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Molecular Cloning of β-Galactosidase Gene from *Neisseria lactamica* 2118 into *Escherichia coli* MC 1061

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Neisseria lactamica 2118의 β-galactosidase 유전자의 대장균으로의 클로닝

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Neisseria lactamica 2118 의 β-galactosidase 유전자를 Southern hybridization 과 colony hybridization 을 통하여 *Escherichia coli* MC 1061에 클로닝 시켰다.

β-Galactosidase 유전자를 함유하는 6.5 Kb EcoR 1 단편과 7.2 Kb BamH 1 단편들을 pMC 1871의 lac Z 유전자를 probe로 한 Southern hybridization으로 얻고 이들을 pBR 322에 삽입한후 *Escherichia coli* MC 1061에 형질전환 시키고 이들 형질전환체들을 동일 probe로 colony hybridization 시켜 최종적으로 3주의 β-galactosidase positive clone들을 얻었다.

이들의 재조합 plasmid에는 *Neisseria lactamica* 2118 염색체 DNA의 약 7.2Kb BamH 1단편이 삽입되어 있음을 확인 하였고 probe와 상동성이 가장 강한것으로 추정되는 pNL 24에 대한 제한효소지도를 작성 하였다.

The gene coding for β-galactosidase of *Neisseria lactamica* 2118 was cloned into *Escherichia coli* MC 1061. The isolated 6.5 Kb EcoR 1 fragment and 7.2 Kb BamH 1 fragment of chromosomal DNA in Southern hybridization were ligated to a vector plasmid pBR322 and then transformed into *Escherichia coli* MC 1061 cells. Finally, I obtained three clones as β-galactosidase positive clone by colony hybridization and Southern hybridization(β-galactosidase probe; lac Z gene of pMC1871). Three recombinant plasmids(pNL13, 17 and 24) were found to contain the 7.2Kb BamH 1 fragment originated from *Neisseria lactamica* 2118 chromosomal DNA by Southern hybridization and pNL 24 was showed high homology to probe especially and also its physical map was constructed.

Keywords : Molecular cloning, β-galactosidase, *Neisseria lactamica* 2118, Southern hybridization, colony hybridization

Introduction

I previously reported on purification and properties of β-galactosidase from *Neisseria lactamica* 2118 by p-aminophenyl-β-D-thiogalactopyranoside agarose affinity chromatography.[1] The enzyme was constitutive enzyme, not induced by lactose and IPTG. Optimal activity of the purified enzyme was observed at 35℃ and pH 7.5, and was stable at the range of pH 6.0-9.0 and at temperature below 50℃.

β-Galactosidase(EC 3.2.1.23) which hydrolyses β-1,4-D-galactosidic linkage of lactose is important in dairy industry[2] and medical industry[3] since it has been widely used for producing lactose-free milk and hydrolysing lactose in whey to produce glucose and galactose, and to cure indigestion by lactose intolerance etc.[4~5]

Meanwhile, *Neisseria lactamica* is pathogenic bacterium which cause a frank meningitis or a septicema[6] and also involves in several disease process in children,[7~8] but it have a β-galactosidase activity and lactose fermentability.

In order to secure a new β-galactosidase gene and produce a large amount of the gene, I cloned a β-galactosidase gene of *Neisseria lactamica* 2118 into *Escherichia coli* MC 1061.

Materials and Methods

1. Bacterial strains and media

Neisseria lactamica 2118 described previously[1] was used as donor strain of β-galactosidase gene and cultured in HIY-C medium under strict anaerobic condition at 37℃. *Escherichia coli* MC 1061 which was not able to produce β-galactosidase, was used as host strain in transformation with recombinant DNA and cultured in LB broth(tryptone 10g, yeast ext.5g, NaCl 10g per liter, pH 7.0) at 37℃ on reciprocal shaker.

pBR 322 and pUC 13 were used as a cloning vectors, and pMC 1871 containing β-galactosidase gene was used as a probe in Southern hybridization and colony hybridization(Table 1).

