Characterization of *Rrh4273I*, a restriction–modification system of *Rhodococcus rhodochrous* ATCC 4273 (*Nocardia corallina*) which recognizes the same sequence as the *Streptomyces albus* G *SalI* restriction–modification system

M. J. YEBRA, I. S. NOVELLA, C. BARBÉS, J. F. APARICIO, C. G. MARTIN, C. HARDISSON and J. SÁNCHEZ*

Area de Microbiología, Departamento de Biología Funcional, Facultad de Medicina, Universidad de Oviedo, Oviedo 33006, Spain

(Received 29 October 1990; revised 31 January 1991; accepted 7 February 1991)

Rhodococcus rhodochrous ATCC 4273 (*Nocardia corallina*) has a restriction–modification system with the same recognition sequence, methylation site and cleavage site as the *SalI* restriction–modification system. Both the restriction endonuclease and the DNA-methyltransferase (DNA-MTase) have been partially purified and characterized. The nuclease has requirements for activity similar to *SalI*, and a native M_r of about 46 000. The DNA-MTase is a protein with an M_r of about 67 000. No DNA homology was detected between the cloned *SalI* restriction–modification genes of *Streptomyces albus* and *R. rhodochrous* chromosomal DNA.

Introduction

Many restriction endonucleases have been isolated from actinomycetes (Roberts, 1990) but only one adenine DNA-methyltransferase (DNA-MTase), which does not appear to be associated with a restriction system has been purified from *Streptomyces antibioticus* (Barbés *et al.*, 1988).

The *SalI* endonuclease of *Streptomyces albus* G has been studied biochemically (Arrand *et al.* 1978; Maxwell & Halford, 1982*a, b, c*) and genetically (Chater & Wilde, 1976, 1980), and the gene has been cloned (Rodicio & Chater, 1988*a*). Isoschizomers of *SalI* have been found in different strains belonging to the genera *Nocardia*, *Rhodococcus* (*Rhodococcus* sp. and *R. rhodochrous* ATCC 4276, ATCC 14349 and ATCC 21664), *Herpetosiphon* and *Xanthomonas* (Arrand *et al.*, 1978; Kröger *et al.*, 1984; Roberts, 1990). Hybridization between the *SalI* restriction–modification genes of *S. albus* G and DNA from some but not all of the bacteria producing *SalI* isoschizomers was observed by Rodicio & Chater (1988*a*).

In this work we describe a restriction–modification system from *R. rhodochrous* ATCC 4273 which recog-

nizes the same sequence as the *S. albus* G restriction–modification system. Both the *Rrh4273I* endonuclease and the DNA-MTase have been partially purified and characterized.

Methods

Bacterial strains, plasmids and phages. The strain used in this study was *R. rhodochrous* ATCC 4273. λ , pBR322 and calf thymus DNA were supplied by Boehringer; M13mp19 single-stranded DNA was isolated from *Escherichia coli* (Sambrook *et al.*, 1989); P27 and R4 phage DNA were purified as described in Sánchez *et al.* (1985), and ϕ A7 is described by Díaz *et al.* (1989). Chromosomal DNA of *Rhodococcus* and *Streptomyces* was isolated as described by Hopwood *et al.* (1985).

Chemicals. *S*-Adenosyl-L-[methyl- ^3H]methionine [15 Ci mmol $^{-1}$ (0.56 TBq mmol $^{-1}$)] (^3H -SAM) was purchased from Dupont. M_r markers for gel filtration comprising ovalbumin, BSA and cytochrome *c* were obtained from Sigma. The following materials were used for column chromatography: phosphocellulose (Pharmacia), hydroxylapatite (Bio-Gel) HT, Bio-rad, Sephadex G-100 (Pharmacia), Blue Sepharose CL-6B (Pharmacia) and DNA cellulose (double-stranded DNA) (Sigma). Aquacide II was obtained from Calbiochem. Restriction endonucleases (*SalI*, *TaqI* and *HaeIII*) were obtained from Boehringer and used as indicated by the suppliers.

