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SHORT COMMUNICATION

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The *malEFG* gene cluster of *Streptomyces coelicolor* A3(2): characterization, disruption and transcriptional analysis

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Abstract The *malEFG* gene cluster of the Gram-positive mycelial actinomycete *Streptomyces coelicolor* A3(2) was cloned and sequenced. MalEFG show only limited similarity to homologues involved in maltose and maltodextrin transport in other bacteria. Disruption of *malE* prevented the utilization of maltose as carbon source. Transcription of *malE* was induced by maltose and repressed by glucose.

Key words *Streptomyces coelicolor* A3(2) · *malEFG* · Glucose repression · Maltose utilization

Introduction

The maltose transport system of both Gram-positive and Gram-negative bacteria belongs to the family of ABC transporters (Higgins 1992) and is responsible for the import of maltose and maltodextrins (for a review see Boos and Lucht 1996). In the Gram-negative *Escherichia coli*, maltose and maltodextrins pass through the outer membrane via the porin LamB. They are then bound by the maltose-binding protein, MalE, and are transported across the inner membrane by a complex containing MalF, MalG, and two copies of MalK, an ATP-binding protein (Nikaido 1994). The genes encoding these proteins are organized in two adjacent and divergently transcribed operons, *malEFG* and *malK-lamB* (Bedouelle and Hofnung 1982). In the Gram-positive bacterium *Streptococcus pneumoniae*, *malXCD* are homologues of *malEFG*, respectively, and are required for maltotetraose transport (Puyet and Espinosa 1993); while a *malK* homologue remains to be identified, the absence of a *lamB* homologue is consistent with the lack of an outer membrane in Gram-positive bacteria. Here we report the isolation and analysis of the *malEFG* gene cluster of *Streptomyces coelicolor* A3(2), a member of the high GC Gram-positive actinomycetes, which are phylogenetically distinct from *St. pneumoniae*.

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Results and discussion

The *malEFG* gene cluster of *S. coelicolor*

Analysis of the nucleotide sequence upstream of *malR*, a *lacI*/*galR*-like repressor gene of *S. coelicolor* (van Wezel et al. 1997), revealed four open reading frames (ORFs) whose products (MalE, MalF, MalG and AglA) resemble proteins involved in maltose and maltodextrin transport and utilization in other bacteria (Fig. 1). In *E. coli*, *malE* encodes a 396 amino acid (aa) propeptide with a 26 aa signal sequence at its N-terminus that is removed during translocation of MalE to the periplasmic space (Duplay et al. 1984); in *St. pneumoniae*, *malX* encodes a 423 aa protein whose N-terminal signal sequence is probably not cleaved, possibly providing a membrane anchoring domain (Puyet and Espinosa 1993). The MalE/X homologue of *S. coelicolor* (MalE, 423 aa) also possesses a likely signal peptide (residues 1–17 as predicted using PSORT; Hoffman and Stoffel 1993) but shares only 25% and 30% sequence identity with MalE of *E. coli* and MalX of *St. pneumoniae*, respectively, with the similarity spread throughout the length of the proteins. A tyrosine residue (Y210 of the mature protein), implicated in the interaction of MalE with MalF in *E. coli* (Hor and Shuman 1993), is also present in MalE of *S. coelicolor*, but is replaced by glycine in MalX.

MalF of *S. coelicolor* shares 31% and 28% sequence identity with MalF of *E. coli* and MalC of *St. pneumoniae*,

