Volume 300, number 3, 254–258 FEBS 10893 April 1992

Escherichia coli leucine-responsive regulatory protein (Lrp) controls lysyl-tRNA synthetase expression

Myriam Gazeau, Florence Delort, Philippe Dessen, Sylvain Blanquet and Pierre Plateau

Laboratoire de Biochimie, Unité de Recherche Associée 240 du Centre National de la Recherche Scientifique, Ecole Polytechnique, 91128 Palaiseau Cedex, France

Received 2 January 1992; revised version received 20 February 1992

Using random Tn*10* insertion mutagenesis, we isolated an *Escherichia coli* mutant strain affected in the regulation of *lysU*, the gene encoding the inducible form of lysyl-tRNA synthetase. The transposon giving rise to the altered expression of *lysU* was found inserted within *lrp*. The latter gene codes for the leucine-responsive regulatory protein (Lrp) which mediates a global response of the bacterium to leucine. An involvement of Lrp in the regulation of *lysU* was searched for by using a *lysU–lacZ* operon fusion. The following conclusions were reached: (i) inactivation of *lrp* causes an increased activity of the *lysU* promoter, whatever the growth conditions assayed, (ii) insertion of a wild-type *lrp* gene into a multi-copy plasmid significantly reduces *lysU* expression, and (iii) sensitivity of the *lysU* promoter to the presence of leucine in the growth medium is abolished in the *lrp* context.

Lysyl-tRNA synthetase; Leucine-responsive regulatory protein; *lysU*; *lrp*; Leucine; *Escherichia coli*

1. INTRODUCTION

In *Escherichia coli*, lysyl-tRNA synthetase (LysRS) is unique since it occurs as two species encoded by two distinct genes, *lysS* and *lysU* [1–3], the regulations of which are very different. While *lysS* seems to be constitutively expressed [1,2], *lysU* expression is sensitive to either the composition [4], the pH [5,6], the temperature [7] or the oxygenation [6] of the growth medium. In rich medium, *lysU* expression is induced by anaerobiosis, low external pH or growth during late-log phase at a temperature higher than 37°C [6]. In minimal medium, a high expression of *lysU* occurs if the culture is supplemented with alanine [8,9], leucine [8] or various leucine-containing dipeptides [10]. Finally, *lysU* is described to belong to the heat-shock regulon [7], since (i) its expression increases upon temperature shift from 28°C to 42°C and (ii) the effect of temperature depends on the presence of a functional *rpoH* (*htpR*) gene [7]. However, the *lysU* promoter region resembles standard *E. coli* promoters rather than a σ^{32}-specific sequence [11].

Recently, *lysS* null mutants were observed to grow slowly before 37°C [6]. The temperature-dependent *lysU* expression accounts for such a phenotype [6]. One such mutant was used in the present study to select a Tn*10* insertion mutation causing a high expression of *lysU* at 30°C. The characterization of this insertion mutant indicates that *lysU* is negatively regulated by Lrp [12], a protein involved in the global response of the bacterium to leucine.

Correspondence address: P. Plateau, Laboratoire de Biochimie, Ecole Polytechnique, 91128 Palaiseau Cedex, France. Fax: (33) (1) 69 33 30 13

2. MATERIALS AND METHODS

2.1. *Enzymes and substrates*

DNA restriction and modification enzymes were purchased from Boehringer (Mannheim, Germany), Bethesda Research Laboratory (Rockville, Maryland, USA), or Pharmacia (Uppsala, Sweden). L-amino acids were from Merck (Darmstadt, Germany). L-glycyl-L-leucine was from Sigma (St. Louis, MO). [γ-^{32}P]ATP (111 TBq/mmol) was from NEN (Cambridge, MA). L-[^{14}C]lysine (12 GBq/mmol) was from the Commissariat à l'Energie Atomique (Saclay, France). Pure unfractionated *E. coli* tRNA was from Boehringer.

Strains were grown either in LB medium [13] or in MOPS minimal medium [14] supplemented with glucose (0.4%), proline (40 μg/ml), methionine (40 μg/ml) and isoleucine (75 μg/ml). Isoleucine was added to the MOPS medium to avoid growth inhibition in experiments involving leucine or glycyl-leucine [10]. To assay growth on L-serine as sole carbon source, bacteria were plated on M9 medium [13] supplemented with serine (2 mg/ml), proline, methionine, valine and isoleucine (40 μg/ml each). Anaerobic conditions were insured by the use of GasPaks (from BioMérieux, Craponne, France) in a hermetically closed jar.

tRNA aminoacylation and β-galactosidase activities were measured in crude cell extracts obtained by sonication, as already described [15]. The total amount of protein in the extract was estimated by using the BioRad protein assay. One unit of enzymatic activity is defined as the amount of enzyme capable of producing 1 nmol of aminoacyl-tRNA or of *o*-nitrophenol per min.