Partial purification and characterization of the *R. rhodochrous* *Rrh4273I* restriction endonuclease. *R. rhodochrous* was grown at 30 °C with aeration for 24 h (to early stationary growth-phase) in 6 litres of

Abbreviation: MTase, methyltransferase.

NBG medium (de los Reyes Gavilán *et al.*, 1988) and harvested by centrifugation. Approximately 33 g of wet cells were resuspended in 148 ml phosphate buffer (Greene *et al.*, 1978) containing 0.15 M-NaCl and disrupted by passing them once through a French pressure cell (Apex) followed by 20 min sonication (30 s periods) at 14 µm amplitude. The extract was clarified by 10 min centrifugation at 15000 r.p.m. followed by 90 min centrifugation at 25000 r.p.m. (75000 g) at 4 °C. The supernatant was then applied to a phosphocellulose column (bed volume 100 ml) and eluted with 700 ml 0.15 to 0.8 M-NaCl. Active fractions were dialysed against buffer A (Barbés *et al.*, 1988), containing 0.1 M-NaCl, applied to a column of DNA cellulose (bed volume 5 ml) and eluted with 30 ml 0.1 to 1 M-NaCl. The enzyme-containing fractions were concentrated to 2 ml by dialysis against buffer A containing 0.1 M-NaCl and 50% (v/v) glycerol, and used to determine the optimal conditions of reaction. A column of Sephadex G-100 was used for M_r determination. Thermostability was investigated by incubating the enzyme preparation for 10 min at different temperatures, between 30 and 60 °C, after which the remaining activity was analysed as described below.

Endonuclease assay. The reaction mixture (30 µl) contained 10 mM-Tris/HCl (pH 8), 1 mM-DTT, 10 mM-MgCl₂, 125 mM-NaCl, 0.5 to 1 µg DNA substrate and 3 µl enzyme extract. Incubation was at 37 °C for 30 min to 1 h. After the reaction was stopped (Sánchez *et al.*, 1985), samples were analysed by electrophoresis on 2% (w/v) agarose gels, stained in ethidium bromide (1 µg ml⁻¹), and observed under UV light.

Identification of the *Rrh4273I* cleavage site. This was done according to the enzymic sequencing approach described by Brown & Smith (1980). M13mp18, which contains the 5'-GTCGAC-3' sequence in the polylinker, was used for enzymic sequencing reactions starting with a 5'-phosphorylated M13 universal sequencing primer. In a parallel reaction, the same primer, end-labelled with T4 polynucleotide kinase and [γ -³²P]ATP, was annealed to the template, and the labelled primer was extended by treatment with Klenow polymerase and all four dNTPs through the *Rrh4273I* site. The double-stranded DNA was used as a substrate for *Rrh4273I* and *SalI* to produce 5'-end-labelled DNA fragments comparable to the sequencing ladder. Samples were analysed by electrophoresis and subsequent autoradiography.

Purification and characterization of the modification MTase *M.Rrh4273I*. Cell-free extract was prepared as described above, but in this case 130 g cells were used and resuspended in 500 ml phosphate buffer (Greene *et al.*, 1978) containing 0.2 M-NaCl. The extract was applied to a phosphocellulose column (bed volume 200 ml) and eluted with a 1500 ml gradient of 0.2 to 0.8 M-NaCl. The enzyme-containing fractions were diluted in the above buffer to adjust the NaCl concentration to 0.1 M, applied to a column of hydroxylapatite (bed volume 50 ml) and eluted with a 350 ml gradient of 0.01 to 0.5 M-KH₂PO₄. The active fractions were dialysed against buffer A (Barbés *et al.*, 1988) containing 0.1 M-NaCl, applied to a column of Blue Sepharose CL-6B (bed volume 14 ml) and eluted with a 120 ml gradient of 0.1 to 2.2 M-NaCl. The active fractions from this column were diluted to 0.1 M-NaCl, applied to a column of DNA cellulose (bed volume 5 ml) and eluted with a 30 ml gradient of 0.1 to 0.7 M-NaCl. Finally, the enzyme-containing fractions were concentrated to 3 ml in Aquacide II and dialysed against buffer A containing 0.1 M-NaCl and 50% (v/v) glycerol. A column of Sephadex G-100 (bed volume 130 ml) was used to determine the molecular weight. Thermostability of the enzyme was analysed as described above for the nuclease.

