Volume 16 Number 22 1988 Nucleic Acids Research

Identification of *psiB* genes of plasmids F and R6-5. Molecular basis for *psiB* enhanced expression in plasmid R6-5

Marie Dutreix, Assar Bäckman[1], Jérome Célérier, Mira M.Bagdasarian[1], Suzanne Sommer, Adriana Bailone, Raymond Devoret* and Michael Bagdasarian[1+]

GEMC, Enzymologie, Centre National de la Recherche Scientifique, F-91190 Gif-sur-Yvette, France and [1]Department of Microbiology, Michigan State University, Michigan Biotechnology Institute, PO Box 27609, Lansing, MI 48909, USA

Received July 11, 1988; Revised and Accepted October 24, 1988 Accession nos X12462, X12463

ABSTRACT

PsiB protein of plasmid R6-5 inhibits the induction of the SOS pathway. The F sex factor also carries a psiB gene homologous to that of R6-5. Yet, it fails to inhibit SOS induction. In order to solve this difference, we characterized the psiB genes of R6-5 and F. We found that (i) the sequences of the two psiB genes share extensive homology the predicted amino acid sequences of the two proteins differing by 5 residues, (ii) the expression of R6-5 psiB is 4 times higher than F psiB gene, (iii) in plasmid R6-5, a Tn10 transposon upstream from the psiB gene enhances psiB expression. Hence, the F sex factor may be unable to prevent SOS induction for two non-exclusive reasons: (i) F PsiB protein, being slightly different from R6-5, may be less active, (ii) the level of synthesis of F PsiB protein may be insufficient to prevent SOS induction.

INTRODUCTION

Damage to chromosomal DNA or inhibition of a component of the replisome triggers the SOS pathway (1, 2, 3, 4). This pathway is regulated by RecA protein which is activated to cleave LexA and phage repressors, among other proteins, when there are single-stranded DNA stretches resulting from DNA lesions or aborted DNA replication (5, 6). When DNA lesions are repaired, RecA protein activation ceases, cleavage of the repressor proteins is no longer catalyzed and repression of the LexA regulon and prophage genes is restored (3).

A mechanism inhibiting the activation of RecA protein and, thus, many functions exerted by RecA protein has been described (7, 8, 9). Such inhibition, defined as Psi (Plasmid SOS Inhibition), is caused by plasmid R6-5 psiB gene, which specifies a polypeptide with an apparent molecular weight of about 12 kDa.

A gene homologous to R6-5 psiB is carried by several conjugative plasmids including the F sex factor (10). Nevertheless, contrary to plasmid R6-5, wild type F sex factor fails to prevent SOS induction. The F psiB gene had to be cloned on a multicopy plasmid to prevent SOS induction.

In order to elucidate why PsiB protein activities from R6-5 and F are different, we established the extent of homology of the two psiB sequences. We also showed that R6-5 psiB is expressed 4 times higher than F psiB and we demonstrated that a Tn10 transposon upstream from the psiB gene in plasmid R6-5 enhances psiB expression.

MATERIALS AND METHODS

Bacterial strains, plasmids and gene fusions

Bacterial strains, growth conditions, and test of PsiB activity were described previously (8).

Plasmids pMMB175 (8), pMMB177 (8), pMMB182 (Fig. 1) and pGY7568 carry the R6-5 psiB gene. Plasmid pMMB182 was constructed as follows. Plasmid pMMB135 expressing the psiB gene (8), was linearized at the NruI site, treated with Bal31 nuclease, digested with EcoRI and the fragments smaller than 2 kb were inserted between the EcoRI and SmaI sites of the vector pMMB66HE (12). One of the resulting plasmids, pMMB182, carrying a 1200 bp insert contained the psiB gene and expressed the PsiB polypeptide in maxi-cells (8). Plasmid pGY7568 was constructed by inserting into the bla gene of vector pACYC177 (13) a 3.8 kb HindII fragment of plasmid R6-5 encoding the PsiB polypeptide.

The mutants psiBam30, psiBam22 and psiB2 are described in ref. 8. In plasmid pMMB177, even though psiB is truncated at codon 126, it encodes a smaller but fully functional PsiB protein.

pKL3 (14) is a pBR322 derivative carrying the BamHI-EcoRI fragment of plasmid F (Fig. 1) encoding the F psiB gene (10).

