Cloning of the Endoglucanase Gene from *Actinomyces* sp. 40 in *Escherichia coli* and Some Properties of the Gene Products

MIN, HAE-KI, YUN-JAIE CHOI*, KWANG-KEUN CHO, JONG-KYU HA AND JUNG-HEE WOO

Department of Animal Science and Technology, College of Agriculture and Life Sciences, Seoul National University, Suweon 441-744, Korea

The β-1,4-endoglucanase gene from *Actinomyces* sp. 40 was cloned into *Escherichia coli* DH5α with pUC19. Chromosomal DNA from *Actinomyces* sp. 40 was cleaved with the restriction enzyme *Sau*3AI and ligated into pUC19 for the transformation of *Escherichia coli* DH5α. Positive clones of β-1,4-endoglucanase gene were detected as the clear zones on a medium supplemented with carboxymethylcellulose (CMC). This transformant possessed a single plasmid, designated pDS1, which contained the vector DNA and a 3.5 kilobase (kb) *Sau*3AI insertion fragment encoding endoglucanase. The size of the cloned fragment was reduced to 2.0 kb. The endoglucanase activity produced by the *E. coli* DH5α (pDS6) was higher than that of *Actinomyces* sp. 40 strain. The optimum pH and temperature of the cloned enzyme were pH 4.0~5.0 and 55°C, respectively. The cloned enzyme was stable at 55°C or below and in buffer ranging from pH 4.0 to 7.0. The enzyme degraded CMC but did not degrade xylan, cellobiose, and methyl-umbelliferylcellobiopyranoside (MUC).

The rumen is one of the most abundant sources of cellulolytic bacteria. *Ruminococcus flavefaciens, Ruminococcus albus, Butyrivibrio fibrisolvens*, and *Bacteriodes succinogenes* are the four major cellulolytic bacteria found in the rumen (4). A number of cellulolytic systems in the bacteria have partially been characterized. *Ruminococcus flavefaciens* is one of the most important species of the rumen bacteria and is capable of degrading the plant cell walls (23). Also, *Ruminococcus flavefaciens* degrades crystalline cellulose more efficiently than *Ruminococcus albus* (23). *Bacteriodes succinogenes* possess the ability to hydrolyze cellulosic materials such as cotton fibers and straw (9).

The prospect of using genetically manipulated microorganisms in programes aiming to achieve biomass conversion or improvement in the animal production has led to the interest in cloning cellulase genes. Recently, there have been reports on the molecular cloning of cellulolytic rumen bacterial cellulase into *Escherichia coli*; including *Ruminococcus albus* (10, 21), *Ruminococcus flavefaciens* FD-1 (2), and *Bacteriodes succinogenes* (26). *Actinomyces* sp., an anaerobic cellulolytic rumen bacterium, was found to be a predominant cellulolytic bacterium present in the rumen of Korean native goat (K.M. Park. 1986. Ph. D. dissertation, University of Sung Kyun Kwan, Seoul). This bacterium possessed the ability to hydrolyze cellulosic material such as carboxy methylcellulose (CMC). To obtain data for the effective production and use of the *Actinomyces* sp. 40 endoglucanase, we have cloned an β-1,4-endoglucanase gene of the bacterium into *Escherichia coli* and also studied the enzymatic properties of the cloned endoglucanase.

MATERIALS AND METHODS

Bacterial Strains, Media, and Plasmid

Actinomyces sp. 40 (20) was maintained as described by Bryant and Robinson (5). It was grown anaerobically at 39°C in a medium 10 broth (6) containing 0.4% sugar as the fermentable carbohydrate source. The host strain was *E. coli* DH5α [F-(φ80*dlacZ*△M15) Δ (*lacZYA-argF*) U169 *deoR recA1 endA hsdR17 supE44* λ-*thi*-1 *gyrA*96 *relA1*]. It was grown in a Luria-Bertani (LB) medium by incubation at 37°C (16). To select the transformants, The LB medium was supplemented with ampicillin (50 μg/ml), 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and 5-bromo-4-chloro-3-indolyl-β-D- galactoside (X-gal) at 40 μg/ml. Plasmid pUC19 (21) was used as the vector throughout this study.