SDS-PAGE. Samples from the different chromatographic steps were analysed by SDS-PAGE using 12% (w/v) polyacrylamide gels, according to Laemmli (1970). Proteins were silver-stained, as described by Morrissey (1981). Protein standards for M_r estimations were obtained from Pharmacia.

Preparation of a cell-free extract from *S. albus* G. About 12 g cells, grown in NBG medium were resuspended in 50 ml buffer A and ruptured by passing through a French pressure cell. The extract was centrifuged as described for the *Rrh4273I* endonuclease, precipitated by addition of solid ammonium sulphate (Suri *et al.*, 1984) and dialysed against buffer A.

MTase assay. The reaction mixture (30 µl) using P27 DNA as substrate is described by Barbés *et al.* (1988). The ability of *M.Rrh4273I* to protect DNA against cleavage by *SalI* was tested as follows. After incubation for 1 to 2 h at 37 °C, the MTase was inactivated by heating the samples for 10 min at 70 °C and cooling in ice. Then 5 µl of restriction buffer and 1 µl of *SalI* restriction enzyme were added as described by the manufacturers and incubated for 1 h at 37 °C. Agarose electrophoresis was carried out and DNA bands were observed under UV light.

Base analysis of methylated DNA. Methylated bases were analysed after formic acid hydrolysis of the labelled methyl-³H-labelled pBR322 DNA (2 µg), as described by Günthert *et al.* (1976), except that the DNA was resuspended in 1.5 ml formic acid (90%, v/v) and incubated for 120 min at 165 °C.

Analysis of the sequence recognized by *M.Rrh4273I*. One microgram of pBR322 DNA was digested for 2 h with *SalI* (5 U) or *TaqI* (8 U) under two different conditions: unmethylated, and after modification with *M.Rrh4273I* and ³H-SAM for 1 h; fluorography was carried out following the method described by Suri *et al.* (1984), using 5% (w/v) polyacrylamide. In the other experiment, pBR322 DNA (2 µg) was methylated with *M.Rrh4273I* and ³H-SAM for 30 min and digested for 2 h with *HaeIII* (5 U), before fluorography.

Hybridization between *SalI* restriction-modification genes and DNA from *R. rhodochrous*. Plasmids pIJ4435 and pIJ4442 containing the *SalI* restriction-modification genes (Rodicio & Chater, 1988a) were used for hybridization with *R. rhodochrous* chromosomal DNA. The hybridization procedure was performed as described by Hopwood *et al.* (1985). DNA restriction endonuclease fragments were transferred to a nylon membrane (Hybond-N, Amersham) by the method of Southern (Hopwood *et al.*, 1985) and fixed by exposing the membrane to low doses of UV irradiation (254 nm). The prehybridization and hybridization were carried out for 5 and 16 h, respectively, at 70 °C in 3 × SSC (1 × SSC is 0.15 M-NaCl, 0.015 M-trisodium citrate, pH 7.0), 4 × Denhardt's solution (1 × Denhardt's solution is 0.02% BSA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), and 100 µg denatured herring sperm DNA ml⁻¹.

The membrane was washed twice for 30 min at 70 °C in 2 × SSC/0.1% SDS. In these conditions probes with higher homology than 68% remain bound (Hopwood *et al.*, 1985).