Plasmid pLC1983 (15), a derivative of pMC1403, is a vector designed for isolating LacZ protein fusions (16). In pLC1983, the lacZ gene lacks promoter and ribosome binding sites as well as the first eight codons, it does not produce beta-galactosidase. When the 5' end of a gene containing expression signals and

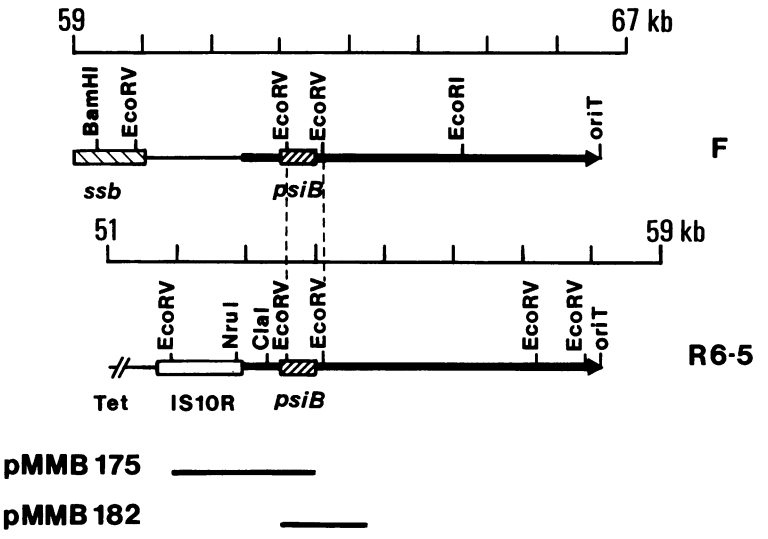

Fig. 1. Physical map of psiB genes in the leading regions of F and R6-5 plasmids. Homology between F and R6-5 plasmids is indicated by a solid bar and psiB homology by a striated box. The coordinates of oriT, ssb, psiB, IS10-R, and of the restriction sites are from this work and from refs. 27, 28, 29, and 8. The arrow shows the polarity of DNA transfer.

encoding the NH_2-terminal portion of a protein is placed upstream from this lacZ sequence, in the proper reading frame, synthesis of beta-galactosidase is restored. To determine promoter strength, we measured in a Δlac host the activity of beta-galactosidase of chimeric psiB::lacZ gene fusions.

Analytical methods.

Methods of in vitro DNA manipulations and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) were described previously (12). Plasmid-encoded polypeptides were visualised in an in vitro transcription-translation system (17).

Nucleotide sequence determination.

The fragments to be sequenced were inserted into M13mp9 in both orientations; overlapping fragments suitable for sequence determination were generated from the single-strand DNA isolated from the recombinant phages (18). Overlaps were selected such that the sequence of each nucleotide was determined at least twice and both DNA strands were sequenced. Sequencing was done by