Materials

Restriction enzymes, T4 DNA ligase, and calf intestinal

*Corresponding author
Key words: *Actinomyces* sp. 40, endoglucanase

alkaline phosphatase were purchased from either Kosco Biotech (Korea) or Promega Biotech (Madison, Wis, USA). DNA labelling and detection kit were purchased from Boehringer Mannheim Biochemical Co. Nitrocellulose BA-85 (0.85 μm) was obtained from Schleicher & Schuell, Keene, N.H. Glucose assay kit, agarose, lysozyme, CMC, MUC, and the other chemicals were purchased from the Sigma Chemical Co. (St. Louis, Mo. USA).

Isolation of DNA and Restriction Enzyme Digestion

Chromosomal DNA was isolated from *Actinomyces* sp. 40 cells by the method of Saito and Miura (24). Plasmid DNAs were prepared by the alkaline lysis method. The purification of the plasmid DNAs by centrifugation to equilibrium in cesium chloride-ethidium bromide has been described by Maniatis *et al.* (16). For the restriction endonucleases, the conditions for the reaction were the same as described by the supplier.

Construction of Recombinant Plasmid DNAs and Transformation

A 50 μg sample of the chromosomal DNA from *Actinomyces* sp. 40 was partially digested with *Sau*3AI and the fragments of 2 to 10 kb were obtained by the sucrose density gradient centrifugation (16). These fragments were ligated into *Bam*HI-digested pUC19. The transformation of *E. coli* DH5α cells with recombinant plasmids was carried out following the competent cell method described by Maniatis *et al.* (16).

Screening of Recombinants for Endoglucanase Activity

Ampicillin-resistant colonies were transferred with tooth-picks to LB agar plates containing 50 μg/*ml* of ampicillin, 0.5% CMC in 50 mM potassium phosphate buffer, pH 7.0. The plates were incubated at 37°C overnight, stained for 1 h with Congo red solution (1 mg/*ml*), and destained with 1 M NaCl for at least 15 min. The endoglucanase activity was detected as the clear zones around the colonies.

Subcloning of Insert Fragment

The plasmid DNA (pDS1) was purified and completely digested with *Sma*I or *Pst*I, partially with *Eco*RI. After the separation of the DNA fragments, the inserted DNA fragments were electroeluted and purified. The inserted DNA fragments were ligated to CIP (calf intestinal alkaline phosphatase)-treated pUC19 with T_4 DNA ligase. The ligated plasmid DNA was used to transform $CaCl_2$-treated competent *E. coli* DH5α cells (16). The transformants were cultured on LB plates which contained 50 μg/*ml* ampicillin and 0.5% CMC. The subclones were tested for the expression of the CMCase activity by the modified Congo-red method (13).

Southern Hybridization

A nonradioactive DNA labeling and detection kit were used to detect chromosomal DNA fragments homologous to the cloned endoglucanase. To prepare the DNA probe, plasmid pDS1 was digested with *Hin*dIII. The DNA probe for the Southern hybridization was labeled by priming randomly with digoxigenin-11-dUTP as described by the supplier. *Actinomyces* sp. chromosomal DNA was digested with *Hin*dIII. For the hybridization with labeled probes, the DNA was transferred to nitrocellulose by the method of Southern (25) and was hybridized by the method of Maniatis *et al.* (16).

Enzyme Assays

1 *ml* of the culture supernatant (crude enzyme solution) was mixed with 1 *ml* each of 0.5% CMC solution in 0.5 M potassium phosphate buffer, pH 6.5. The mixture was incubated for 1 h at 40°C, then the reaction was stopped by boiling the mixture for 10 min. The reducing sugar produced was measured by the DNS reagent (19). One unit of enzyme activity was defined as that forming 1 μmole of glucose equivalent of reducing sugar per minute under the standard conditions. Specific activity was defined as units per milligram of protein. To find the substrate specificity, the following concentrations of substrates were incubated with 1 *ml* each of crude enzyme solution in 0.5 M potassium phosphate buffer, pH 6.5, at 40°C for 1 h: xylan, 1%; cellobiose, 1%; CMC, 1%. The enzymatic activities on methylumbelliferyl-β-D-cellobiopyranoside (MUC) were determined by the method of Faure *et al.* (8). The xylose and glucose produced were measured by the Somogyi-Nelson method (1) and the glucose assay kit, respectively. Protein concentration was determined by the method of Lowry *et al.* (15).

