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CHARACTERIZATION OF THE REPLICON FROM PLASMID pAC1 FROM *Acetobacter pasteurianus*

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A panel of recombinant plasmids pACK5 and pACT7 was prepared by introducing kanamycin and tetracycline resistance into the partially split plasmid pAC1 which contained replicon isolated from *Acetobacter pasteurianus*. The replicon in plasmid pAC1 is compatible with the ColE1 replicon. Compared to pBR322, the plasmid had more than 30 copies per chromosome in *Escherichia coli* cells. Plasmids were transformed into *E. coli* DH1, *Acetobacter pasteurianus* 3614, *Acetobacter aceti* 3620, *Shigella*, *Citrobacter*, and *Brevibacterium flavum* cells, and the stability of plasmid DNA was tested after cultivation in nonselective conditions.

Acetobacter spp. are Gram-negative bacteria many of which have industrial applications. These bacteria can utilize different saccharides, such as glucose and sorbitol. They metabolize glucose to yield acetic acid and sorbose metabolism produces gluconic acid.

Several strains of *A. aceti*, *A. albuminosum*, *A. xylinus*, *A. aurrantius* and *A. pasteurianus* were reported to have a panel of plasmid DNA [1]. A cell may contain one to eight different molecules of plasmid DNA with a molecular wight of 0.9-17x10^6. Plasmids pMV101 and pMV102 were isolated from two *A. aceti* strains [2]. Plasmid pAC1 was isolated and characterized from *A. pasteurianus* [3]. These plasmids are suitable for the preparation of cloning vectors for which cryptic plasmids pAT5001A and pTA5001B had been utilized [1].

This paper presents the results obtained in the preparation of recombinant plasmids by introducing genes responsible for kanamycin or tetracycline resistance and the replicon from the pAC1 plasmid isolated from *A. pasteurianus*.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and plasmids.
E. coli DH1 [4] and *Shigella* were cultivated in LB medium [5] at 37 °C, *A. pasteurianus* 3614 and *A. aceti* 3620 in YPG culture medium (containing g/l: peptone 3 g, yeast extract 5 g and glucose 20 g) [1], at 28 °C. *Citrobacter* sp. and *B. flavum* ATCC 21474 strain were grown in LB media at 30 °C. Cultivation medium were supplemented at the following concentrations: ampicillin at 50 μg/ml, kanamycin at 15-30 μg/ml, and tetracycline 15 μg/ml. Plasmids used are listed in Table 1.

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Table 1

Plasmids used in this work

Plasmid	Phenotype	References
pAC1		Grones [2]
pACK5	Km^r	this paper
pACT7	Tc^r	this paper
pUC4-KAPA	Ap^r, Km^r	Barany [12]
pBR322	Ap^r, Tc^r	Bolivar et al. [13]
pLAFR1	Tc^r	Friedman et al. [14]

Transformation.
E. coli DH1 cells were transformed according to the method of Mandel and Higa [6]. *A. pasteurianus* and *A. aceti* were transformed by the method of Grones and Turňa [7] and *Shigella, Citrobacter* and *B. flavum* were transformed by electroporation.

Electroporation.
Bacterial cells in the logarithmic phase were harvested from liquid culture by centrifugation. The pellet either was resuspended in the small volume of sterile water to yield concentrated cells for use in electroporation experiments or was washed three times in water prior to electroporation. Cells were suspended in 1/100 volume to original culture volume. Electroporation of plasmid DNA was done according to Kilbane and Bielaga [8].

Isolation of plasmid DNA.
Plasmid DNA was isolated from *E. coli* DH1, *Shigella*, and *Citrobacter* cells according to the method of Birnboim and Doly [9] and from *Acetobacter* cells using the method of Grones and Turňa [7]. Plasmid DNA from *Brevibacterium* was isolated by the method of Jucovič et al. [10].

Determination of vector stability.
The stability of recombinant plasmids prepared in *E. coli, Acetobacter, Citrobacter, Shigella* and *Brevibacterium* cells was evaluated as follows. Cells containing plasmids were allowed to grow on liquid selective medium. Grown cultures were ground in solid media with antibiotic. One colony was used to inoculate liquid medium without the antibiotic and cultivated 24 h at 30 °C to 37 °C. Culture was repeated by transferring inoculum from the culture without the antibiotic at 24-h intervals. After this, 100 colonies that failed to grow were counted. Plasmid DNA was isolated from all positive colonies.