2. DNA preparation and manipulation

Chromosomal DNA of *Neisseria lactamica* 2118 was prepared according to the method of Marmur[9] and Thomas et al.[10]

Plasmid DNA was prepared rapidly by the method of Birnboim and Doly,[11] and according to the method of Maniatis et al.[12] CsCl-ethidium bromide equilibrium density gradient centrifugation

Table 1. Bacterial strains and plasmids used in this study

Strains and plasmids	Genotype	Source or reference
Neisseria lactamica 2118	β-Galactosidase(+)	Natl.Res.Coun.Canada Biotech.Res.Inst.
Escherichia coli MC 1061	Δ(lac IPOZYA)X74 gal U^-,gal K^-, str A^r, hsd R^-, ara D 139, hsm^+, Δ(ara-leu)7697,hsr^-	
pBR 322	Ap^r, Tc^r	Bolivar,F.et al Gene 2 : 95(1977)
pMC 1871	β-Galactosidase probe in Southern and colony hybridization	Shapira,S.K.et al Gene 25 : 71(1983)

was used to prepare and purify plasmid DNA in a large scale. Gene clean kit(BIO 101 Inc., USA) was used for DNA purification from agarose gel.

Digestion of DNA with restriction endonuclease, electrophoresis(0.6-1.0% agarose gel) and transformation were performed as described by Maniatis et al.[12] β-Galactosidase-producing transformanants were detected as ampicillin resistant and blue colony from LB agar plate containing 100μg per ml of ampicillin, 2.0% 5-bromo-4-chloro-3-indolyl-β-D-galactoside(X-gal) and 10^{-3}M isopropylthio-β-D-galactopyranoside(IPTG).

3. Southern hybridization and colony hybridization

β-Galactosidase probe(lac Z of pMC 1871) was labelled with ($\alpha - {}^{32}$p)-dCTP by a random primed DNA labelling kit from Boehringer.[13]

Restriction enzyme digestions, Southern[13] and colony hybridization were performed as described in Maniatis et al[12] using Transfer Membranes(Hybond-N™) and Millipore Filter(HATF type).

4. Enzyme assay

β-Galactosidase activity was estimated as reported previously.[1]

Results and Discussion

1. Cloning of β-galactosidase gene of *Neisseria lactamica* 2118

Neisseria lactamica 2118 chromosomal DNA was partially digested with Hind III and EcoR 1, and ligated to dephosphorylated Hind III-cut pUC 13 and EcoR 1-cut pBR 322 with T_4 DNA ligase and transformed into *Escherichia coli* MC 1061.

I obtained 59,690 transformanants in Hind III fragments inserted in pUC 13 and 36,259 transformanants inserted in pBR 322. But I failed to obtain a β-galactosidase positive clone(blue colony) in selective medium which supplemented with ampicilline, X-gal and IPTG. So, I attempted to cloning the β-galactosidase gene by Southern and colony hybridization.

Chromosomal DNA of *Neisseria lactamica* 2118 was partially digested with BamH 1, EcoR 1 and Hind III, and all of fragments were hybridized with the end-labelled β-galactosidase probe. 6.5Kb EcoR 1 fragment and 7.2 Kb BamH 1 fragment were hybridized to probe(Fig.1).

Their fragments were ligated to pBR 322 and transformed into *Escherichia coli* MC 1061. Out of 2,800 colonies transformed, I obtained three clones finally as β-galactosidase positive clones by colony and Southern hybridization. The autoradiogram of positive clones are shown in Fig. 2, 3and 4.

I identified to insert the 7.2Kb BamH 1 fragments of *Neisseria lactamica* 2118 chromosomal DNA into BamH 1 site of three recombinant plasmids(pNL 13,17 and 24)(Fig.5) and among them,pNL 24 was showed high homology to β-galactosidase probe.

2. Restriction endonuclease map of recombinant plasmid pNL 24

For mapping restriction sites of the insert, pNL 24 was digested with Acc 1, Ava 1, Bgl 1, Hind II, Rsa 1 and BamH 1. The fragments were electrophoresed on 0.8% agarose gel and then hybridized with β-galactosidase probe. The 7.2Kb fragment had a single restriction site for Acc 1, Ava 1, Bgl 1 and Hind II, but the site for Rsa 1 was not found(Fig.6).

The restriction endonuclease map of the fragment is shown in Fig.7.

3. Subcloning of pNL 24 β-galactosidase gene

For subcloning of 7.2Kb Hind II fragment of pNL 24, the fragment was digested with Hind II and ligated with pUC 13 and transformed into *Escherichia coli* MC 1061, and I being further investigated on base sequence of the inserted β-galactosidase gene.