Distribution of Enzymes

The fractionation of extracellular, periplasmic, and intracellular enzymes were performed by using a modification of the methods of Cornelis *et al.* (7). The culture broth was centrifuged at 8,000 × g for 10 minutes at 4°C and washed twice with an equal volume of original broth containing 0.9% NaCl. The cells harvested at 4°C were washed in 0.5 M potassium phosphate buffer (pH 6.5) and suspended in a half volume of original broth with 25% (W/V) sucrose solution in potassium phosphate buffer (pH 6.5)-1 mM ethylenediaminetetraacetic acid (EDTA) at room temperature. After 10 minutes of constant and gentle shaking, the cells were sedimented by centrifugation. The extracellular fraction was calculated from the sum of the culture broth supernatant, the two washes, and the EDTA treatment supernatant. It was then suspended in the same volume of ice-cold distilled water and shaken for another 10 minutes. The cells were centrifuged and the resulting supernatant fluid was used as the periplasmic fraction. The cells sonicated

in the same buffer were centrifuged and the supernatant was used as the intracellular fraction.

RESULTS

Cloning of *Actinomyces* sp. 40 Endoglucanase Gene

A genomic library of *Actinomyces* sp. 40 was prepared in *E. coli* DH5α by using pUC19 as the vector. Among approximately 3,000 ampicillin-resistant colonies obtained, two colonies had CMCase activity on the CMC-Congo red plate. Recombinant plasmid DNAs of two the transformants were designated pDS1 and pDS101. Plasmid pDS1 and pDS101 contained, in addition to the vector DNA, 3.5 and 4.0 kb DNA, respectively. These plasmids digested with *Sma*I contained a common 2.7 kb DNA fragment (data not shown). Thus, plasmid pDS1 was used for further study. The isolated plasmid pDS1 was digested with several restriction enzymes and analyzed by the agarose gel electrophoresis. A restriction map of plasmid pDS1 is shown in Fig. 1. No *Hin*dIII site was detected inside the insert. On the contrary, five *Eco*RI sites were found in this fragment. *Sma*I, *Pst*I, and *Cla*I all have a single restriction site.

In order to localize the essential region of the gene coding for endoglucanase in the 3.5 kb insert fragment, plasimd pDS1 was digested with *Sma*I and religated (*Sma*I had one cleavage site in pUC19 and in the 3.5 kb insert). The derivative pDS2 deleted 0.8 kb *Sma*I DNA fragment from pDS1 (Fig.1). The plasmid pDS2 expressed CMCase activity on the CMC-Congo red plate. Another derivative pDS3 prepared in a similar manner, deleted 1.5 kb *Xba*I-*Pst*I DNA fragment from pDS1, and showed no CMCase activity. pDS2 (2.7 kb) was further digested partially with *Eco*RI and religated. As a result, only the 2.0 kb *Eco*RI fragment, designated pDS6, was found to show CMCase avtivity (Fig. 1), indicating that the CMCase gene was located on the 2.0 kb *Eco*RI fragment of pDS1. Attempts to obtain shorter fractions than pDS6 with positive CMCase activity ended in failure. The CMCase activity of the transformants harboring the plasmid derivatives made from pDS1 is shown in Fig. 2. The plasmid pDS6 showed slightly higher CMCase activity than pDS1 when measured by the Congo-red method. But pDS5 showed no activity on the LB plate, suggesting that the expression of the gene is effective when placed under the direct control of the *lac* promoter.

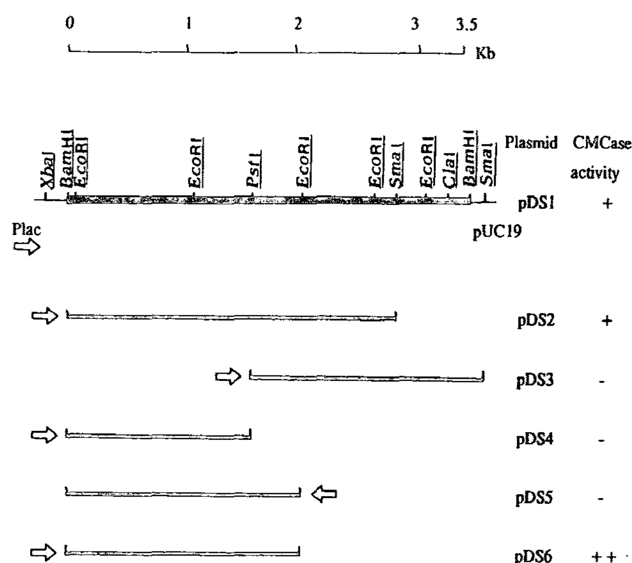

Fig. 1. Restriction endoglucanase map of plasmid pDS1 carrying the CMCase gene.