Results and Discussion

Presence of a SalI-isoschizomer restriction system in R. rhodochrous

Cell-free crude extracts of *R. rhodochrous* showed a type II endonuclease activity which gave the same fragments as *SalI* with λ , ϕ A4 and ϕ A9 DNA. After double digestion of ϕ A9 DNA with *SalI* and *Rrh4273I* no new bands appeared. Chromosomal DNA from *R. rhodochrous* was not cleaved by *SalI* or *Rrh4273I*,

Fig. 1. Determination of cleavage site (arrow) for *Rrh4273I* on M13mp18. The last two lanes contain *SalI*- and *Rrh4273I*-cut DNA.

indicating that *Rrh4273I* might contain a specific DNA methylase.

Partial purification and characterization of the Rrh4273I restriction endonuclease

Endonuclease activity eluted in a unique peak at 0.6 M-NaCl from a phosphocellulose column and at about 0.3 M-NaCl from DNA cellulose. The enzyme preparation thus obtained was free from other contaminating nucleases as tested by overnight hydrolysis of several DNA substrates.

The M_r of *Rrh4273I*, determined with a Sephadex G-100 column, was about 46000. Mg^{2+} was absolutely required for the reaction and could not be replaced by Ca^{2+} . Substitution of Mg^{2+} by Mn^{2+} caused loss of specificity of *Rrh4273I* but not of *SalI*. This effect has also been reported for other restriction endonucleases (Hsu & Berg, 1978). Endonuclease activity was observed between 20 and 200 mM-NaCl and between pH 6.2 and 9.0. Addition of phenol (50 mM) or dimethylsulphoxide (20%, (v/v)) to the reaction mixture inhibited the activity by about 70%. The enzyme was stable at 45 °C for

10 min. At 50 °C about 50% of the activity was lost within 10 min and complete loss of activity was observed after 10 min at 60 °C. It can be concluded that the general requirements and the optimal conditions for *Rrh4273I* are similar to those described for *SalI* (Maxwell & Halford, 1982a).

Identification of the cut position within the Rrh4273I recognition site

Double-stranded M13mp18 samples (see Methods) treated with *Rrh4273I* or *SalI* produced identical single bands of the sizes expected for cleavage between G and T in the *SalI* recognition sequence 5'-G↓TCGAC-3' (Fig. 1).

Purification and characterization of M.Rrh4273I

DNA-MTase activity eluted in single peaks between 0.35 and 0.45 M-NaCl from phosphocellulose, between 0.15 and 0.25 M- KH_2PO_4 from hydroxylapatite, between 1.4 and 1.6 M-NaCl from blue Sepharose and as a sharp peak at about 0.35 M-NaCl from DNA cellulose. The results of this purification are summarized in Table 1. SDS-PAGE of the purest enzyme preparation showed two major protein bands (Fig. 2c). SDS-PAGE analysis together with the measurement of MTase activity from each of the fractions from the DNA cellulose column suggested that the *M.Rrh4273I* enzyme corresponded with the upper band, with an M_r of about 67000 (Fig. 2c, d). A native M_r of about 65000 was obtained after Sephadex G-100 filtration (data not shown), indicating that the enzyme is monomeric, as are most of the DNA-MTases that have been characterized (Lauster, 1989).

Purification of *M.SalI* from *S. albus* G has proved to be difficult (M. J. Yebra, unpublished results), which makes *R. rhodochrous* a possible alternative source of this enzyme. However, loss of *M.Rrh4273I* activity in the last step of purification (see Table 1) is considerable. This, could be due to the relative instability of the enzyme at this high degree of purification. The addition of BSA (100 $\mu g\ ml^{-1}$) to this preparation improved the thermal stability of the enzyme (data not shown).

Table 1. *Purification of DNA-MTase from R. rhodochrous*

Purification step	Protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Purification (-fold)	Yield (%)
Cell-free extract	1860	8000	4.3	1	100
Phosphocellulose	51	21250	416	96.7	255.6
Hydroxylapatite	20.4	10000	490	113.9	125
Blue Sepharose	5.3	4750	892	207.4	59.4
DNA cellulose	1.3	833	634.9	147.6	10.4

Fig. 2. SDS-PAGE analysis of samples obtained during the *M.Rrh4273I* purification process (*a*, *b*, *c*) and DNA-MTase activity of different fractions obtained after the last step of purification (*d*). (*a*, *b*) Pool of active fractions after (*a*) phosphocellulose chromatography and (*b*) Cibacron Blue Sepharose chromatography. (*c*, *d*) PAGE analysis (*c*) and activity (*d*) of four active fractions after DNA cellulose chromatography. The activity was measured as the capability to protect P27 DNA (0.5 µg) against cleavage by *SalI*, as indicated in Methods. Positions of M_r standards are shown on the left.

