大腸菌における，マルトースオペロンの遺伝子発現は，高圧力により，阻害される

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大腸菌におけるラムダファージによるプラークの形成が，加圧に従い抑制されることが観察され，30MPaの加圧下において，完全に抑制された。これは，ラムダファージの受容蛋白質である，LamB蛋白質が加圧下にて，量的に減少していくことに起因するものであった。そこで，本蛋白質の遺伝子発現を制御している，マルトースオペロン（malB領域）のプロモーターの活性を調べたところ，やはり，加圧下においてネガティブに制御されていることが明らかとなり，加圧は，本オペロンの遺伝子発現を阻害することを明かとした。

キーワード：高圧力，大腸菌，マルトースオペロン，ラムダファージ，遺伝子発現，圧力制御

The *malB* Regulated Interval of *Escherichia coli* Is Repressed Under High Pressures

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The formation of plaques by λ phage in *Escherichia coli* was repressed by elevated hydrostatic pressures ; phage plaques were not detected at 30 MPa. This phenomena is due to the deduced expression of λ receptor protein, LamB under the elevated pressures. Promoter fragments derived from the *malB* operon were also repressed by elevated hydrostatic pressures. Our findings suggest that high pressure affects gene expression directed by the *malB* regulatory interval.

Key words : High pressure, *malB* operon, LamB, *Escherichia coli* λ phage

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1 Introduction

High pressure influences several gene and protein expression as reviewed by Bartlett et al[1]. A ressure-regulated promoter was cloned and sequenced from a barophilic bacterium strain DB6705 isolated from deep-sea. Gene expression by this promoter was enhanced by elevated hydrostatic pressures at the transcription level[23]. The studies of thermophilic methanogen *Methanococcus thermolithotropicus* show that the growth of it at 50MPa results in dramatic changes in total cellular amino acid composition, and 2-D PAGE analysis has indicated levels of a series of basic proteins between 38-70kDa[4]. We reported previously the effect of pressures up to 30 MPa on gene expression in *Escherichia coli*[5]. A pressure of 30 MPa activated the *lac* promoter region and induced gene expression controlled by this promoter on a plasmid. *E. coli* belongs to the Proteobacteria γ-subgroup together with the barophilic strains[6], so they are related genetically. It seems advantageous to use the *E. coli* system which is known well to study the mechanisms of pressure affect on microorganisms.

When λ phage infects *E. coli*, the phage binds to a λ receptor protein, LamB, located in the outer membrane[7]. The LamB protein is expressed from the *lamB* gene located in the *malB* region which is composed of two operons, *malEFG* and *malK-lamB*, transcribed divergently from an inter-operon regulatory interval[8-10]. Both operons of the *malB* region are positively controlled by the *malT* gene product through its interaction with an inducer, maltose[11]. However, no information exists about the expression of this region under high pressure, Rutberg[12] reported that high pressure treatment($\sim$100MPa)of lysogenic *E. coli* caused the induction of bacteriophage, but the ability of the phage to infect cells under high pressure was not tested. In this paper, we discribe that high pressure prevents the formation of plaques by λ phage in *E. coli*, and indicate that the phenomenon is due to the deduced production of the λ receptor protein, LamB, in the outer membrance under high pressures.

2 Materials and methods
2.1. Bacterial strains, phage, and plasmids

E. coli JM109(recA1 supE44 endA1 hsdR17 gyr A96 relA1 thi Δ (lac-proAB)[F'traD36proAB lac I^q lacZΔM15])was used as the host for the plasmids, and was also used for infection with phage λ ZAP II (λ ZAP II kit, purchased from Toyobo, Osaka, Japan). The phage plasmid, pUC118 (Takara, Otsu, Japan), and the promoter-probe vector, pKK232-8 (Pharmacia P-L Biochemicals, Milwaukee, WI, USA), were used for DNA sequence and gene expression studies involving the *malB* regulatory interval region, respectively. Kohara bacteriophage λ #634(1F8), from the *E. coli* genomic library[13], containing the *malB* operon at around 92min. on the chromosomal DNA, was kindly provided by the National Institute of Genetics(Mishima, Japan). The plasmid pTS4 was used as a positive control for studies of gene expression at high pressure[5].