The CMCase activity expressed by each derivative is indicated on the right side as either positive (+) or negative (−). The open arrows indicate the orientation of the *lac* promoter of the pUC19 vector.

Southern Hybridization

To confirm that the cloned DNA fragment originated from *Actinomyces* sp. 40 chromosomal DNA, the *Actinomyces* sp. 40 DNA were digested with *Hin*dIII and hybridized with Dig-dUTP labeled, 6.2 kb *Hin*dIII fragment of pDS1 used as a probe. As shown in Fig. 3, the *Hin*dIII fragment of pDS1 gave positive hybridization with *Hin*dIII-digested *Actinomyces* sp.40 chromosomal DNA (Fig. 3B, lane 2) but no hybridization was detected with *E. coli* DNA digest (Fig. 3B, lane 4). This result demonstrates that the cloned DNA fragment was derived from *Actinomyces* sp. 40 chromosomal DNA.

Enzymatic Properties of Cloned Endoglucanase

To examine the effect of pH on enzyme activity and stablilty, the transformant harboring pDS6 was cultivated in LB broth at 37°C, and the endoglucanase activity in the culture supernatant (crude enzyme) was measured. The enzyme activity was assayed at 40°C

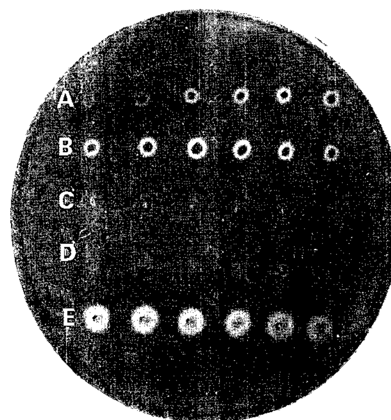

Fig. 2. CMC-Degrading activity of the transformants harboring plasmid derivatives made from pDS1.

Symbols: A, pDS1; B, pDS2; C, pDS3; D, pDS5; E, pDS6.

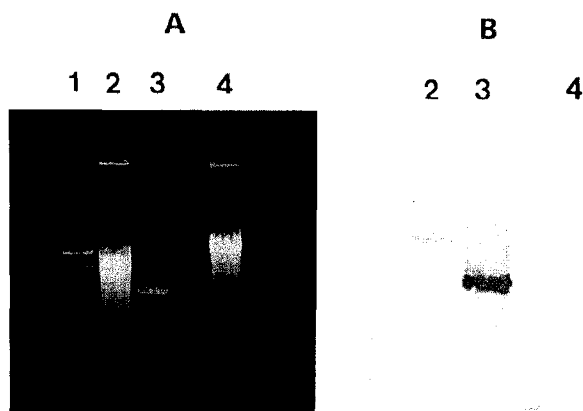

Fig. 3. Southern hybridization showing homology between cloned DNA and *Actinomyces* sp. 40 chromosomal DNA digested with *Hin*dIII.

(A) Ethidium bromide-stained agarose gel patterns of DNAs. Lanes: 1, molecular weight size markers (lambda-*Hin*dIII); 2, Chromosomal DNA of *Actinomyces* sp. digested with *Hin*dIII; 3, pDS1 DNA fragment digested with *Hin*dIII; 4, Chromosomal of DNA of *E. coli* DH5α digested with *Hin*dIII. (B) Detection of DNA transferred to nylon membrane and hybridized with the digoxigenin-dUTP labeled *Hin*dIII fragment. The lanes are the same as those in (A).