respectively, with most of the similarity occurring towards the carboxyl ends of the proteins. MalF of *S. coelicolor* (334 aa) is 180 aa shorter than MalF from *E. coli* (SWISSPROT P02916), *Salmonella typhimurium* (P26467) and *Enterobacter aerogenes* (P18812) (all 514 aa), and 96 aa shorter than MalC (430 aa; Puyet and Espinosa 1993). The N-terminal 144 aa of the enterobacterial MalFs, which encode the first three of eight membrane-spanning domains (Froshauer et al. 1988), and the first 86 aa of MalC of *St. pneumoniae*, which encode the first two of nine predicted membrane-spanning domains (determined using the membrane protein topology prediction program TMPRED; Hoffman and Stoffel 1993), are absent from MalF of *S. coelicolor*. However, a cluster of charged residues thought to be involved in anchoring MalF of *E. coli* to the inner side of the cytoplasmic membrane (Froshauer et al. 1988) is also present in MalF of *S. coelicolor*: more than half of residues 6–27 are charged. Analysis of MalF with TMPRED (Hoffman and Stoffel 1993) suggests that the streptomycete homologue spans the cytoplasmic membrane six times.

Two genes, *malG* and *aglA*, occur downstream of *malF* (Fig. 1). MalG (302 aa), is a homologue of MalG (296 aa) of *E. coli* (34% identity) and of MalD (277 aa) of *St. pneumoniae* (28% identity), with most of the similarity occurring in the c-terminal two-thirds of the proteins. AglA resembles α-glucosidases found in other bacteria, e.g. an α-glucosidase from *Thermomonospora curvata* (EMBL U17917) (75% identity over 161 aa), an isomaltase from *Bacillus cereus* (SWISSPROT P21332) (44% identity over 164 aa) and a trehalose-6-phos phohydrolase from *E. coli* (P28904) (48% identity over 161 aa). In contrast to *E. coli* and *S. typhimurium* (Bedouelle and Hofnung 1982), *S. coelicolor* has no *malK* or *lamB* homologue upstream of *malEFG*. The absence of a LamB homologue is not surprising since Gram-positive bacteria lack an outer membrane. However, MalK is an essential component of the inner membrane complex $MalFGK_2$ in *E. coli* and a *malK* homologue is therefore expected elsewhere on the *S. coelicolor* chromosome. The recently identified *msiK*, a *malK* homologue, in the closely related *Streptomyces lividans* (Hurtubise et al. 1995), may fulfill this function, *msiK*, whose apparent homologue in *S. coelicolor* maps at a different location to *malEFG* (Redenbach et al. 1996), is required for both cellobiose and xylobiose uptake in *S. lividans*; its ability to transport disaccharides containing pentoses or hexoses may indicate a general role for MsiK in sugar uptake in streptomycetes, including that of maltose and maltodextrins (the ATP-binding protein MsmK, a homologue of MalK, is also involved in the transport of multiple sugars in *Streptococcus mutans*; Russell et al. 1992).

Both MalF and MalG of *S. coelicolor* contain the conserved EAA cytoplasmic loop (residues 227–248 of MalF and 196–217 of MalG) that is found in all other integral membrane components of binding protein-dependent transport systems (Boos and Lucht 1996), and that may interact with the membrane-associated ATP-hydrolyzing subunit, in this case the hypothetical MalK.

The *malXCD* transcript of *St. pneumoniae* contains a 115 bp intergenic region between *malX* and *malC* with the potential to form a large secondary structure that may be involved in mRNA processing and regulation of *malXCD* expression (Puyet and Espinosa 1993). Inspection of the 118 bp intergenic region between *malE* and *malF* of *S. coelicolor* revealed perfect inverted repeats of 14 bp separated by 12 bp that may function in a similar manner, or that may act as a transcription termination site (Richardson and Greenblatt 1996) to modulate the levels of *malFG* expression (in *E. coli*, the level of MalE exceeds that of MalF by 30–100 fold; di Guan et al. 1988; Froshauer et al. 1988).