Table 2. Methylation of several plasmids and phage DNA substrates with *M.Rrh4273I*

Substrate DNA (1 µg)	Radioactivity incorporated into DNA (d.p.m.)*	D.p.m. per molecule ($\times 10^{-7}$)	No. of <i>SalI</i> sites in DNA
P27	43716	18.44	> 30 (M. J. Yebra, unpublished results)
R4	29832	16.60	> 30 (Chater & Carter, 1979)
λ	2227	1.19	2
pBR322	17185	0.82	1
ϕ A7	≤ 100	—	0 (Diaz <i>et al.</i> , 1989)
P27†	≤ 100	—	> 30
P27‡	5893	2.48	> 30
λ ‡	152	0.08	2
M13mp19§	≤ 100	—	1

* The data represent the means of three experiments.

† DNA first cleaved with *SalI* and then methylated.

‡ DNA heat-denatured.

§ Single-stranded form.

The requirements for the reaction were studied. The optimal NaCl concentration was about 25 mM. At 50 mM-NaCl the activity was reduced to 50% and no activity was observed at 100 mM-NaCl. The enzyme obeyed Michaelis-Menten kinetics with respect to both SAM, and P27 DNA (not shown). The K_m for SAM was 4.6 µM, and for P27 DNA 0.42 nM.

M.Rrh4273I was active between pH 6.2 and 9.0, with maximum activity at pH 8.0. The maximum velocity of the reaction was observed at 37 °C. Under these optimal conditions the reaction was linear for 60 min. Addition of Mg^{2+} (100 µM) or ATP (1 mM) to the buffer had no

effect on the reaction. The enzyme was stable at 35 °C for 10 min, but at 45 °C all activity was lost.

DNA from different sources was tested with *M.Rrh4273I* (Table 2). The amount of radioactivity incorporated into the DNA was in agreement with the amount expected from the number of *SalI* restriction sites in the DNA molecules tested. It seems that the enzyme does not methylate single-stranded DNA. It has been suggested that ability to methylate single-stranded DNA could be a characteristic of some monomeric methylases which may interact asymmetrically with the recognition site of only one strand of the DNA (Smith &

Fig. 3. Sensitivity of pBR322 to *SalI* (a) and *TaqI* (b) after *in vitro* methylation by *M.Rrh4273I* for 1 h. Lanes: 1, pBR322 DNA; 2, pBR322 DNA cleaved with *SalI* and subsequently methylated for 1 h with *M.Rrh4273I*; 3, DNA methylated for 1 h with *M.Rrh4273I* before digestion with *SalI*; 4, 5, 6, fluorographic detection of the [^3H]methyl groups in the above substrates; 7, 8, *TaqI* digests of pBR322 before and after modification with *M.Rrh4273I*. Bands affected by the modification are indicated by arrowheads.

Kelly, 1984). Chromosomal DNA isolated from *R. rhodochrous* or *S. albus* G was not a substrate for *M.Rrh4273I*, while DNA from *S. incarnatus* NRRL 8089 and *S. antibioticus* ETHZ 7451 was efficiently methylated.