2.2 λ phage infection of *E. coli* under high pressure

E. coli JM109 was cultivated at 37℃, with shaking, in LB medium containing 0.2%(v/v) maltose and 10mM $MgSO_4$, until exponential phase, and then the cell was collected by centrifuged at 8000rpm for 10 min. The cells were washed in 10mM $MgSO_4$ solution, and the cell suspension was adjusted to an O.D.at 660nm of 0.5. A 20 μl aliquot of diluted λ ZAP II solution (in SM buffer [0.58% NaCl, 0.2% $MgSO_4$, 50mM Tris-HCl pH7.5, 0.01% gelatin], 2.5×10^2 plaque forming unit/ml) and 400 μl of *E. coli* JM109 solution were mixed, and incubated for 20 min. at 37℃. The infected cell suspension was mixed with 3ml of NZY medium (0.5%NaCl, 0.2% $MgSO_4$, 0.5%Bacto yeast extract, 1% NZ Amine [ICN Pharm. Costa Mesa, Calif. USA]) containing 0.7% soft agar at 40℃, and the inoculated medium was poured onto LB agar medium supplemented with 0.2% (v/v) maltose and 10mM $MgSO_4$ in a sterilized polystyrene plate (35mm diameter ; 25000 DISH 35N, Iwaki glass, Chiba, Japan). When the soft agar had solidified (10min.), the plate was placed into a sterilized vinyl pouch, cut to 5cm

×5cm (PT pouch ; Sakami Co. Osaka, Japan), containing 3ml of oxygen saturated fluorinert (FC-72; Sumitomo 3MM, Tokyo, Japan), and the pouch was sealed excluding air, using a polysealer. Each pouch was placed into a pressure vessel (titanium ; Rigosha, Tokyo, Japan) and incubated for 14hr at 37℃ under defined pressure conditions (0.1 MPa to 30 MPa). As control experiments, λ phage solution was pressurized up to 30 MPa at 37℃ for 1hr, then infected to E, coli. The result showed that no significant difference was detected for plaque forming ability and plaque numbers compared with the normal condition. We also checked λ phage adsorption to E. coli cells under high pressure up to 30 MPa at 37℃ for 30min., the results indicated that no significant difference was detected. These suggested that the pre-treated high pressure condition was not effective for infection and adsorption of λ phage.