The *malEFG* cluster is essential for maltose utilization in *S. coelicolor*

To assess whether the *malEFG* gene cluster is essential for maltose utilization in *S. coelicolor*, *malE* was disrupted by insertion of *aadA* (Prentki and Krisch 1984), which confers spectinomycin (Spc) resistance. *S. coelicolor* M145 (Hopwood et al. 1985) was transformed with pIJ2571 (Fig. 1) isolated from the methylation-deficient *E. coli* strain ET12567 (MacNeil et al. 1992) (*S. coelicolor* possesses a methyl-specific restriction system that drastically reduces transformation frequencies; MacNeil 1988). Since pIJ2571 cannot replicate in *Streptomyces*, thiostrepton (Thio)-resistant colonies were most likely to arise by single crossover integration of the plasmid into *malE*. A $Thio^R$, Spc^R transformant, shown by Southern analysis to contain pIJ2571 integrated in *malE*, was put through three rounds of sporulation on SFM plates (Floriano and Bibb 1996) containing 50 μg/ml Spc but lacking Thio, to allow a second crossover to occur; the result of the required excision event is shown in Fig. 1. DNA was extracted from a Spc^R $Thio^S$ isolate, designated M544, digested separately with *Sal*I and *Xho*I, and subjected to Southern analysis (Sambrook et al. 1989; van Wezel et al. 1991) using the 2.2 kb *Xho*I fragment containing *malE* and part of *malF* (Fig. 1) as probe. The 2.8 kb *Sal*I and 2.2 kb *Xho*I hybridizing fragments of M145 DNA were replaced by 4.5 kb *Sal*I and 3.9 kb *Xho*I hybridizing fragments in M544 DNA, confirming disruption of *malE* by *aad*.

M544 and its parental strain, M145, were grown in liquid minimal medium (SMM lacking casamino acids; Strauch et al. 1991) containing various carbon sources (1% w/v). The *malE* mutation had no effect on the utilisation of glucose, maltodextrin, arabinose, mannitol or glycerol, but only M145 grew on maltose, albeit poorly (while M544 showed no growth on maltose, M145 reached an OD_{450} of 0.1 after 16 h of incubation at 30° C starting from an initial OD_{450} of 0.02, confirming an earlier report that maltose is a relatively poor carbon source for *S. coelicolor*; Hodgson 1982).

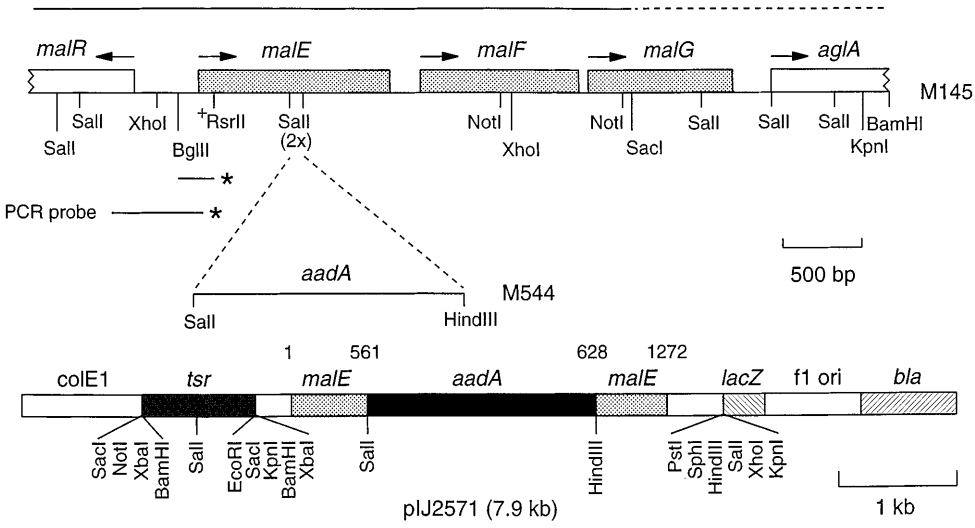

Fig. 1 *Top.* Restriction map of the *malEFG* cluster of *S. coelicolor* M145. *Arrows* indicate the direction of transcription. The fragments used as probes for S1 nuclease mapping (*asterisks* indicate labelled ends) and as templates for the in vitro transcription assays are shown by *horizontal lines.* The location of the inserted *aadA* in the *malE* disruptant M544 is shown *below* the map. Sequence determined on both DNA strands (EMBL accession number Y07706) is shown by the *solid line* above the map; the region marked by the *dotted line* indicates that only part of the sequence was determined on both strands. + indicates the presence of additional unmapped *Rsr*II sites. *Bottom.* Restriction map of pIJ2571. Numbers refer to nucleotide positions in *malE*, with the translation start site chosen as 1. ColE1 and f1 ori, origins of replication; *aadA*, spectinomycin resistance gene; *bla*, ampicillin resistance gene; *tsr*, thiostrepton resistance gene; *lacZ*, segment containing the *lacZ* promoter and encoding the α-fragment of β-galactosidase