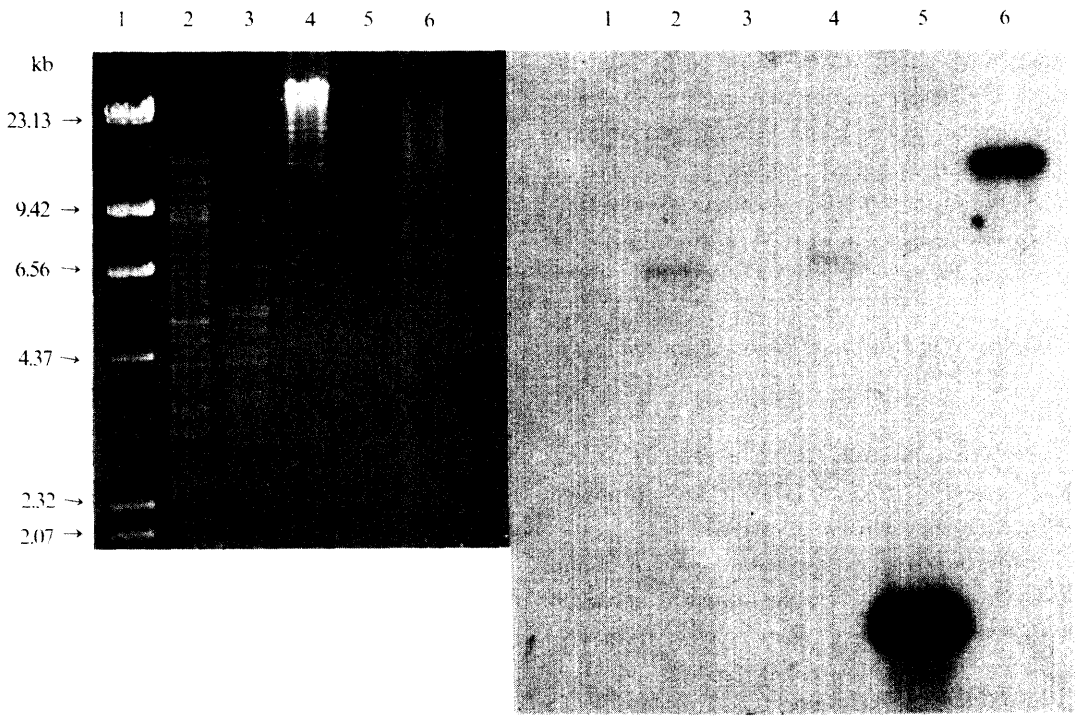

Fig.1. Homology between lac Z gene of pMC 1871 and chromosomal DNA of *N. lactamica* 2118

(A) Agarose gel(0.5%) electrophoresis of DNA digests.

Lane 1. λ DNA digested with Hind III as size marker.

2. *N. lactamica* 2118 chromosomal DNA digested with EcoR 1.

3. 〃 〃 Hind III.

4. 〃 〃 Bam H 1.

5. probe of lac Z of pMC 1871.

6. *E. coli* HB 101 plasmid DNA digested with EcoR 1.

(B) Hybridization analysis of the Southern transfer of the DNAs from gel A.

Lane 1 through 6 are the same as described in (A). The 32 p-labelled 3. 3kb EcoR 1 fragment(lac Z) of pMC 1871 was used as probe.