2.2. *Recombinant DNA techniques*

General genetic and cloning techniques were as previously described [16]. Southern blot analysis was performed by the unblot method of Wallace and Miyada [17]. DNA probes were labeled by phosphorylating oligonucleotides in the presence of [γ-^{32}P]ATP [16]. DNA sequencing was performed on single- or double-stranded DNA by the dideoxy chain termination method [18]. Computer analyses of nucleic acid

Published by Elsevier Science Publishers B.V.

sequences were carried out using the DNAid program on a Macintosh computer [19] and the data bases and facilities of the Centre Inter-Universitaire de Traitement de l'Information (CITI2, Paris) [20].

2.3. *Strains and plasmids*

The strains used in this study are listed in Table I. Strain GE1031 and plasmid pNK972 [21] were kindly provided to us by Dr. M. Springer. Strain PALSΔK5 was mutagenized by insertion of the Tn*10* transposon at random positions of its chromosome. For this purpose, Tn*10* transposon from strain GE1031 was transduced into a strain which overproduced transposase (XA103(pNK972)), selecting for tetracycline resistance. Then, a P1 lysate prepared on a pool of such transductants was used to transduce the tetracycline resistance into the strain PALSΔK5.

To obtain plasmid pTP8H2, chromosomal DNA from mutant strain PALTP8 was digested by *Hin*dIII and ligated with pBluescript(+)KS DNA previously cut with *Hin*dIII. The ligation mixture was used to transform strain IBPC111, and tetracycline-resistant clones were selected. The plasmid harbored by one of them was named pTP8H2.

To obtain plasmid pC941, an oligonucleotide probe (5'-CATCAACCAGACGGCAAACAGGACAATAAGGATCAGC3') was deduced from part of the chromosomal DNA carried by pTP8H2. From Southern blot analyses of various restriction digests of PALSΔK5 chromosomal DNA, we concluded that the probe specifically hybridized to a 3.6 kbp *Pst*I-*Eco*RI fragment. Then, chromosomal DNA from PALSΔK5 was digested by *Pst*I and *Eco*RI enzymes and the resulting DNA fragments were separated by high performance size exclusion chromatography [22]. Aliquots of the collected fractions were electrophoresed on a 0.8% agarose gel and hybridized to the labeled probe. The fraction displaying the strongest hybridization signal was ligated to pBluescript DNA and strain JM101TR was transformed with the ligation mixture by selecting ampicillin resistance. The transformants which strongly hybridized with the probe were identified by colony hybridization. The plasmid harbored by one of them was named pC941.

Plasmid pH9 and pC20 were derived from pC941 by removing the *Hin*dIII(1)-*Hin*dIII(2) or the *Cla*I(2)-*Cla*I(3) fragment, respectively (Fig. 1). As pC20 was obtained through a limited *Cla*I digestion, an inversion of the *Cla*I(1)-*Cla*(2) fragment could have occurred during the digestion-ligation process leading to this plasmid. It was verified by DNA sequencing that the *Cla*(1)-*Cla*(2) fragment had been maintained in the same orientation in pC20 and pC941. The *Hin*dIII(1)-*Hin*dIII(2), *Hin*dIII(1)-*Bgl*II and *Bgl*II-*Hin*dIII(2) fragments of pC941 were inserted into pBluescript(+)KS to make p16, pBB3 and pBB6, respectively (Fig. 1). Plasmid pBSTNAV was already described [23]. This plasmid is a pBluescript derivative in which *lacZ'* is interrupted by a tRNA gene.