M.Rrh4273I recognizes the same sequence and methylates the same base as *M.SalI*

The sensitivity of pBR322 to *SalI* before and after modification with *M.Rrh4273I* is shown in Fig. 3(a). The methylated DNA (lanes 3 and 6) is not cleaved by *SalI*. *TaqI* recognizes the sequence TCGA and is sensitive to adenine methylation (Nelson & McClelland, 1989). Fig. 3(b), lane 8, shows that, as expected *M.Rrh4273I* protects only the TCGA (*TaqI*) sequence within the *SalI* (GTCGAC) recognition site from being cleaved by *TaqI*. The results proved that the sequences recognized by *M.Rrh4273I* and *M.SalI* are identical. Restriction of methylated pBR322 with *HaeIII* and fluorography confirmed that methylation occurs exclusively in the DNA fragment containing the *SalI* site (Fig. 4). Thin-layer chromatography of hydrolysed pBR322 DNA methylated with *M.Rrh4273I*, or crude cell-free extracts of *S. albus* G, showed that adenine, but not cytosine, was methylated *in vitro*. Adenine also seems to be modified *in vivo* in *S. albus* G, as suggested by the above results showing a lack of methylation of *S. albus* G chromosomal DNA by *M.Rrh4273I*. This corroborates the previously reported indirect evidence that the *S.*

Fig. 4. Location of the *M.Rrh4273I*-specific modification site on pBR322. Lanes: 1, PAGE of modified pBR322 cleaved with *HaeIII*; 2, fluorographic detection of [^3H]methyl group incorporation. The band affected by modification (indicated by an arrow) contains the *SalI* site.

albus G modification enzyme protects its own DNA by methylation of adenines within the recognition sequence (Rodicio & Chater, 1988b).

Homology between *SalI* restriction-modification genes and DNA from *R. rhodochrous*

No homology between total DNA from *R. rhodochrous* and the cloned *SalI* restriction-modification genes was detected using low-stringency conditions (see Methods) and long autoradiographic exposure (data not shown). This fact, together with the similar specificity between *R. rhodochrous* and *S. albus* G enzymes, raises the question of which parts of these proteins are conserved. Cloning and sequence analysis of the *R. rhodochrous* restriction-modification genes will facilitate these studies.

We thank Dr T. A. Trautner for his valuable comments on the manuscript. M. J. Yebra was the recipient of a predoctoral fellowship of the Ministry of Education and Science, Spain. Research was supported by grant BI089-0014 from the Plan Nacional de Investigación Científica y Desarrollo Tecnológico, Comisión Interministerial de Ciencia y Tecnología, Madrid, Spain.

References

- ARRAND, J. R., MYERS, P. A. & ROBERTS R. J. (1978). A new restriction endonuclease from *Streptomyces albus* G. *Journal of Molecular Biology* **118**, 127–135.
- BARBÉS, C., HARDISSON, C., NOVELLA, I. S., YEBRA, M. J. & SÁNCHEZ, J. (1988). DNA methyltransferase activities in *Streptomyces antibioticus*. *FEMS Microbiology Letters* **55**, 59–64.
- BROWN, N. L. & SMITH, M. (1980). A general method for defining restriction enzyme cleavage and recognition sites. *Methods in Enzymology* **65**, 391–404.