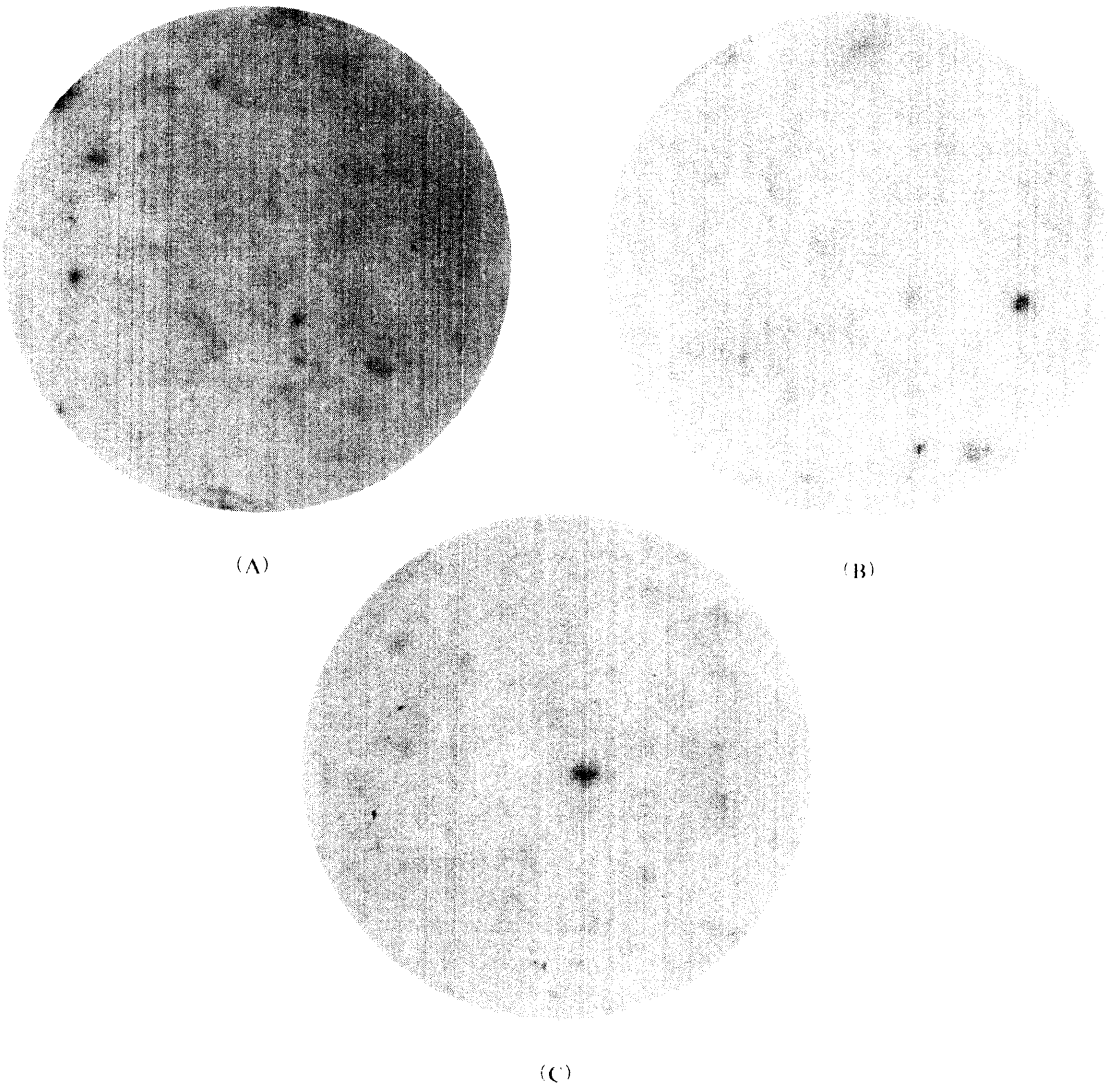

Fig.2. Homology between lac Z gene of pMC 1871 and DNA of transformanants.
(A)－(C) : 6.5kb EcoR 1 fragment of *N. lactamica* 2118 chromosomal DNA was cloned in pBR 322 and transformed into *E. coli* MC 1061.
The 32 p-labelled 3.3 kb EcoR 1 fragment(lac Z) of pMC 1871 was used as probe.