Table I

E. coli strains used in this study

Strain	Genotype or relevant characteristics	Source or reference
JM101TR	*supE thi* Δ(*lac-pro*) *recA56 srl-300*::Tn*10* F'(*traD36 lacI*^{q} *proAB lacZM15*)	39
XA103	F$^-$ Δ(*lac-pro*) *gyrA rpoB metB argE*(Am) *ara supF*	40
XA1035[a]	XA103 (λRS45 *lysU*::*lacZ*)	6
PALSΔK5[a]	XA103 *lysS*::*kan* (λRS45 *lysU*::*lacZ*)	6
PALTP8	PALSΔK5 *lrp*::Tn*10d*-Tet	This work
GE1031	*supE42 zaf-1831*::Tn*10d*-Tet	M. Springer
IBPC111	F$^-$ Δ(*lac-pro*) *gyrA rpoB metB argE*(Am) *supE ara recA1*	41

[a] In our previous work [6], strains XA1035 and PALSΔK5 were named XA103(λXU5) and PAL3103SΔK(λXU5), respectively.

3. RESULTS AND DISCUSSION

3.1. *Isolation of a mutant affected in* lysU *regulation*

PALSΔK5 is an *E. coli lysS* null mutant carrying a *lysU-lacZ* operon fusion (Table I). Upon plating at 30°C on LB-Xgal medium, this strain forms small white colonies, because of the reduced expression of *lysU* at this temperature. To obtain mutants affected in *lysU* regulation, we plated on this medium a pool of PALSΔK5 cells mutagenized by random insertion of the Tn*10* transposon into the bacterial chromosome. After incubation at 30°C, most colonies were small and white. However, one mutant, named PALTP8, formed a large blue clone.

The Tn*10* insertion from PALTP8 was transduced back into strain PALSΔK5. All the transductants, selected for tetracycline resistance, formed large blue colonies at 30°C. Then, LysRS and β-galactosidase specific activities were measured in crude extracts of mutant PALTP8 and parental strain PALSΔK5. After aerobic growth at 30°C in LB medium until the stationary phase, the activities in the mutant (18 and 1,100 U per mg of total protein, respectively) were significantly higher than those in the parental strain (1.43 and 120 U/mg, respectively). Therefore, it was likely that the insertion mutation in PALTP8 had caused a higher expression of *lysU* at 30°C.

3.2. *Localization of the mutation on the* E. coli *chromosome*

To locate the above insertion mutation on the *E. coli* genome, a ~3-kbp *Hin*dIII fragment carrying the tetracycline resistance gene was subcloned from the mutant strain PALTP8 into plasmid pBluescript. In addition to the tetracycline resistance gene, the resulting plasmid (pTP8H2) carried a ~550-bp fragment from *E. coli* chromosomal DNA (Fig. 1).

To identify the *E. coli* DNA region corresponding to the insertion mutation, part of the chromosomal DNA carried by pTP8H2 was sequenced and an oligonucleotide probe was accordingly synthesized. Southern blot analyses of PALSΔK5 DNA revealed that a 3.6- kbp *Pst*I-*Eco*RI fragment was specifically recognized by the probe. This fragment was partially purified by HPLC, ligated with pBluescript DNA and transformed into strain JM101TR by selecting ampicillin resistance. Three out of 800 transformants hybridized particularly well to the probe. Each of the three clones carried a plasmid with a 3.6-kbp insert. One of them, named pC941, was used in further studies.

The sequencing of several region of pC941 revealed the presence of two already known genes: (1) between the *Hin*dIII(1) and *Hin*dIII(2) sites (Fig. 1), a total of 300 bp were sequenced. In this region, the DNA sequence was found exactly identical to that of *trxB*, the gene for thioredoxin reductase [24]; and (ii) around the *Bgl*II site, the DNA sequence conformed to that of *lrp*. The

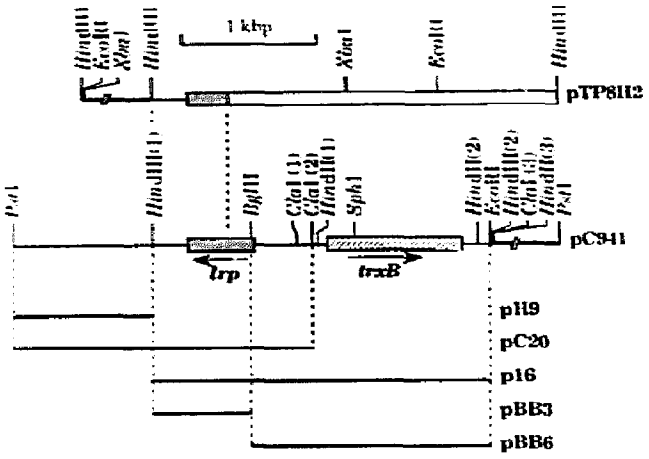

Fig. 1. Structures of plasmids used in this study. pBluescript vector is indicated by a heavy line in the pTP8H2 and pC941 structures. The *lrp* and *trxB* genes (shaded and hatched box, respectively) are shown on the cloned DNA with their orientations (arrows). The open box at the top symbolizes DNA from the Tn*10* transposon. Below the pC941 structure are indicated as heavy lines the various fragments of pC941 subcloned into pH9, pC20, p16, pBB3 or pBB6. In pTP8H2, the junction between chromosomal and transposon DNA was precisely mapped by DNA sequencing.