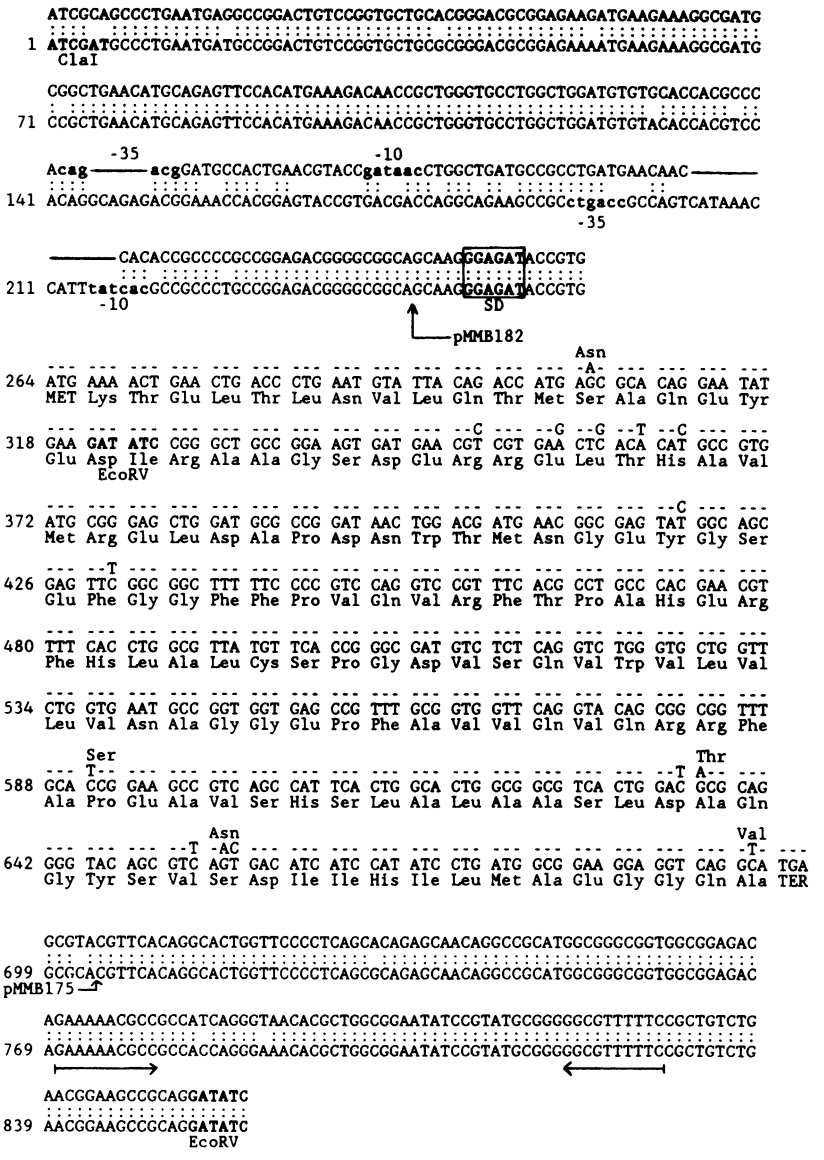

Fig. 2. Nucleotide sequences of R6-5 and F psiB genes. The nucleotide sequence starts at the ClaI site of the ClaI-EcoRV fragment of R6-5 (859 bp). The F psiB sequence (upper sequence) is aligned with the R6-5 sequence (lower sequence). Putative -35 and -10 sequences upstream of R6-5 and F psiB genes are shown in bold lower case; a Shine-Delgarno-like sequence is boxed. Inverted repeats are shown as well as the insert left end in plasmid pMMB182 and the insert right end in plasmid pMMB175.

the dideoxy chain termination method (19) and sequences were analyzed by programs such as described in ref. 20.

RESULTS

A. Nucleotide sequence of the R6-5 psiB gene.

The psiB gene was mapped physically on plasmid R6-5 (Fig. 1). A minimal R6-5 psiB coding region can be defined by an overlapping DNA fragment common to plasmids pMMB175 and pMMB182, which express PsiB function and produce PsiB protein (8, data not shown). We sequenced the minimal psiB coding region as well as the regions upstream and downstream (Fig. 2). There is no psiB promoter in plasmid pMMB182, the psiB gene being expressed from an external tac promoter (data not shown).

There is only one open reading frame in the minimal R6-5 psiB coding region, it starts at position 264 and contains 144 codons. Two amber mutations psiBam21 and psiBam30 can be positioned at codons 11 and 63 (7)(Fig. 2)(Table 1). The calculated molecular weight of R6-5 PsiB protein is 15741 Da (Fig. 2) corresponding roughly to the size of the PsiB polypeptide seen on gels (see Discussion). In the region upstream from the psiB gene, there are two promoter sequences (21), -35 at position 191 and -10 at position 215, and a Shine-Delgarno-like sequence, GGAGAT, 7 bases upstream from the ATG initiation codon.

Table 1. Location of mutations in the psiB gene of plasmid R6-5

Mutant	Codon number	Nucleotide change	Amino-acid change
psiBam30	11	CAG --> TAG	Gln --> Stop
psiBam21	63	CAG --> TAG	Gln --> Stop
psiB2	34	CAT --> TAT	His --> Tyr
psiB177	126-145	deletion	

Codons are numbered starting with the translation initiation codon (position 264 in Fig. 2).

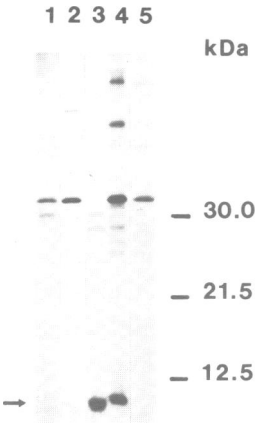

Fig. 3. Proteins encoded by R6-5 psiB and F psiB genes. Purified DNA of recombinant plasmids pGY7568 and pKL3, containing the psiB gene of R6-5 and F respectively, was used as template in an in vitro transcription-translation system (17). Labelled proteins were separated on SDS-PAGE and autoradiographed. Tracks: 1, pACYC177; 2, pBR322; 3, pGY7568; 4, pKL3; 5, pKL3 digested by EcoRV. Molecular standards were (in kilodaltons): carbonic anhydrase (Mr 30), soybean trypsin inhibitor (Mr 21.5), and cytochrome C (Mr 12.5). The arrow shows the position of F and R6-5 PsiB polypeptides.