As described earlier (Redenbach et al. 1996), the 2.2 kb *Xho*I fragment containing *malE* and part of *malF* (Fig. 1) hybridized to cosmid 10B7 (derived from *Ase*I fragment C) of the ordered cosmid library of the *S. coelicolor* chromosome, locating *malEFG* at approximately 11 o'clock on the circular form of the combined physical and genetic map.

malE is transcribed from a single, maltose-inducible, glucose-repressible promoter

Transcription of *malE* was analysed by S1 nuclease mapping using RNA isolated from exponential phase cultures of M145 grown in SMM containing mannitol (which serves as a non-repressing carbon source for *S. coelicolor*; Virolle et al. 1988), maltose, glucose, or a combination of maltose and glucose as carbon sources (all 1% w/v; for techniques see Strauch et al. 1991). A 549 bp PCR probe (Fig. 1) was made using a ^{32}P 5′ end-labelled (Sambrook et al. 1989) oligonucleotide corresponding to nucleotides 33–13 of *malE* (mal02; Fig. 2) and an unlabelled oligonucleotide (5′-TCGAGGGCGGCGAGCACGGACT-3′) corresponding to nucleotides 125–104 of *malR*. While prolonged exposure of the autoradiograph was needed to reveal a low basal level of *malE* transcription in cultures grown on mannitol, transcription of *malE* was readily detected in the presence of maltose, and strongly repressed by glucose (Fig. 3A). These data were confirmed using RNA from independently grown cultures and the *Bgl*II-*Rsr*II fragment (Fig. 1) uniquely ^{32}P-labelled at the 5′ end of the *Rsr*II site as probe. These results suggest that maltose acts as an

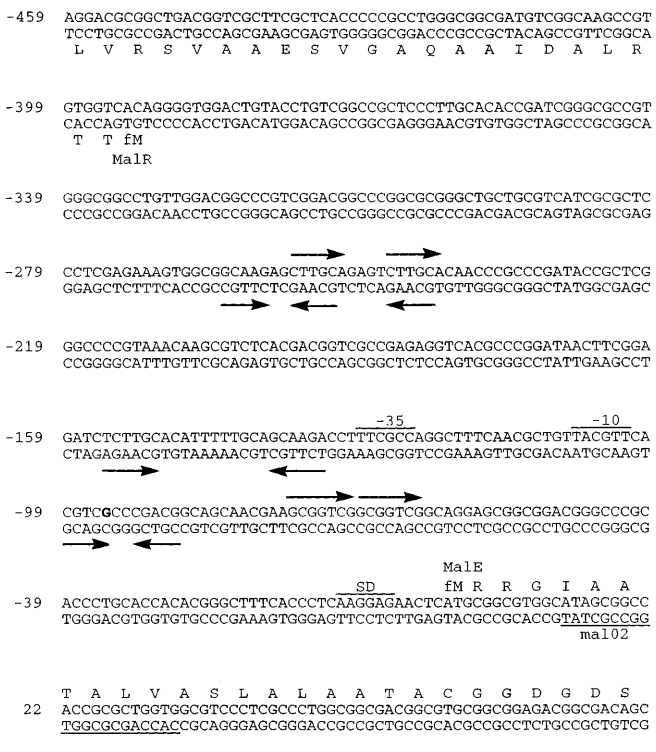

Fig. 2 Nucleotide sequence of the region upstream of *malE*. The *malE* transcription start site is shown in *bold face* at nucleotide position −95; putative −35 and −10 regions, and a Shine-Dalgarno sequence (SD) presumably involved in ribosome-binding are indicated *above* the appropriate nucleotide sequences. The sequence corresponding to oligonucleotide mal02 used in the PCR and for sequencing is *underlined.* Direct repeats and inverted repeats are indicated by *arrows* above and below the sequence, respectively

