

# CELLULOSE-DEPENDENT AND PENICILLIN-RESISTANT PLASMIDS ISOLATED FROM *RUMINOCOCCUS ALBUS*

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## Introduction

*Ruminococcus albus*, a rumen anaerobe, is found to be a potent cellulolytic bacteria dominant in the rumen of cattle which are important economically because they supply us a large amount of meat, milk, leather etc. with high quality by taking cellulosic materials only. One of the growth-limiting factors of cattle is considered to be the cellulose degrading rate of rumen microorganisms. But cellulolytic rate of *R. albus*, *in vitro*, is quite variable depending on cultivation conditions such as presence or absence of insoluble cellulose in the medium, speculating the presence of plasmids in *R. albus* connected with some factors concerning cellulose degradation. However, little is known about plasmids of *R. albus*. In the present study, isolation and specification of plasmids in *R. albus* has been described.

## Materials and Methods

*R. albus* F-40, isolated from bovine rumen, was cultivated in an anaerobic medium containing 1.5% ball-milled cellulose (BMC medium) or 1% cellobiose (cellobiose medium) at pH 6.8 and 37°C. The host strain *Escherichia coli* HB101 and a plasmid pACYC 184 were used. Transformants were cultivated in LB broth containing antibiotics at 37°C, pH 7.5 under aeration. Plasmid of *R. albus* was isolated by the method of Birnboim and Doly (1977). Restriction endonucleases and other manipulation enzymes were purchased from Boehringer Mannheim (Tokyo). Separation and molecular size estimation of DNA fragments were performed by electrophoresis with agarose gel (0.7%) for DNA.

## Results and Discussion

### Isolation of plasmid from *R. albus*

In a plasmid preparation from *R. albus* culti-

vated in BMC medium, two plasmids were detected and denoted as pRAB and pRAC, respectively.

### Restriction maps of plasmids

The restriction maps of pRAB and pRAC were determined as shown in figure 1. The sizes of pRAB and pRAC were evaluated to be 7.4 and 15.3 kb, respectively.

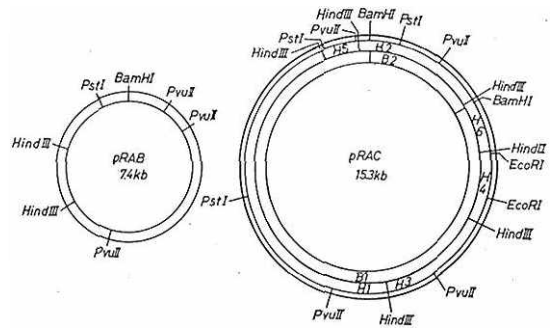


Figure 1. Restriction maps of pRAB and pRAC. H1 ~ H6 are the *Hind* III fragments of pRAC. B1 and B2 are the *Bam*HI fragments of pRAC.

### Cellulose dependent plasmid

Plasmid preparations B and C were isolated from *R. albus* adapted well in the media containing ballmilled cellulose and cellobiose, respectively. Preparation BC was obtained from the organism transferred from BMC medium into cellobiose one and cultivated overnight. These preparations B, BC and C were digested with *Bam*HI and analyzed electrophoretically as shown in figure 2. In the preparations B, and BC, three DNA bands with 11.8. (arrow 1), 7.4 (arrow 2) and 3.5 kb (arrow 3) were present (figure 2-B and 2-BC) and only two bands with 11.8 and 3.5 kb were present in the preparation C. From these results, it is clearly shown that the band with 7.4 kb, pRAB, was excluded from the preparation

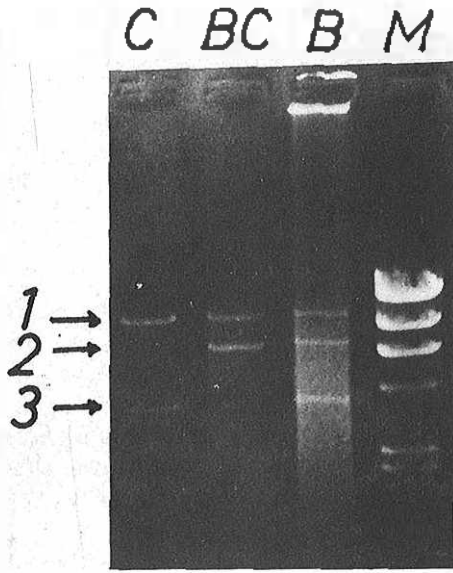


Figure 2. Agarose gel electrophoregrams of plasmid preparations of B, BC and C, all of which were digested with *Bam*HI. M is the size marker ( $\lambda$  DNA/*Hind*III).

B. This suggests that *R. albus* lose pRAB when cultivated in the cellobiose medium. Therefore, pRAB was a cellulose dependent plasmid in

*R. albus*

#### Penicillin resistant plasmid

pRAC was completely hydrolyzed with *Hind*III or *Bam*HI. Each of the hydrolyzates was ligated into pACYC 184 for preparing gene bank of pRAC. With the gene bank, transformants were cultivated in LB broth containing chloramphenicol (30  $\mu$ g/ml) and penicillin (100  $\mu$ g/ml). The transformant having a fragment B2 of pRAC (figure 1) could grow well in the medium containing both antibiotics. From this result, it is clearly shown that pRAC contains a penicillin resistant factor in the fragment B2.

In conclusion, from *R. albus*, we could isolate two novel plasmids, pRAB and pRAC encoding a cellulose dependent factor and a penicillin resistant one, respectively.

(Key Words: Plasmid, Cellulose, *Ruminococcus Albus*)

#### Literature Cited

- Birnboim, H.C. and J. Doly. 1976. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7:1513-1523.