B. The F psiB gene sequence is almost identical to that of R6-5.

The F plasmid psiB gene was located by Southern hybridization (Fig. 1)(10). Amplification of the psiB homologue carried by F results in the inhibition of SOS induction (10). The F psiB region produced a polypeptide with the same mobility in SDS-PAGE as the R6-5 PsiB protein (Fig. 3). When F psiB gene was digested with EcoRV endonuclease, the presumptive F PsiB polypeptide disappeared (Fig. 3).

As R6-5 psiB gene, F psiB gene is 435 nucleotides long and shares extensive sequence homology with it (Fig. 2). The two protein sequences of 144 amino acids are homologous, they differ by only 5 amino acids. In F psiB, Asn replaces Ser_{14}, Pro Ser_{110}, Thr Ala_{125}, Asn Ser_{131}, and Val Ala_{144} (Fig. 2). The nucleotide sequence upstream from the psiB ATG codon is similar in plasmids R6-5 and F except for the F putative promoter region found at positions 142 (-35) and 166 (-10) (Fig. 2).

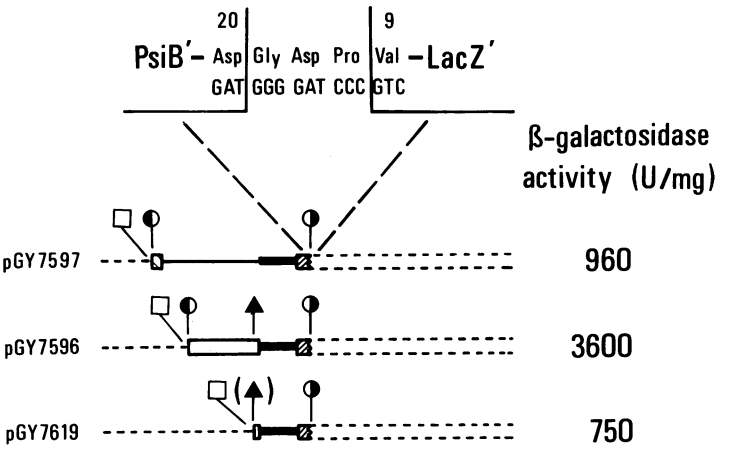

Fig. 4. Structures of the regions upstream from psiB genes and expression of fused lacZ gene. Plasmids pGY7597 and pGY7596 carry in the pLC1983 vector (broken line) F and R6-5 psiB gene ending at codon 20 followed by a polylinker of 3 codons and the lacZ gene (dotted bar, the first 8 codons are missing). Symbols for IS10-R, ssb, and psiB are as in Fig. 1. Plasmid pGY7619 was derived from pGY7596 by deleting the NruI-EcoRI fragment. Ligation of the blunt NruI hemisite to a filled EcoRI hemisite creates a novel EcoRI site. Restriction sites are: EcoRI (squares), NruI (triangles), SmaI (open circles), EcoRV (closed circles). Values on the right show the level of expression of psiB genes.

C. Expression of R6-5 psiB gene is higher than F psiB.

To compare the expression of psiB gene from R6-5 and F plasmids, each psiB gene was fused to a promoterless lacZ gene and the amount of beta-galactosidase produced was determined. To make the R6-5 psiB::lacZ fusion, we inserted into the SmaI site of plasmid pLC1983 an 1.6 kb EcoRV fragment starting upstream from the psiB reading frame and terminating at the 20th codon (Fig. 4). Similarly, the F 2.2 kb EcoRV fragment derived from plasmid pKL3 was inserted into the same site of pLC1983 to give rise to the F psiB::lacZ fusion (Fig. 4). Cells carrying pGY7596 (R6-5 psiB::lacZ) produced 3500 u/mg of beta-galactosidase whereas cells containing pGY7597 (F psiB::lacZ) produced only 960 u/mg, indicating that R6-5 psiB gene is expressed more efficiently than F psiB gene.