2.3. Construction of the recombinant plasmids containing the promoter regions of the malB operon

Preparation of plasmid DNA, and recombinant DNA work were performed as described by Sambrook et al[14]. Recombinant plasmids containing the promoter regions of the malB operon were constructed as shown in Fig.1. Kohara phage λ #644 was digested with EcoR I and BglⅡ, and a 0.9kb EcoR I -BglⅡ fragment containing the malB regulatory interval was purified using a Gene Clean Kit (Bio101 Co., La Jolla, CA, USA). The purified fragment was ligated with the phage plasmid pUC118, digested with EcoR I and BamH I, then E. coli JM109 was transformed with the recombinant plasmid. The DNA sequence of the fragment derived from the recombinant plasmid, pYNMALB1, was determined using the DNA Sequencer Model 373A system (Perkin-Elmer／Applied Biosystems Div. Foster City,
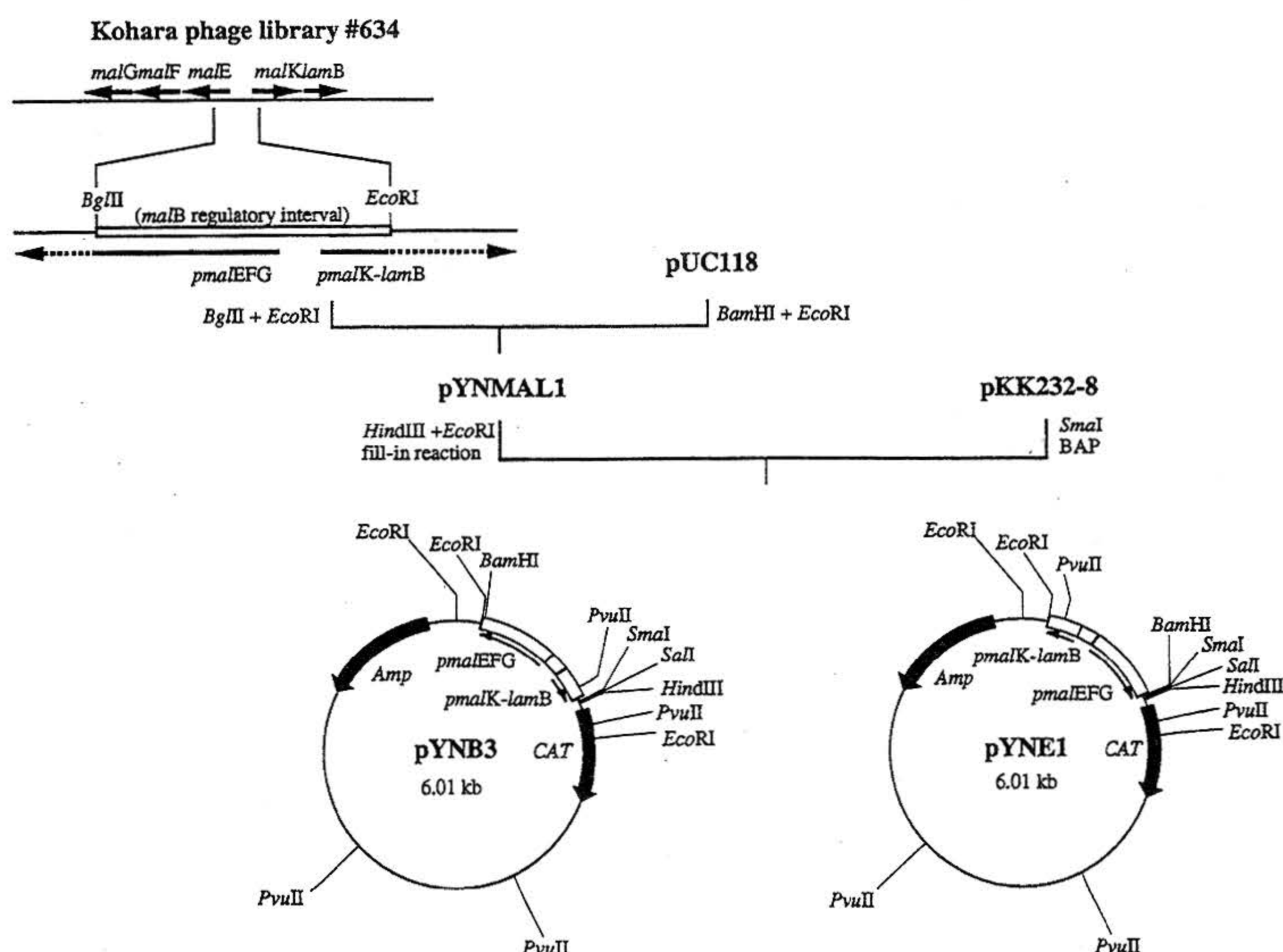

Fig. 1　Construction of the plasmids containing the malB regulatory interval from Kohara phage library #634, and physical maps of the plasmids, pYNB3 and pYNE1. The thin line represents the vector and gray arrow boxes show the β-lactamase gene (Amp) and the CAT gene (CAT). The white boxes and line arrows represent the malB regulatory interval, and the direction of promoters in the malEFG operon (pmalEFG) and the malK-lamB operon (pmalK-lamB).