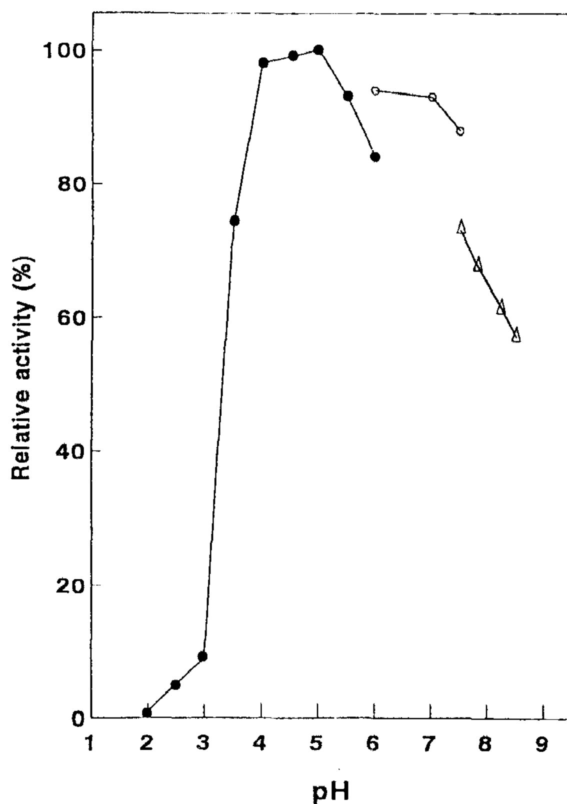

Fig. 4. Effect of pH on the CMCase activity of *E. coli* DH5α (pDS6).

Symbols: citrate buffer, ●; potassium phosphate buffer, ○; HEPES, △.

for 1 h in 0.5 M citrate, 0.5 M potassium phosphate, and 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-ethane-sulfonic acid) buffer with a pH range of 2.0 to 8.5. The crude enzyme solution was allowed to stand for 1 h at 40°C and then assayed for the CMCase activity. The optimum pH was between 4.0 and 5.0 (Fig. 4). The crude enzyme solution was kept for 24 h at room temperature and the residual activity was assayed under the standard assay conditions. This enzyme was stable at pH range between 4.0 to 7.0 (Fig. 5). The optimum temperature for the CMCase activity was 55°C, At temperature above 60°C, there was a sharp decrease in the activity (Fig. 6). Thermostability was investigated by measuring the residual activity after incubation at temperatures ranging from 35 to 70°C at various stages of the incubation. At 45°C, less than 10% of the activity was lost after 6 h incubation period (Fig. 7). A significant loss in the activity was exhibited at temperatures above 65°C. The residual activity was 50% at 65°C after 1 h. However, there was a 100% loss at 65°C after 70 min and 70°C after 40 min.

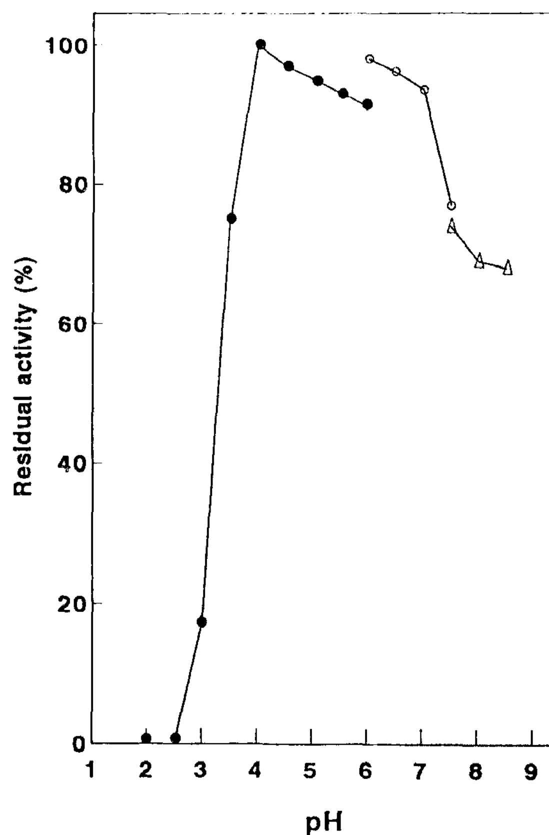

Fig. 5. Stability of pH on the CMCase activity of *E. coli* DH5α (pDS6).

Symbols: citrate buffer, ●; potassium phosphate buffer, ○; HEPES, △.