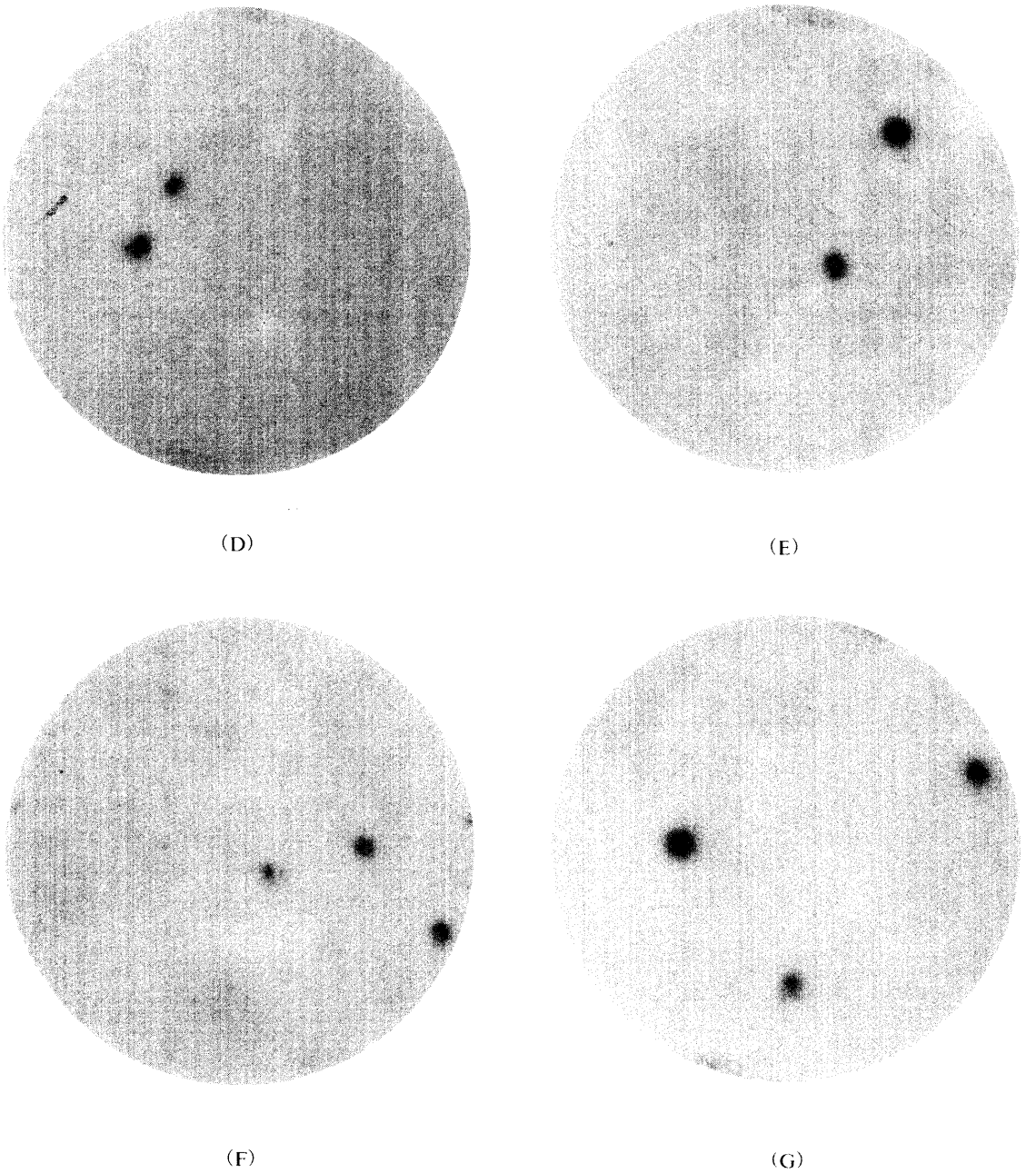

Fig.3. Homology between lac Z gene of pMC 1871 and DNA of transformanants.
(D)－(G) : 7.2 kb Bam H 1 fragment of *N. lactamica* 2118 chromosomal DNA was cloned in pBR 322 and transformed into *E. coli* MC 1061.
The 32 p-labelled 3.3 kb EcoRI fragment(lac Z) of pMC 1871 was used as probe.