CA, USA). The *mal*B regulatory interval region was purified from pYNMALB1, digested with *Eco*R I and *Hind*Ⅲ, and filled in using Klenow DNA polymerase. The promoter-probe vector, pKK232-8, was digested with *Sma* I, dephosphorylated by bacterial alkaline phosphatase (BAP), and ligated to the purified fragment containing the *mal*B regulatory interval. *E. coli* JM109 was transformed with the ligated DNAs and two recombinant plasmids from these transformants, extracted by the method of Holmes and Quingley[15], were used in further studies. In one of these plasmids, pYNB3, the reporter gene encoding chloramphenicol acetyltransferase (CAT) was oriented in the direction of the *mal*K-*lam*B operon's promoter, while in the other plasmid, pYNE1, the CAT gene was oriented in the direction of the *mal* EFG operon's promoter. Gene expression initiated from these promoters was regulated well by the inducer (0.2% maltose), in *E. coli* carrying either of the recombinant plasmids, pYNB3 or pYNE1.

2.4. Expression study of the CAT gene

An expression study of the CAT gene on the plasmids pYNB3 and pYNE1 was carried out under various pressure conditions (0.1 to 40 MPa) as described previously[5]. Protein concentrations were determined using a Protein Assay Kit (Bio Rad, Hercules, CA, USA) with bovine serum albumin (BSA) as the protein standard. CAT activity was assayed by standard methods[16]. One unit of CAT activity was defined as 1 nmol of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) reduced per minute at 37°C[17].

2.5. Detection of LamB protein under the elevated pressures.

Urea-sodium dodecyl sulfate polyacylamide gel electrophoresis (SDS-PAGE) was used to detect the LamB protein expressed under elevated pressures. *E. coli* JM109 was cultivated in LB medium containing 10mM MgSO₄, with or without 0.2%(v/v) maltose at 37°C under defined pressures as metioned above. After 14hr cultivation, cells were collected and washed with 1M KCl, 0.1M Tris.HCl pH7.8 buffer, and resuspended in 0.1M Tris.HCl pH7.8 solution.

The suspensions from diffirent pressures were ajusted to the same concentration. Cells were broken by ultrasonication. Outer membrane proteins were isolated by treatment with sodium lauryl sarcosinate (Sarkosyl) according to the method of Nikaido[18], and the protein compositions were analyzed by Urea-SDS-PAGE, as discribed by Bollag et al[19].

3. Results

3.1. Effect of pressure on phage λ infection in *E. coli*

Fig. 2 shows that the size of phage plaques on *E. coli* decreased with elevated hydrostatic pressures. At 30 MPa, no phage plaques were detected, and so high pressure appeared to prevent λ phage plaque formation in *E. coli*.