The cloned endoglucanase could hydrolyze CMC, which contained β-1,4-linkages but no detectable enzyme activity was observed for xylan, MUC, and cellobiose. The enzyme activity (U/mg protein) of DH5α (pSD6) and *Actinomyces* sp. were 3.83 and 0.19, respectively. *Escherichia coli* DH5α (pDS6) had activity 20 times higher than that of *Actinomyces* sp. 40.

Localization of CMCase in *E. coli* DH5α (pDS6)

To determine the localization of endoglucanase, *E. coli* DH5 (pDS6) was cultivated in LB broth at 37°C.

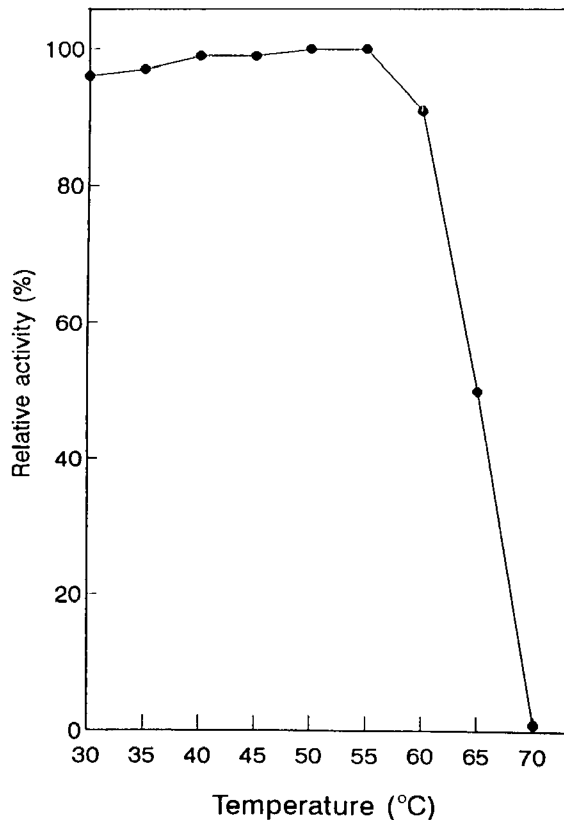

Fig. 6. Effect of temperature on the CMCase activity of *E. coli* DH5α (pDS6).

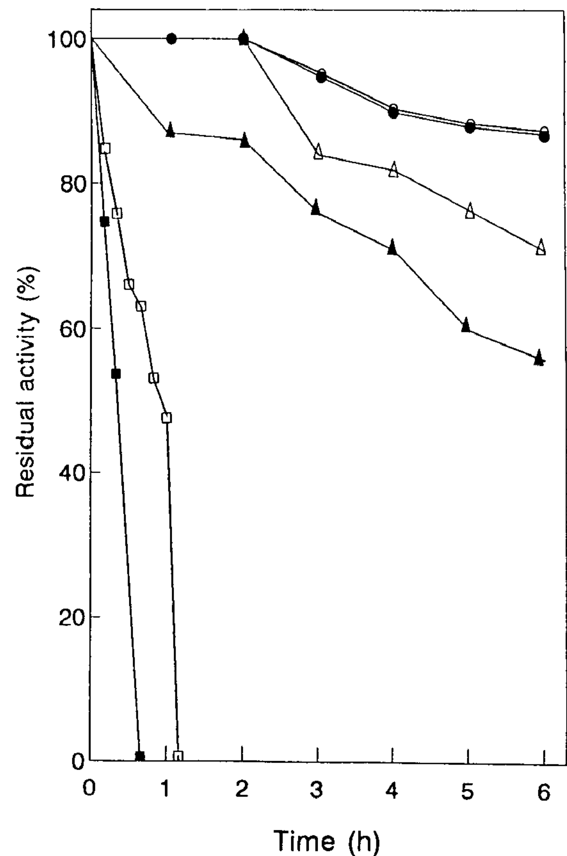

Fig. 7. Stability of CMCase activity of *E. coli* DH5α (pDS6) at various temperatures.
Symbols: 35°C, ○; 45°C, ●; 55°C, △; 60°C, ▲; 65°C, □; 70°C.

The CMCase activity in the extracellular, periplasmic, and intracellular fractions were 55, 20, and 26%, respectively.