RESULTS AND DISCUSSION

Construction of recombinant plasmids.

Large plasmid pAC1 isolated from *A. pasteurianus* [1] was used to prepare new recombinant plasmids. Plasmid pAC1 was cleaved with restriction endonuclease EcoRI and the fragment containing replicon was ligated with the EcoRI fragment coded with

kanamycin resistance from the pUC4-KAPA cassette vector or EcoRI-PvuII fragment coded tetracycline gene from pBR322. Recombinant plasmids pACK5 with kanamycin resistance and pACT7 with tetracycline resistance were prepared (Fig 1). A panel of the recombinant plasmids pACK5 and pACT7 was chosen with a view to the molecular weight.

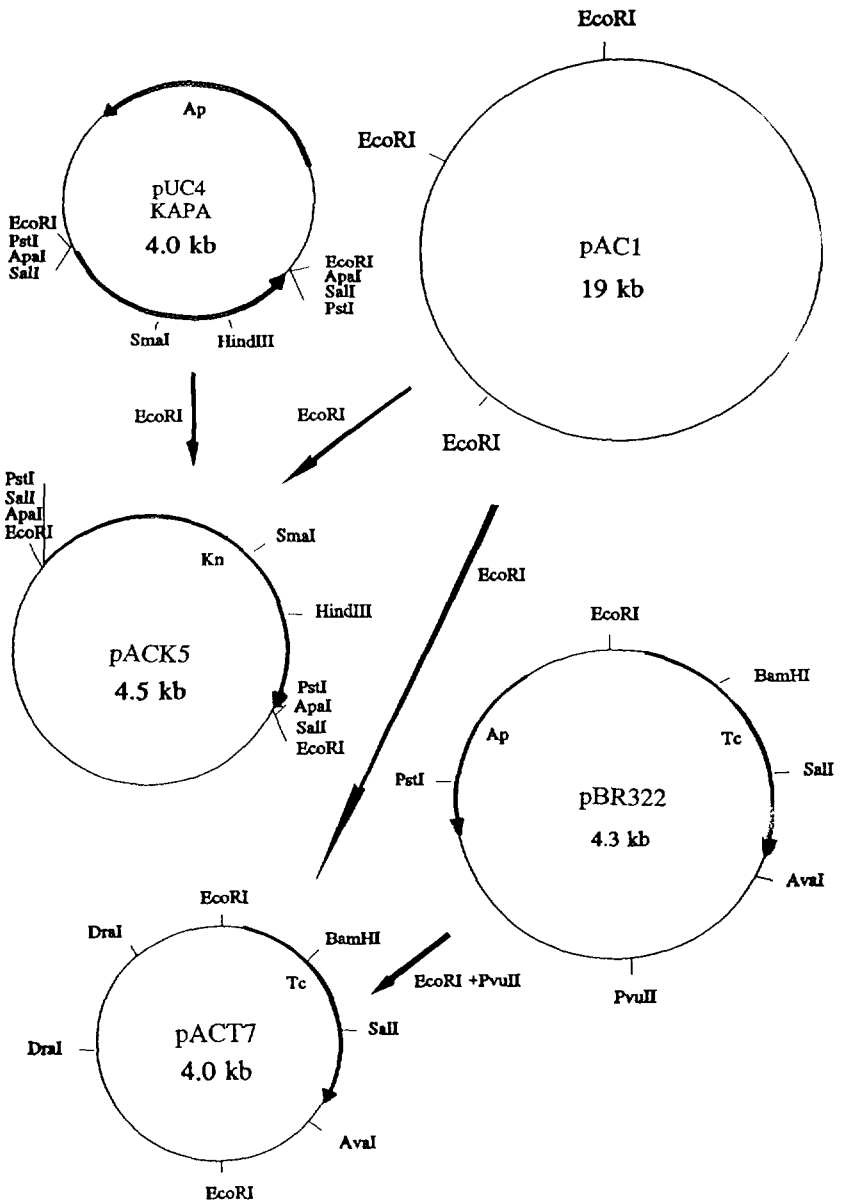

Fig. 1.
Schematic representation of the construction of recombinant plasmids from plasmid pAC1 from *Acetobacter pasteurianus* cells.

Determining the number of plasmid copies.