3.2. Effect of pressure on gene expression directed by the *mal*B regulatory interval region

E. coli JM109 carrying the plasmid, pYNB3 or pYNE1, was cultivated under various pressure conditions ranging from atmospheric pressure (0.1MPa) up to 40 MPa, and CAT activity was assayed. Table 1 shows that the CAT activity of *E. coli* carrying pYNB3 or pYNE1 decreased when cells were grown at elevated hydrostatic pressure in the presense of maltose. This result suggested that high pressure inhibited gene expression derected by the *mal*B regulatory interval.
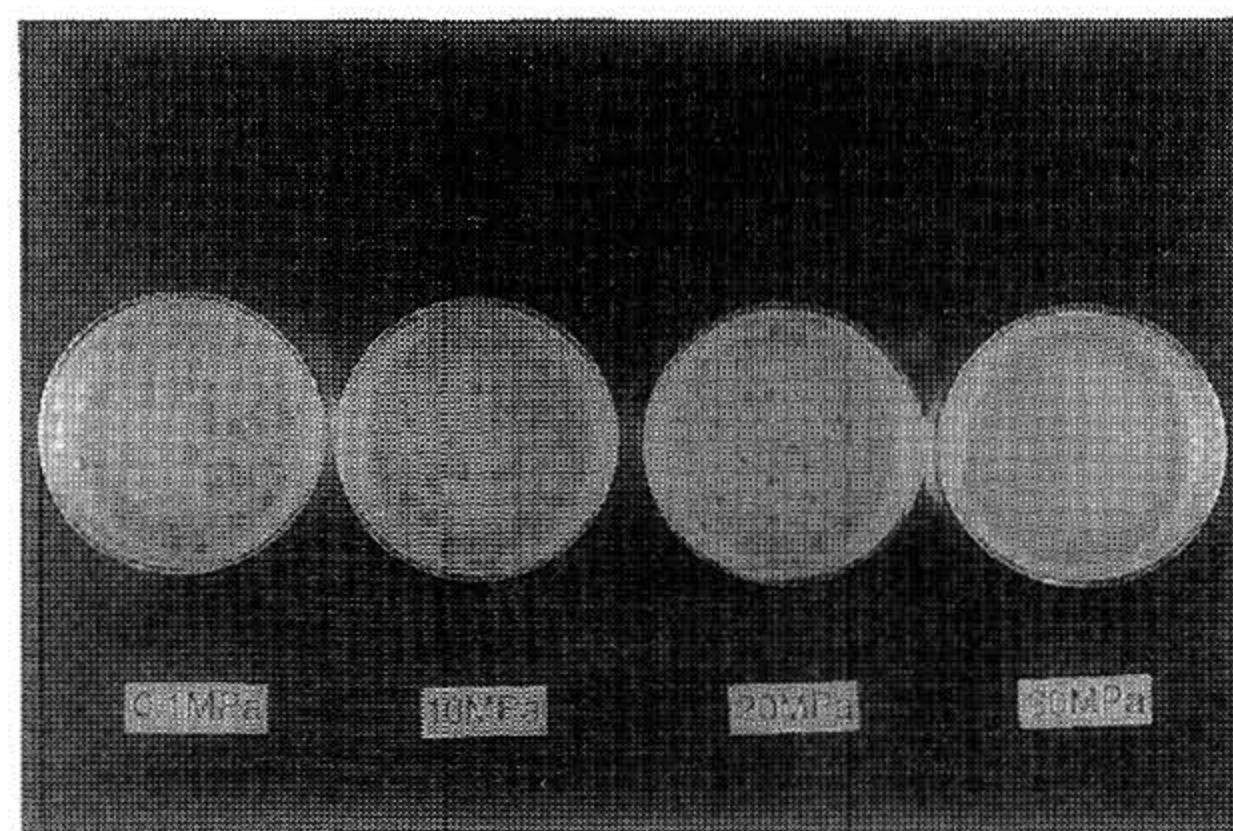

Fig. 2 The ability of λ phage, ZAP Ⅱ, to infect *E. coli* JM109 and form plaques under high pressure conditions. Incubation pressures are indicated in the figure.

Table 1 Comparison of CAT activity encoded by different plasmids in *E. coli* JM109 grown at elevated hydrostatic pressures (MPa).

Plasmid	Inducer	Enzyme activity (unit/mg)				
		0.1MPa	10MPa	20MPa	30MPa	40MPa
pYNB3	+	173.9	64.8	26.7	10.9	10.8
	−	20.5	14.3	15.9	25.1	9.3
pYNE1	+	326.4	205.1	57.2	17.9	18.3
	−	27.5	22.4	27.9	37.9	16.9

Inducer was presented at 0.2% maltose; -, no inducer.

3.3. Effect of pressure on the expression of LamB protein.

Outer membrane proteins of *E. coli* JM109 grow at elevated pressures were demonstrated on a urea-SDS-PAGE (Fig.3). The expression of LamB protein was induced by maltose. There are a strong LamB protein band appeared at the atomsphere pressure condition (0.1 MPa), and the expression of LamB protein deduced with the pressure increased. No LamB protein was detected at the pressure of more than 30MPa even with the inducer, maltose. This results approve that high pressure repress the gene expression directed by the *mal*B regulatory interval, thereby preventing the synthesis of LamB protein.