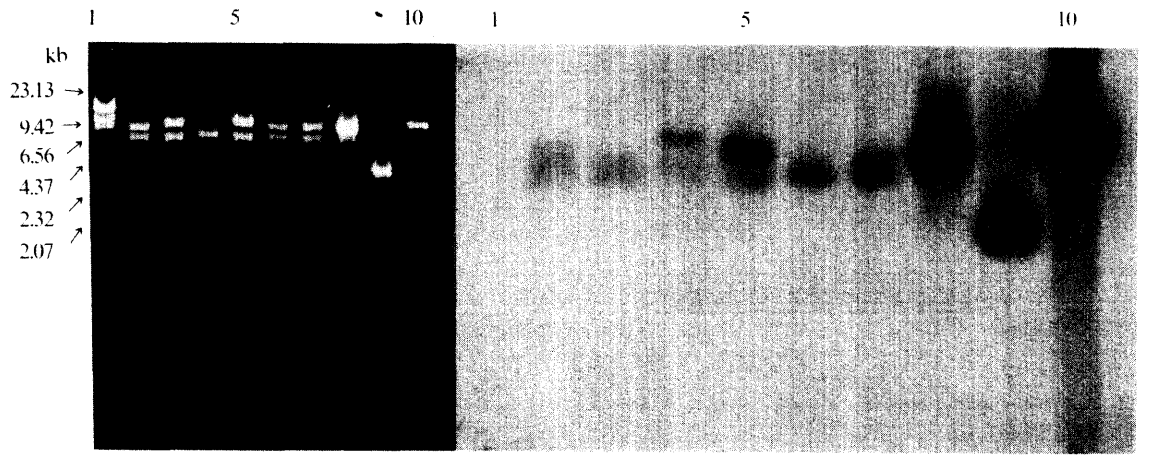

Fig.5. Homology between lac Z gene of pMC 1871 and plasmid DNA of cloned cells.

(A) Agarose gel(0.5%) electrophoresis of DNA digests.

Lane 1 : λ DNA digested with Hind III as size marker.

2 : plasmid DNA of #4 clone digested with Bam H 1.

3 : 〃 #8 〃 〃 .

4 : 〃 #13 〃 〃 .

5 : 〃 #17 〃 〃 .

6 : 〃 #19 〃 〃 .

7 : 〃 #23 〃 〃 .

8 : 〃 #24 〃 〃 .

9 : pUC 13 undigested.

10 : pSKS 104 lac Z gene.

(B) Hybridization analysis of the Southern transfer of the DNAs from gel A. Lane 1 through 10 are the same as described in (A). The 32 p-labelled 3.3 kb EcoR 1 fragment(lac Z) of pMC 1871 was used as probe.

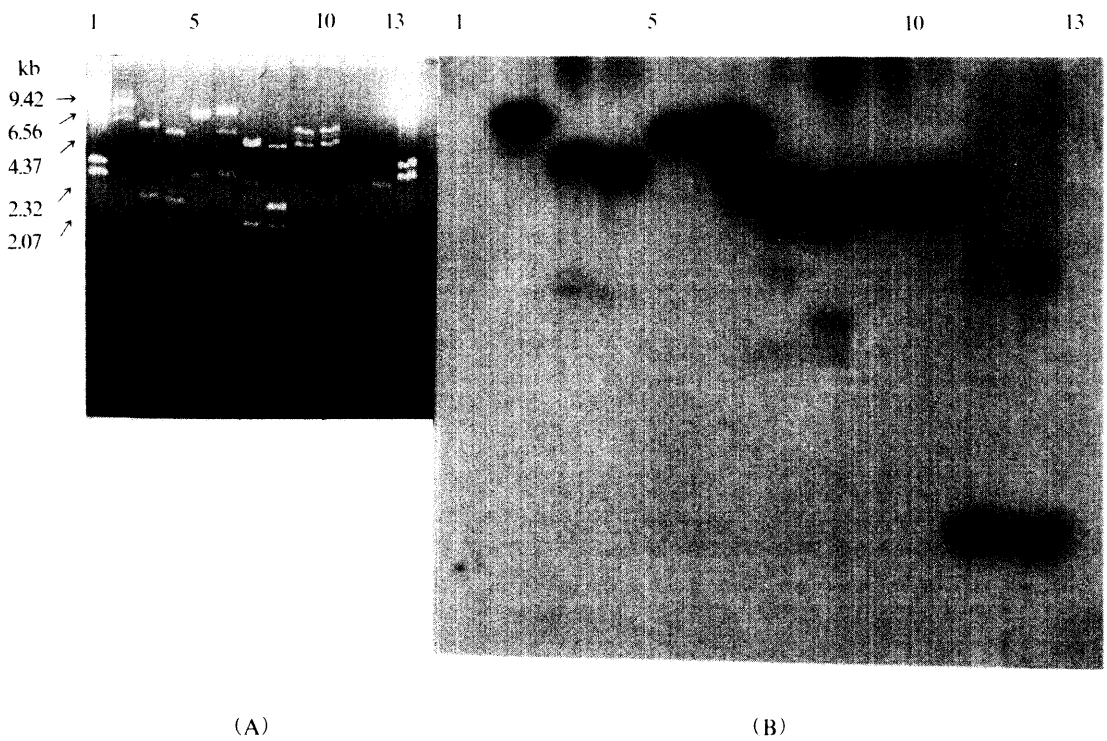

Fig.6. Homology between lac Z gene of pMC 1871 and plasmid DNA of #24 clone(pNL 24).