The number of plasmid copies was evaluated by comparing a known plasmid standard with a tested plasmid. Plasmid pBR322, which is compatible with pACK5 and has an average of 18 copies per *E. coli* chromosome [11], was used as the reference standard. Plasmid DNA isolated from *E. coli* DH1 cells (pBR322, pACK5) was separated by electrophoresis in 0.7 % agarose gel. Having scanned the electrophoretogram negative at 560 nm, we determined that plasmid pACK5 had more than 30 copies per chromosome compared to pBR322.

Determining the stability of recombinant plasmids.

The stability of recombinant plasmids was tested in *E. coli* DH1, *A. pasteurianus* 3614, *A. aceti* 3620, *Citrobacter, Shigella,* and *Brevibacterium* according to the procedure described in Materials and Methods. Cells were transformed by plasmids pACK5 and pACT7 and cosmid pLAFR1. As shown in Fig 2 pACK5 was the most stable after 120 h cultivation on media in the absence of antibiotics. Plasmid pACK5 with the kanamycin resistance gene was stabile in *A. pasteurianus* 3614, *E. coli* DH1, *Citrobacter, Shigela*, and *B. flavum.* Plasmid pACK5 had lower stability in *A. aceti* 3620 cells. Vector pACT7 with the tetracycline resistance gene was stabile in *E. coli* DH1, *A. pasteurianus* 3614, *A. aceti* 3620, *Citrobacter, Shigella,* and *B. flavum* with less than 80 % stability in *A. aceti* 3620. The stability of cosmid pLAFR1 was as high as 80 % in *E. coli* DH1, *A. pasteurianus* 3614, and *A. aceti* 3620, *Citrobacter a*nd less than 80 % in *Shigella* and *B. flavum* as shown in Fig 2.

Purified plasmid DNA from *A. aceti* cells was used to prepared shuttle vectors. They were replicated into *E. coli* and *Acetobacter* spp cells. These vectors had the ColE1 replicon from pBR322 or the replicon from pACYC174 and the replicon from *A. aceti* plasmid [2].

Recombinant plasmids pACK5 and pACT7, presented in this paper, have the replicon from pAC1 only. Plasmids were transformed and replicated in different bacterial strains. The replicon is very strong, because after transformation into different cells it replicated and was very stable after cultivation under nonselective conditions. The stability of recombinant plasmids transformed into the cells was dependent on the type of bacterial cell. Vectors were the most stable in *A. pasteurianus* and *E. coli* cells. The possibility of transforming and replicating pACK5 and pACT7 plasmids by electroporation into *Brevibacterium* cells and the plasmid stability in these cells are very interesting. The