-Maltose +Maltose

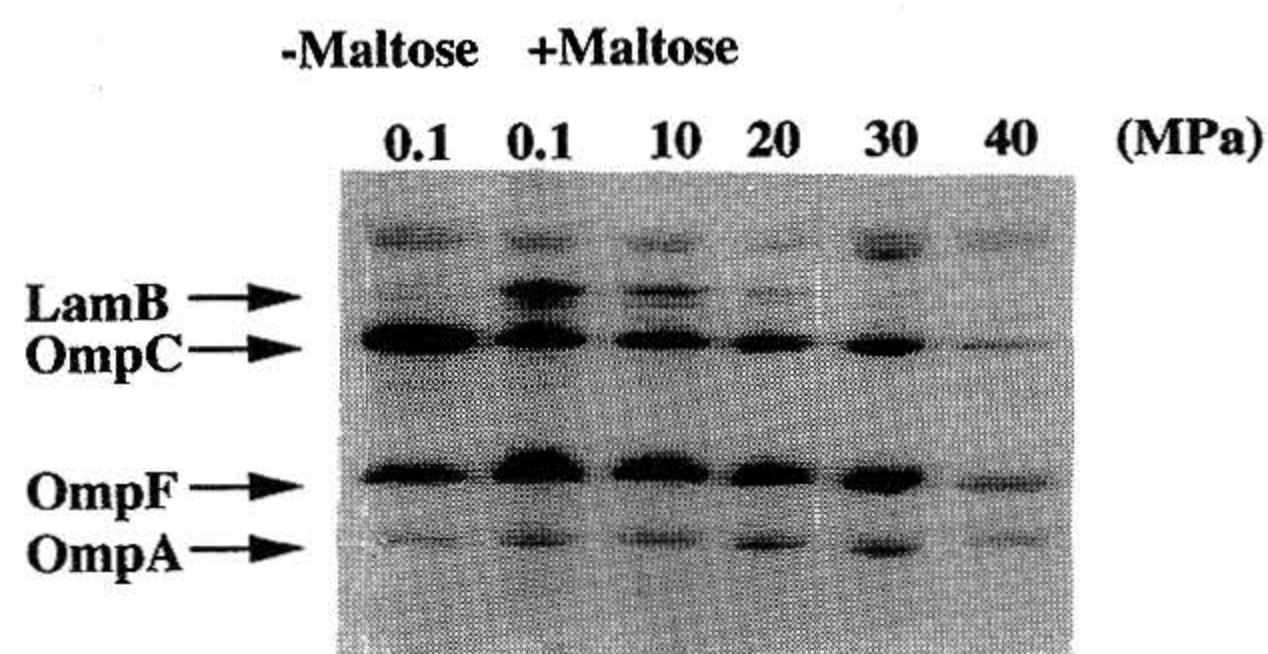**0.1 0.1 10 20 30 40 (MPa)**

LamB →
OmpC→

OmpF →
OmpA→

Fig. 3 The expression of LamB protein in *E. coli* JM109 with or without maltose under elevated pressures. Incubation pressures are indicated in the figure.

4. Discussion

We have found that high pressure prevents the formation of plaques by λ phage in *E. coli*. High pressure repressed gene expression directed by the *mal*B regulatory interval in the presence of inducer, and therefore the λ receptor protein was not synthesized under high pressure conditions. Nakashima et al[20]. have reported that the extent of expression of OmpX, one of the outer membrane proteins of *E. coli*, is reduced under high pressure conditions. Since the N-terminal amino acid sequence of OmpX is identical to that of the λ phage receptor protein, LamB, OmpX is LamB. These observations are very well fit with our foundings, lead us to conclude that prevention of plaque formation by phage λ under high pressure conditions is due to a paucity of the λ receptor protein, LamB.

Bartlett et al[21]. reported that an outer membrane protein, OmpH, from the deep-sea bacterium *Photobacterium* sp., strain SS9, was a major pressure inducible protein, and transcription of the *omp*H gene was controlled by hydrostatic pressure. A study of high pressure signal transduction in the same strain, showed that the abundance of a second outer membrane protein, OmpL, decreased, while the amount of OmpH increased with elevated pressure[22]. Therefore, deep-sea bacteria may have a special mechanism for regulation of *omp*H and *omp*L under high pressure, which differs from the osmo-regulation system in *E. coli*, It is very interesting that the expression of genes directed by the *mal*B regulatory interval was reduced under high pressure, similar to the *omp*L expression system.

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