DISCUSSION

Isolation and identification of *Actinomyces* sp. from Korean native goat, an anaerobic cellulosic rumen bacterium, was first reported by Park (K.M. Park. 1986. Ph. D. dissertation, University of Sung Kyun Kwan, Seoul). This bacterium was found predominantly in the rumen of Korean native goat and showed high CMCase activity in the culture supernatant. The endoglucanase gene from *Actinomyces* sp. 40 was cloned into *E. coli* DH5α using pUC19. Although the endoglucanase genes from anaerobic ruminal bacteria have been reported to be cloned and expressed in *E. coli* (3, 18, 22), but *Actinomyces* sp., has not been reported for the cloning and expression in *E. coli*. *We obtained two transformants which carried plasmids containing Actinomyces* sp. 40 endoglucanase gene. The two plasmids shared a common 3.5 kb fragment, which may contain the endoglucanase gene. Recently, we also isolated an independent gene from *Actinomyces* sp. 40 by the Southern analysis with pDS6 insert as the probe (data not shown). By partial digestion of pDS2 with *Eco*RI, we were able to obtain an endoglucanase positive clone contained on a 2.0 kb fragment. The subcloned plasmid (pDS6) possessed slightly higher CMCase activity than the cloned pDS1.

The reported optimum temperature of the cloned endoglucanases from the cellulolytic rumen bacteria is below 50°C (12, 25). The cloned endoglucanases from *R. albus* (11), *Bacteriodes succinogenes* (26) and other cellulolytic rumen bacteria are unstable above 55°C. Therefore, the results of the present study indicate that the cloned endoglucanase of *Actinomyces* sp. 40 is slightly more thermostable and active at higher temperatures. The optimal pH for the cloned enzyme activity and the pH range for enzyme stability of the cellulolytic rumen bacteria are in the range of pH 6.0 to 7.0. For example, the cloned endoglucanase enzyme from *R. albus* (12) and *Bacteriodes ruminicola* B14 (17) are stable pH 6.0 and 6.7, respectively. As for the enzyme stability reported from other sources, the cloned endoglucanases are stable between pH 7.5 and 10.5 (11), and at pH 7.0 (14). On the other hand, the cloned endoglucanase of *Actinomyces* sp. 40 showed stability and high activity at pH 5.0 to 7.0. In detecting enzymatic degradation of different substrate, the cloned enzyme degraded CMC but did not degrade xylan, cellobiose, and MUC. This result suggests

that the cellulolytic enzyme expressed by the cloned gene may be an endoglucanase.

The endoglucanases of *Bacteriodes succinogenes* (9), *R. albus* (26), and *R. flavefaciens* (2) were reported to be secreted into the supernatant. In the recombinant *E. coli* carrying pDS6, 54% of the enzyme activity was found in the extracellular fraction. Ohmiya *et al.* (21) reported that 20% of the cellulase activity was secreted into the medium in *E. coli* HB101 transformed with pRA1.

We are presently carrying out further characterization of the endoglucanase, structure of gene, and cloning of exoglucanase and β-glucosidase in addition to β-1,4-endoglucanase for the effective bioconversion of cellulosic materials.

Acknowledgement

This research was supported by the Animal Resources Research Center, Kon-Kuk University, Seoul, Korea.