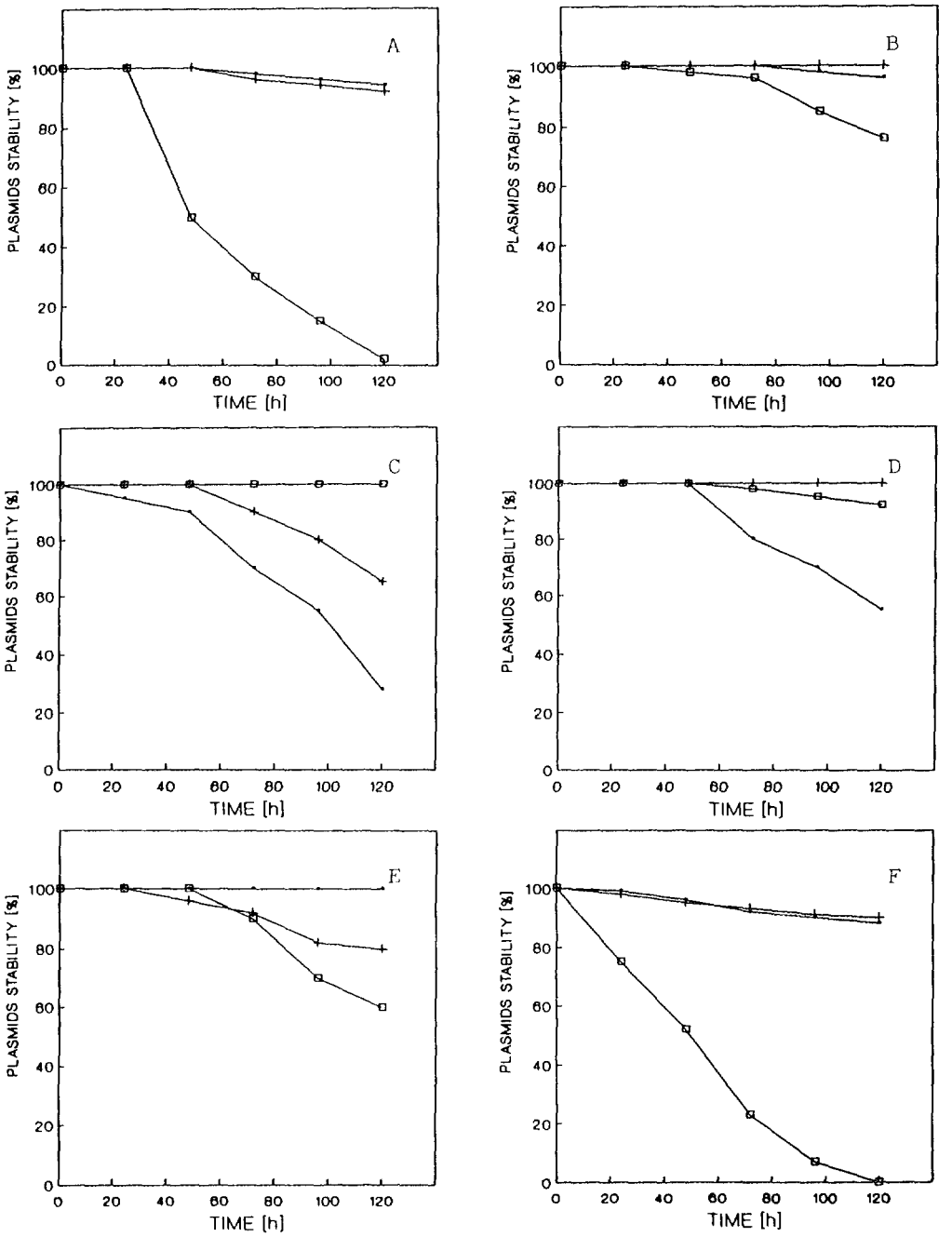

Fig.2.
Stability of recombinant plasmids -⊙- pACK5, -+- pACT7 and -□- pLAFR1 in nonselective media in different strains; A - *Escherichia coli* DH1, B - *Acetobacter pasteurianus* 3614, C - *Acetobacter aceti* 3620, D - *Citrobacter*, E - *Shigella*, F - *Brevibacterium flavum* 21474.

stability of these recombinant plasmids in *B. flavum* cells under nonselective conditions was similar to the stability of the vector used for cloning into *Brevibacterium* cells [10].

The new replicon from pAC1 had a higher copy number and higher stability after cultivation of cells in nonselective media.

REFERENCES

1. Okumura, H., Uozomi, T., and Beppu, T. (1985) Agric. Biol. Chem., 1011-1017.
2. Fukaya, M.,Okumura,T., Masai, H., Ouzumi,T., and Beppu, T. (1985) Agric. Biol. Chem. 49, 2083-2090.
3. Grones, J., Škereňová, M., Baderková, K., and Turňa, J. (1989) Biológia (Bratislava) 44, 1191-1196.
4. Low, G. (1968) Proc. Natl. Acad. Sci.U.S.A. 60, 160-168.
5. Davis, R.W., Botstein, D., and Roth, J.R. (1980) Advance Bacterial Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
6. Mandel, M., and Higa, A. (1970) J. Mol. Biol. 53, 154-174.
7. Grones, J., and Turňa, J. (1992) Folia Microbiologica 37, In press.
8. Kilbane II, J.J., and Bielaga, B.A. (1991) Biotechniques 354-365.
9. Birnboim, H.C., and Doly, J. (1979) Nucl.Acid Res. 7, 1513-1523.
10. Jucovič, M., Timko, J., Barák, I., Bučko, M., and Zelinka, J. (1987) Biológia (Bratislava) 42, 1149-1153.
11. Balbas, P., Somerso, X., Merini, E., Zurita, M., Lomeli, H., Vale, F., Flores, N., and Bolivar, F. (1986) Gene 50, 3-40.
12. Barany, F. (1985) Gene 37, 111-119.
13. Bolivar, F., Rodriguez, R.L., Greene, P.J., Belach, N.C., Heynecker, H.L., Boyer, H.W., Crosa, J.H., and Folkow, S. (1977) Gene 2, 95-106.
14. Friedman, A.M., Long, S.R., Brown, S.E., Buikema, W.J., and Ausubel, F.M. (1982) Gene 18, 289-296.