D. Transposon-driven expression of the R6-5 psiB gene.

Plasmid R6-5 carries, upstream from the psiB gene, a Tn10 transposon that is absent in plasmid F (Fig. 1). Since Tn10, when appropriately located, can provide a promoter for transcription of adjacent genes (22), we reasoned that the Tn10 portion carrying an IS10-R p-OUT promoter (23) may enhance the expression of the downstream R6-5 psiB gene. Indeed, by deleting the Tn10 IS10-R p-OUT promoter of plasmid pGY7596, we found a reduced expression of the downstream psiB::lacZ$^+$ fusion. This was established as follows. We eliminated most of the IS10-R sequence, including the p-OUT promoter, by cleaving plasmid pGY7596 with NruI and EcoRI and filling in the single-stranded protrusion of the EcoRI hemisite with Klenow DNA polymerase. The blunt ends were religated, giving rise to plasmid pGY7619. Plasmid pGY7619 produced five times less beta-galactosidase than cells carrying the parental plasmid pGY7596 (Fig. 4). We conclude that, in plasmid R6-5, the strong Tn10 p-OUT promoter is responsible for the enhanced expression of psiB gene.

DISCUSSION

Two lines of evidence confirm the proposed psiB open reading frame: (i) two psiB amber mutations create stop codons in the proposed reading frame (ii) the construction of a chimeric gene, comprising the first 20 codons of the psiB gene linked to the ninth codon of the lacZ gene, results in normal beta-galactosidase activity.

The apparent molecular weight of F and R6-5 PsiB proteins on SDS-PAGE is about 12 kDa whereas that calculated from the sequence data is 15741 Da. This discrepancy might be due to some post-translational modification of the PsiB proteins, e. g., proteolytic processing. Yet, we have no evidence for high molecular weight precursors of the PsiB proteins being synthesized in vivo (8) or in vitro (Fig. 3). The amino acid composition of the PsiB proteins may influence their mobilities in SDS gel electrophoresis. Indeed, the apparent molecular weight of a protein deduced from SDS gel electrophoresis does not always reflect the molecular weight calculated from its sequence (24, 25).

In the predicted amino acid sequences for F and R6-5 PsiB proteins, 5 over 144 amino acids are not conserved. Three of the differing amino acids (codons 125, 131,and 144) are in the terminal carboxyl part of the protein that can be deleted in plasmid pMMB177 without loss of PsiB function (8). The change of Ser to Asn, at codon 14 of the F sequence, does not change the secondary structure of the PsiB polypeptide as predicted by the Garnier et al. (26) algorithm. In contrast, the presence of Ser_{110} instead of Pro in the F sequence induces a pronounced change in the predicted secondary structure. In the R6-5 PsiB polypeptide, the region spanned by amino acids 100 through 110 consists of coils, turns and extended structure, whereas in F amino acids 101 through 106 form an alpha-helix which presumably confers considerable rigidity to the corresponding part of the molecule.

Despite the extensive homology between R6-5 and F psiB genes, the two plasmids differ in the efficiency of inhibition of SOS induction. Our data support two non-exclusive hypotheses accounting for this difference (1) The observed amino acid differences between F and R6-5 PsiB proteins and in particular the local structural change at codon 110 in the F PsiB protein might be significant to account for a reduced activity of the F PsiB protein. This question will be resolved when the F and R6-5 proteins are purified and their activities determined in vitro. (2) Plasmids R6-5 and F differ in the level of psiB gene expression, plasmid R6-5 carries a Tn10 transposon which enhances the expression of psiB gene. This can account for its efficiency in inhibiting SOS induction.

ACKNOWLEDGEMENTS

The technical assistance of D. Bouillon and M. Pierre is highly appreciated. We thank M. Chandler and E. Golub for the gift of strains. Grants from Euratom (BI6-E-145-F)(R.D.), Association pour la Recherche sur le Cancer(R.D.), Ligue Francaise contre le Cancer (R.D.), Fondation pour la Recherche Médicale (R.D.), Collaboration Franco-Suédoise (R.D. and M.B.) are gratefully acknowledged as well as a Research Excellence and Economic Development Grant from MSU to M. B.