latter gene (also called *ihb*, *oppI* or *rblA* [25,26]) encodes a regulatory protein that mediates a global response to leucine [12]. To confirm the presence of *lrp* on plasmid pC941, 600 bp were sequenced, covering the entire region susceptible to correspond to *lrp*. This sequence exactly matched the *lrp* sequence, with the exception of a T instead of a C at position 444 of the originally published *lrp* coding sequence [12]. This difference does not affect the gene product since it replaces a Val codon by another Val codon. Noteworthy, both *trxB* and *lrp* have been localized near min 20 on the *E. coli* genetic map [24,25].

To precisely localize the insertion of Tn*10* transposon in the DNA of the mutant strain PALTP8, the fragment of chromosomal DNA carried by pTPH8H2 was entirely sequenced. The junction between transposon and chromosomal DNA occurs within the *lrp* gene, upstream of nucleotide 191 in the *lrp* coding sequence [12].

Inactivation of *lrp* in strain PALTP8 was further confirmed by assaying a phenotypic property of *lrp* mutants. *lrp*$^-$ strains are known to be capable of growing on L-serine as sole carbon source, contrary to wild- type *E. coli* [26]. In agreement with the disruption of *lrp* in PALTP8 DNA, the mutant strain PALTP8 could grow on L-serine, while the parental strain PALSΔK5 could not.

From all these data, we concluded that the insertion of Tn*10* transposon in the PALTP8 chromosome resulted in the inactivation of *lrp*.

3.3. *Overexpression of* lrp *inhibits* lysU *expression*

The occurrence of a link between *lysU* expression and the *lrp* product was established as follows. Firstly, we observed that the presence of pC941 in *lysU::lacZ* strain XA1035 reduced ~6-fold the β-galactosidase production from the *lysU* promoter after growth at 42°C in LB medium, as compared to the presence of the control plasmid pBSTNAV (Table II). Then, the gene responsible for this inhibition was precisely localized by constructing various subclones of pC941 and assaying their effect on *lysU* expression. The presence of pC20 and p16 reduced 6- to 10-fold the β-galactosidase activity of strain XA1035, while pH9, pBB3 pBB6 had no effect, as compared to pBSTNAV (Fig. 1 and Table II). Clearly, the occurrence of an inhibition of *lysU* expression correlated with the addition *in trans* of an intact *lrp* gene.

3.4. *The control by leucine of* lysU *expression is lost in the* lrp *mutant PALTP8*

As mentioned above, *lysU* expression is stimulated by the addition of leucine or of various leucine-containing dipeptides in the culture medium [8–10]. This effect was studied in the case of the *lysU::lacZ* strain PALSΔK5 by growing it under various growth conditions and by measuring β-galactosidase activity. This activity was strongly increased (i) by the presence of 10 mM leucine when bacteria were grown aerobically at 42°C or anaerobically at 30°C, and (ii) by the presence of 3 mM glycyl-leucine when bacteria were grown aerobically at 30°C (Table III). In agreement with the hypothesis that the effect of leucine or glycyl-leucine on *lysU* expression was mediated by Lrp, we observed that neither leucine nor glycyl-leucine significantly affected β-galactosidase production in the *lrp* mutant PALTP8 (Table III).

3.5. *Concluding remarks*

An involvement of *lrp* in the mechanism of *lysU* regulation was already suspected [26]. *lysU* expression is increased in a *metK* context [27]. However, transformation of the *metK* mutant, RG62, with a plasmid carrying

Table II

β-Galactosidase activity in *E. coli* strain XA1035 (*lysU::lacZ*) transformed by various plasmids

Plasmid	β-Galactosidase activity (U/mg)
pBSTNAV (control)	800
pC941	140
pH9	950
pC20	130
p16	90
pBB3	1,000
pBB6	1,100

Bacteria were grown aerobically at 42°C in 15-mm test tubes containing 5 ml of LB medium supplemented with 60 μg/ml ampicillin. Cultures were arrested when the stationary phase of growth was reached. β-Galactosidase activity (in units per mg of total protein) was measured from crude extracts obtained by sonication.

the wild-type *metK* allele did not restore normal *lysU* expression [28]. Recently, Lin et al. showed that strain RG62 had acquired a secondary mutation in *lrp* [26] and suggested that the increased expression of *lysU* in RG62 could have, in fact, originated from the *lrp* mutation [26]. The present work clearly supports this hypothesis.