(A) Agarose gel(0.8%) electrophoresis of plasmid DNA digests.

Lane 1 : λ DNA digested with Hind III as size marker.
2 : plasmid DNA of #24 clone digested with Bam H 1.
3 : 〃 〃 Acc 1.
4 : 〃 〃 Acc 1 and Bam H 1.
5 : 〃 〃 Ava 1.
6 : 〃 〃 Ava 1 and Bam H 1.
7 : 〃 〃 Bgl 1.
8 : 〃 〃 Bgl 1 and Bam H 1.
9 : 〃 〃 Hind II.
10 : 〃 〃 Hind II and Bam H 1.
11 : 〃 〃 Rsa 1.
12 : 〃 〃 Rsa 1 and Bam H 1.
13 : DNA digested with Hind III as size marker.

(B) Hybridization analysis of the Southern transfer of the DNAs from gel A.
Lane 1 through 13 are the same as described in (A). The 32 p-labelled 3.3 kb EcoR 1 fragment(lac Z) of pMC 1871 was used as probe.

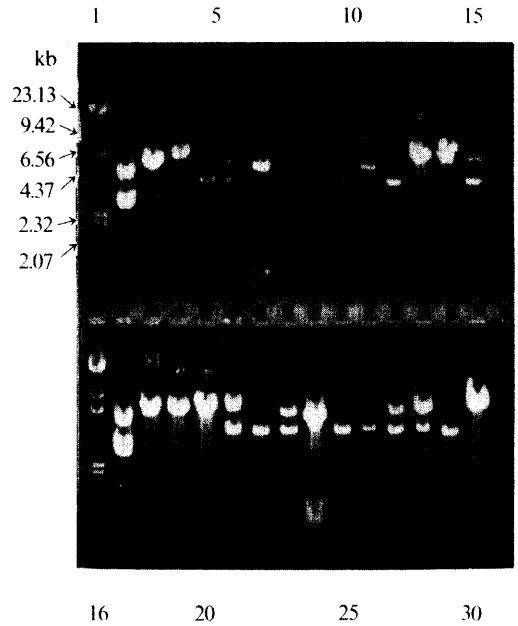

Fig.4. Agarose gel electrophoretic analysis of recombinant DNA of *N. lactamica* 2118.

Lane 1 and 16 : λ DNA digested with Hind III as size marker.
2 and 17 : pBR 322 undigested.
3 – 6 : recombinant DNA digested with Bam H 1.
7 – 12 : recombinant DNA digested with EcoR 1.
13 – 23 : recombinant DNA digested with Bam H 1.
24 – 27 : recombinant DNA digested with EcoR 1.
28 – 30 : recombinant DNA digested with Bam H 1.

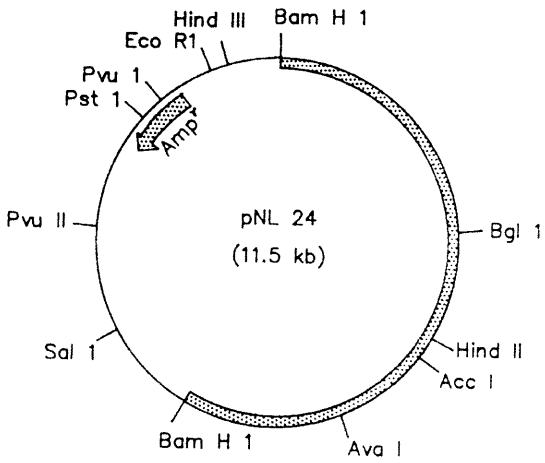

Fig.7. Endonuclease cleavage map of recombinant plasmid pNL 24
A Bam H 1 fragment cotaining β-gal gene fragment(closed thick line) was inserted into plasmid pBR 322.

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