*To whom correspondence should be addressed

+To whom requests for reprints should be addressed

REFERENCES

1. Radman,M. (1975) In Hanawalt,P.C. and Setlow,R.B. (eds), Molecular Mechanisms for Repair of DNA, Plenum Press, New York. pp. 355-367.
2. Witkin,E.M. (1976) Bacteriol. Rev. **40**, 869-907.
3. Little,J.W. and Mount,D.W. (1982) Cell **29**, 11-22.
4. Walker,G.C. (1984) Microbiol. Rev. **48**, 60-93.
5. Little,J.W., Edmiston,S.H., Pacelli,L.Z. and Mount,D.W. (1980) Proc. Natl. Acad. Sci. USA **77**, 3225-3229.
6. Roberts, J.W. and Devoret, R. (1983) In Hendrix, R.W., Roberts, J.W., Stahl, F.W. and Weisberg, R.A. (Eds), Lambda II Chapter VII. Cold Spring Harbor Laboratory, . pp. 123-144.
7. Bagdasarian,M., Bailone,A., Bagdasarian,M.M., Manning,P.A., Lurz,R., Timmis,K.N. and Devoret,R. (1986) Proc. Natl. Acad. Sci. USA **83**, 5723-5726.
8. Bailone,A., Bäckman,A., Sommer,S., Célérier,J., Bagdasarian, M.M., Bagdasarian,M. and Devoret,R. (1988) Mol. Gen. Genet. in press.
9. Célérier,J., Sassanfar,M., Bailone,A. and Devoret,R. (1988) In Friedberg,E.C. and Hanawalt,P.C. (eds), UCLA Symposia on Molecular and Cellular Biology: Mechanisms and Consequences of DNA Damage Processing, Alan R. Liss, New York. in press.
10. Golub,E., Bailone,A. and Devoret,R. (1988) J. Bacteriol. **170**, 4392-4394.
11. Bagdasarian,M., D'Ari,R., Filipowicz,W. and George,J. (1980) J. Bacteriol. **141**, 464-469.
12. Fürste,J.P., Pansegrau,W., Frank,R., Blocker,H., Scholz,P., Bagdasarian,M. and Lanka,E. (1986) Gene **48**, 119-131.
13. Chang,A.C.Y. and Cohen,S.N. (1978) J. Bacteriol. **134**, 1141-1156.
14. Kolodkin,A.L., Capage,M.A., Golub,E.I. and Low,K.B. (1983) Proc. Natl. Acad. Sci. USA **80**, 4422-4426.
15. Linder,P., Churchward,G., Yi-Yi,X.G.Y. and Caro,L. (1985) J. Mol. Biol. **181**, 383-393.
16. Casadaban,M.J., Chou,J. and Cohen,S.N. (1980) J. Bacteriol. **143**, 971-980.
17. Zubay,G. (1980) In Grossman,L. and Moldave,K. (eds), Methods in Enzymology vol.65 part I, Academic Press, . pp. 856-877.
18. Dale,R.M.K., McClure,B.A. and Houchins,J.P. (1985) Plasmid **13**, 31-40.
19. Sanger,F., Nicklen,S. and Coulson,A.R. (1977) Proc. Natl. Acad. Sci. USA **74**, 5463-5467.
20. Harr,R., Fällman,P., Häggström,M., Wahlström,L. and Gustafsson,P. (1986) Nucleic Acids Res. **14**, 273-284.
21. Harley,C.B. and Reynolds,R.P. (1987) Nucleic Acids Res. **15**, 2343-2361.
22. Ciampi,M.S., Schmid,M.B. and Roth,J.R. (1982) Proc. Natl. Acad. Sci. USA **79**, 5016-5020.
23. Halling,S.M., Simons,R.W., Way,J.C., Walsh,R.B. and Kleckner, N. (1982) Proc. Natl. Acad. Sci. USA **79**, 2608-2612.

24. Simon,M.I., Emerson,S.E., Shaper,J.N., Bernard,P.D. and Glazer,A.N. (1977) J. Bacteriol. **130**, 200-204.
25. Noel,D., Nikaido, K. and Ames,G.F. (1979) Biochemistry **18** 4159-4165.
26. Garnier,J., Osguthorpe,D.J., and Robson,B. (1978) J. Mol. Biol. **120**, 97-120
27. Willetts,N. and Wilkins,B. (1984) Microbiol. Rev. **48**, 24-41.
28. Timmis,K.N., Cabello,F. and Cohen,S.N. (1978a) Molec. Gen. Genet. **162**, 121-137.
29. Fee,B.E. and Dempsey,W.B. (1986) J. Bacteriol. **167**, 336-345.