Further studies will be necessary to establish whether the effect of *lrp* is directly or indirectly exerted on *lysU* regulation. However, it has been recently reported that the promoter region of *lysU* displays sequences on the direct and complementary strands (TTTATTAGTGAT and TTTATTCATTAC, respectively) similar to a consensus motif (TTTATTCtNaAT) derived from sequences found in the 5' upstream areas of genes that belong to the leucine regulon [29]. Some of the latter sequences have been shown to be located within the region recognized by the Lrp protein [29,30].

The occurrence of a link between the addition of leucine and the derepression of *lysU* appears as mysterious as the regulation of *lysU* itself. Among amino acids, leucine is unique in its regulatory effects. In addition to its role in the repression of various leucine transport and biosynthetic genes, leucine is also a specific inducer of a number of operons in *E. coli* and *Salmonella typhimurium* [26,31–35]. In some cases, the reason for the regulation by leucine is not immediately apparent. Thus, the *E. coli* genes for L-serine deaminase [36], L-threonine dehydrogenase [37] and for an L-serine transport system [38] are induced by leucine. It was proposed that this special role of leucine originated from the fact that, unlike most amino acids, leucine is not catabolized [31,32,34]. Therefore, it could serve as an indicator of a shortage of amino acids or of an increased protein breakdown [31,32]. It is likely that the control of *lysU* is related to such a general role of leucine in cellular regulations. Moreover, it is possible that a common set of *cis*-acting elements is responsible for the leucine-response as well as for the anaerobiosis, pH or temperature response of *lysU* expression. The consequence would be that any gene controlled by Lrp might be a good candidate to be submitted, in addition, to the effects of anaerobiosis, pH or temperature. Noteworthy, several leucine-regulated genes in *E. coli* are also sensitive to anaerobiosis [35,36] and/or temperature shift [36].

Table III

β-Galactosidase activity in *E. coli* strains PALSdK5 (*lrp*⁻ *lysU'::lacZ*) or PALTP8 (*lrp lysU'::lacZ*) under various growth conditions.

Medium	Temperature (°C)	Oxygenation	Strain	
			PALSdK5 (*lrp*⁻)	PALTP8 (*lrp*)
LB	42	+	1,000	1,400
MOPS	42	+	60	1,100
MOPS + 10 mM Leu	42	+	550	1,100
MOPS + 10 mM Gly	42	+	70	1,200
LB	30	+	120	1,100
MOPS	30	+	60	1,300
MOPS + 10 mM Leu	30	+	200	1,400
MOPS + 3 mM Gly-Leu	30	+	750	1,400
MOPS + 10 mM Gly	30	+	80	1,400
LB	30	−	1,100	1,400
MOPS	30	−	100	1,500
MOPS + 10 mM Leu	30	−	900	1,600

Aerobic conditions were achieved with vigorous shaking of 18-mm test tubes containing 3 ml medium. Anaerobic conditions were insured by the use of GasPaks in a hermetically closed jar. Cultures were arrested when the stationary phase of growth was reached. β-Galactosidase activity (in units per mg of total protein) was measured from crude extracts obtained by sonication, as described previously.