REFERENCES

1. Ashwell, G. 1957. Colorimetric analysis of sugars. *Methods Enzymol.* **3**: 73-105.
2. Barros, M.E. and J.A. Thomson. 1987. Cloning and expression in *Escherichia coli* of a cellulase gene from *Ruminococcus flavefaciens. J. Bacteriol.* **169**: 1760-1762.
3. Berger, E., W.A. Jones, D.T. Jones, and D.R. Woods. 1989. Cloning and sequencing of an endoglucanase (endl) gene from *Butyrivibrio librisolvens* H170. *Mol. Gen. Genet.* **219**: 193-198.
4. Bryant, M.P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**: 1809-1813.
5. Bryant, M.P. and I.M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. *J. Bacteriol.* **84**: 605-614.
6. Caldwell, D.R. and M.P. Bryant.1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. *Appl. Microbiol.* **14**: 794-801.
7. Cornelis, P., C. Digneffe, and K. Willemot. 1982. Cloning and expression of a *Bacillus coagulans* amylase gene *Escherichia coli. Mol. Gen. Genet.* **186**: 507-511.
8. Faure, E., C. Bagnara, A. Belaich, and J.P. Belaich. 1988. Cloning and expression of two cellulase genes of *Clostridium cellulyticum* in *Escherichia coli. Gene* **65**: 51-58.
9. Groleau, D. and C.W. Forsberg. 1981. The cellulolytic activity of the rumen bacterium *Bacteroides succinogenes. Can. J. Microbiol.* **27**: 517-523.
10. Howard, G.T. and B.A. White. 1988. Molecular cloning and expression of cellulase gene from *Ruminococcus albus* 8 in Escherichia coli bacteriophage λ. *Appl. Environ. Microbiol.* **54**: 1752-1755.
11. Ishizaki, A. and H. Kawauchi.1988. Molecular cloning of a thermophilic alkanine cellulase gene in *Escherichia coli. Agri. Biol. Chem.* **52**: 2937-2939.
12. Kawai, S., H. Honda, T. Tanase, M. Taya, S. Iijima, and T. Kobayashi. 1987. Molecular cloning of *Ruminococcus albus* cellulase gene. *Agri. Biol. Chem.* **51**: 59-63.
13. Lee, J.K., K.H. Yoon, B.H. Kim, G.S. Kwon, and S.B. Kim. 1992. Cloning and expression in *Escherichia coli* of cellulase genes from a mesophilic *Clostridium* sp. *J. Microbiol. Biotechnol.* **2**: 50-55.
14. Lejeune, A., S. Courtois, and C. Colson. 1988. Characterization of an endoglucanase from *Pseudomonas fluorescens* subsp. *cellulosa* produced in *Escherichia coli* and Regulation of the expression of its cloned gene. *Appl. Environ. Microbiol.* **54**: 302-308.
15. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein mearsurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
16. Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. "Molecular cloning", Cold Spring Harbor, Laboratory, N.Y., pp. 363-402.
17. Matsushita, O., J.B. Russell, and D.B. Wilson. 1990. Cloning and sequencing of a *Bacteriodes ruminicola* B14 endoglucanase gene. *J. Bacteriol.* **172**: 3620-3630.
18. McGavin, M.J., C.W. Forsberg, B. Crosby, A.W. Bell, D. Dignard, and D.Y. Thomas. 1989. Structure of the cel-3 gene from *Fibrobacter succinogenes* S85 and characteristics of the encoded gene product, endoglucanase 3. *J. Bacteriol.* **171**: 5587-5595.
19. Miller, G.L., R. Blum, W.E. Glennon, and A.L. Burton. 1960. Measurement of carboxymethycellulase activity. *Anal. Biochem.* **1**: 127-132.
20. Min, H.K., Y.J. Choi, C.K. Ha, K.K. Cho, Y.M. Kwon, Y.H. Chang, and S.S. Lee. 1994. Isolation and identification of anaerobic rumen bacterium, *Actinomyces* sp. 40 and enzymatic properties of β-1,4-endoglucanse. *Asian-Australasian J. Animal Sciences.* in press.
21. Ohmiya, K., K. Nagashima, J. Kajino, E. Goto, A. Isukada, and S. Shimizu. 1988. Cloning of the cellulase gene from *Ruminococcus albus* and its expression in *Escherichia coli. Appl. Environ. Microbiol.* **54**: 1511-1515.
22. Ohmiya, K., T. Kajino, A. Kato, and S. Shimizu. 1989. Structure of a *Ruminococcus albus* endo-1,4-β-glucanase gene. *J. Bacteriol.* **171**: 6771-6775.
23. Pettipher, G. and M.J. Latham. 1979. Characteristics of enzymes produced by *Ruminococcus flavefaciens* which degrade plant cell walls. *J. Gen. Microbiol.* **110**: 21-27.
24. Saito, H. and K. Miura. 1963. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta.* **72**: 619-629.
25. Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**: 503-517.
26. Taylor, K.A., B. Crosby, M. McGavin, C.W. Forsberg, and D.Y. Thomas. 1987. Characteristics of the endoglucanase encoded by a *cel* gene from *Bacteroides succinogenes* expressed in *Escherichia coli. Appl. Environ. Microbiol.* **53**: 41-46.

(Received January 28, 1994)