- CHATER, K. F. & CARTER, A. T. (1979). A new, wide host-range, temperate bacteriophage (R4) of *Streptomyces* and its interaction with some restriction-modification systems. *Journal of General Microbiology* **115**, 431–442.
- CHATER, K. F. & WILDE, L. C. (1976). Restriction of a bacteriophage of *Streptomyces albus* G involving endonuclease *Sal*I. *Journal of Bacteriology* **128**, 644–650.
- CHATER, K. F. & WILDE, L. C. (1980). *Streptomyces albus* G mutants defective in the *Sal*I restriction-modification system. *Journal of General Microbiology* **116**, 323–334.
- DIAZ, L. A., HARDISSON, C. & RODICIO, M. R. (1989). Isolation and characterization of actinophages infecting *Streptomyces* species and their interaction with host restriction-modification systems. *Journal of General Microbiology* **135**, 1847–1856.
- GREENE, P. J., HEYNEKER, H. L., BOLIVAR, F., RODRIGUEZ, R. L., BETLACH M. C., COVARRUBIAS, A. A., BACKMAN, K., RUSSEL, D. J., TAIT, R. & BOYER, H. W. (1978). A general method for the purification of restriction enzymes. *Nucleic Acids Research* **5**, 2373–2380.
- GÜNTHER, U., SCHWEIGER, M., STUPP, M. & DOERFLER, W. (1976). DNA methylation in adenovirus, adenovirus-transformed cells and host cells. *Proceedings of the National Academy of Sciences of the United States of America* **73**, 3923–3927.
- HOPWOOD, D. A., BIBB, M. J., CHATER, K. F., KIESER, T., BRUTON, C. J., KIESER, H. M., LYDIATE, D. J., SMITH, C. P., WARD, J. M., SCHREMPF, H. (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich, UK: John Innes Foundation.
- HSU, M. T. & BERG, P. (1978). Altering the specificity of restriction endonuclease: effect of replacing Mg^{2+} with Mn^{2+} . *Biochemistry* **17**, 131–138.
- KRÖGER, M., HOBOM, G., SCHÜTTE, H. & MAYER, H. (1984). Eight new restriction endonucleases from *Herpetosiphon giganteus*—divergent evolution in a family of enzymes. *Nucleic Acids Research* **12**, 3127–3141.
- LAEMMLI, U. K. (1970). Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- LAUSTER, R. (1989). Evolution of Type II DNA methyltransferases. A gene duplication model. *Journal of Molecular Biology* **206**, 313–321.
- MAXWELL, A. & HALFORD, S. E. (1982a). The *Sal*GI restriction endonuclease. Purification and properties. *Biochemical Journal* **203**, 77–84.
- MAXWELL, A. & HALFORD, S. E. (1982b). The *Sal*GI restriction endonuclease. Mechanism of DNA cleavage. *Biochemical Journal* **203**, 85–92.
- MAXWELL, A. & HALFORD, S. E. (1982c). The *Sal*GI restriction endonuclease. Enzyme specificity. *Biochemical Journal* **203**, 93–98.
- MORRISSEY, J. M. (1981). Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. *Analytical Biochemistry* **117**, 307–310.
- NELSON, M. & MCCLELLAND, M. (1989). Effect of site-specific methylation on DNA modification methyltransferases and restriction endonucleases. *Nucleic Acids Research* **17**, 389–415.
- DE LOS REYES-GAVILÁN, C. G., APARICIO, J. F., BARBÉS, C., HARDISSON, C. & SÁNCHEZ, J. (1988). An exocyttoplasmic endonuclease with restriction function in *Streptomyces antibioticus*. *Journal of Bacteriology* **170**, 1339–1345.
- ROBERTS, R. J. (1990). Restriction enzymes and their isoschizomers. *Nucleic Acids Research* **18**, 2331–2365.
- RODICIO, M. R. & CHATER, K. F. (1988a). Cloning and expression of the *Sal*I restriction-modification genes of *Streptomyces albus* G. *Molecular and General Genetics* **213**, 346–353.
- RODICIO, M. R. & CHATER, K. F. (1988b). The *Sal*I (*Sal*GI) restriction-modification system of *Streptomyces albus* G. *Gene* **74**, 39–42.
- SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. (1989). *Molecular cloning. A Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- SÁNCHEZ, J., BARBÉS, C., HERNÁNDEZ, A., DE LOS REYES-GAVILÁN, C. G. & HARDISSON, C. (1985). Restriction-modification systems in *Streptomyces antibioticus*. *Canadian Journal of Microbiology* **31**, 942–946.
- SMITH, H. O. & KELLY, S. V. (1984). Methylases of the type II restriction-modification systems. In *DNA Methylation, Biochemistry and Biological Significance*, pp. 39–71. Edited by A. Razin, H. Cedar & A. D. Riggs. New York: Springer-Verlag.
- SURI, B., SHEPHERD, J. C. W. & BICKLE, T. A. (1984). The *Eco*A restriction and modification system of *Escherichia coli* 15 T⁻: enzyme structure and DNA recognition sequence. *EMBO Journal* **3**, 575–579.