REFERENCES

[1] Hirshfield, I.N., Bloch, P.L., Van Bogelen, R.A. and Neidhardt, F.C. (1981) J. Bacteriol. 146, 345–351.
[2] Hirshfield, I.N., Tenreiro, R., Van Bogelen, R.A. and Neidhardt, F.C. (1984) J. Bacteriol. 158, 615–620.
[3] Lévêque, F., Plateau, P., Dessen, P. and Blanquet, S. (1990) Nucleic Acids Res. 18, 305–312.
[4] Hirshfield, I.N. and Zamecnik, P.C. (1972) Biochim. Biophys. Acta 259, 330–343.
[5] Hickey, E.W. and Hirshfield, I.N. (1990) Appl. Environ. Microbiol. 56, 1038–1045.
[6] Lévêque, F., Gazeau, M., Fromant, M., Blanquet, S. and Plateau, P. (1991) J. Bacteriol. 173, 7903–7910.
[7] Neidhardt, F.C. and Van Bogelen, R.A. (1981) Biochem. Biophys. Res. Commun. 100, 894–900.
[8] Hirshfield, I.N. and Buklad, N.E. (1973) J. Bacteriol. 113, 167–177.
[9] Hirshfield, I.N., Yeh, F.M. and Sawyer, L.E. (1975) Proc. Natl. Acad. Sci. USA 72, 1364–1367.
[10] Buklad, N.E., Sanborn, D. and Hirshfield, I.N. (1973) J. Bacteriol. 116, 1477–1478.
[11] Clark, R.L. and Neidhardt, F.C. (1990) J. Bacteriol. 172, 3237–3243.
[12] Willins, D.A., Ryan, C.W., Platko, J.V. and Calvo, J.M. (1991) J. Biol. Chem. 266, 10768–10774.
[13] Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[14] Neidhardt, F.C., Bloch, P.L. and Smith, D.F. (1974) J. Bacteriol. 119, 736–747.
[15] Brevet, A., Chen, J., Lévêque, F., Plateau, P. and Blanquet, S. (1989) Proc. Natl. Acad. Sci. USA 86, 8275–8279.
[16] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: a Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[17] Wallace, R.B. and Miyada, C.G. (1987) Methods Enzymol. 152, 432–442.
[18] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[19] Dardel, F. and Bensoussan, P. (1988) Comp. Appl. Biosci. 4, 483–486.
[20] Dessen, P., Fondrat, C. and Mugnier, C. (1990) Comput. Appl. Biosci. 6, 355–356.
[21] Elliott, T. and Roth, J.R. (1988) Mol. Gen. Genet. 213, 332–338.
[22] Schmitter, J.M., Mechulam, Y., Fayat, G. and Anselme, M. (1986) J. Chromatogr. 378, 462–466.
[23] Meinnel, T., Mechulam, Y. and Fayat, G. (1988) Nucleic Acids Res. 16, 8095–8096.
[24] Russel, M. and Model, P. (1988) J. Biol. Chem. 263, 9015–9019.
[25] Platko, J.V., Willins, D.A. and Calvo, J.M. (1990) J. Bacteriol. 172, 4563–4570.

[26] Lin, R.T., D'Ari, R. and Newman, E.B. (1990) J. Bacteriol. 172, 4529–4535.
[27] Hirshfield, I.N., Liu, C. and Yeh, F.M. (1977) J. Bacteriol. 131, 589–591.
[28] Matthews, R.G. and Neidhardt, F.C. (1988) J. Bacteriol. 170, 1582–1588.
[29] Rex, J.H., Aronson, B.D. and Somerville, R.L. (1991) J. Bacteriol. 173, 5944–5963.
[30] Ricca, E., Aker, D.A. and Calvo, J.M. (1989) J. Bacteriol. 171, 1658–1664.
[31] Fraser, J. and Newman, E.B. (1975) J. Bacteriol. 122, 810–817.
[32] Newman, E.B., Kapoor, V. and Potter, R. (1976) J. Bacteriol. 126, 1245–1249.
[33] Squires, C.H., DeFelice, M., Wessler, S.R. and Calvo, J.M. (1981) J. Bacteriol. 147, 797–804.
[34] Jamieson, D.J. and Higgins, C.F. (1984) J. Bacteriol. 160, 131–136.
[35] Andrews, J.C. and Short, S.A. (1986) J. Bacteriol. 165, 434–442.
[36] Su, H., Lang, B.F. and Newman, E.B. (1989) J. Bacteriol. 171, 5095–5102.
[37] Fraser, J. and Newman, E.B. (1975) J. Bacteriol. 122, 810–817.
[38] Hama, H., Shimamoto, T., Tsuda, M. and Tsuchiya, T. (1988) J. Bacteriol. 170, 2236–2239.
[39] Hirel, P.H., Lévêque, F., Mellot, P., Dardel, F., Panvert, M., Mechulam, Y. and Fayat, G. (1988) Biochimie 70, 773–782.
[40] Coulondre, C. and Miller, J.H. (1977) J. Mol. Biol. 117, 525–575.
[41] Springer, M., Trudel, M., Graffe, M., Plumbridge, J., Fayat, G., Mayaux, J.F., Sacerdot, C., Blanquet, S. and Grunberg-Manago, M. (1983) J. Mol. Biol. 171, 263–279.