inducer of *malE* transcription. Although it is possible that contaminating maltodextrins (maltotriose may constitute up to 0.2% of the maltose used in these studies) are the real inducers of the maltose regulon, the ability of the *malE* mutant to utilize maltodextrin but not maltose, suggests that *malEFG* are involved in maltose, rather than maltodextrin, transport.

The transcription start site of *malE* was determined by high-resolution S1 nuclease mapping using RNA isolated from exponential phase M145 cultures grown in SMM containing maltose (1% w/v) as carbon source. The 549 bp PCR fragment described above was used as probe, and the protected fragment analysed alongside the DNA sequence ladder produced using oligonucleotide mal02 as sequencing primer with a T7 DNA polymerase sequencing kit (Pharmacia). The transcript starts at the G located 95 bp 5′ of the ATG translation start of *malE* (Fig. 3B), and is preceded by sequences (Fig. 2, TTCGCC-16 bp-TACGTT) that are similar to the proposed consensus sequence (TTGACN-16–18 bp-TAGAPuT; Strohl 1992) for promoters recognised by the major RNA polymerase holoenzyme of *Streptomyces*. In vitro transcription assays (Buttner and Brown 1985) using *S. coelicolor* RNA polymerase with the 236 bp *Bgl*II-*Rsr*II fragment and the previously described 549 bp PCR product as templates gave the expected run-off transcripts of approximately 190 nt and 120 nt, respectively.

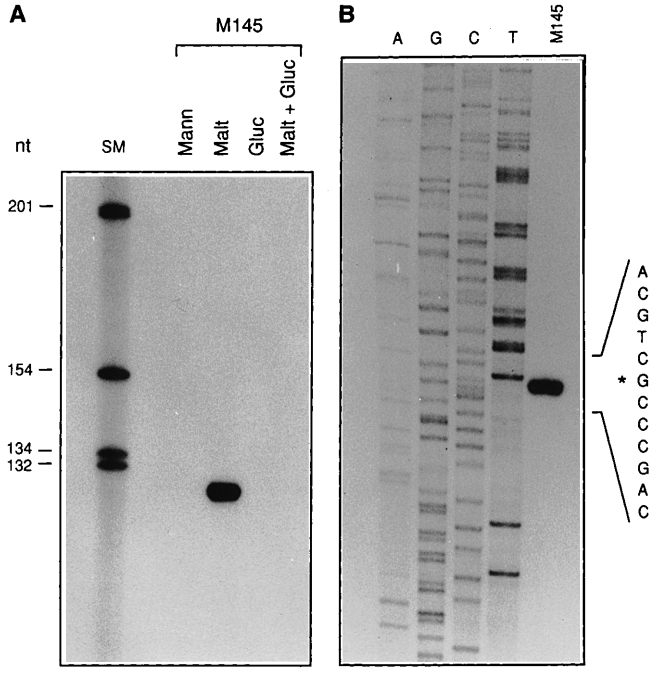

Fig. 3A, B Analysis of *malE* transcription. **A** Transcription of *malE* in SMM-grown cultures of *S. coelicolor* M145 using mannitol (Mann), maltose (Malt), glucose (Gluc) or maltose plus glucose (Malt + Gluc) as carbon sources. SM, ^{32}P end-labelled *Hpa*II-digested pBR322 size markers; nt, nucleotides. **B** Mapping of the transcription start site of *malE*. AGCT, *malE* nucleotide sequence ladder. M145, RNA-protected fragment obtained with RNA isolated from M145 grown in SMM containing maltose; the *asterisk* indicates the most probable transcription start site

Two sets of direct repeats, CTTGCA and GCGGTCG, occur 5′ and 3′ of the *malE* promoter, respectively; the first set also form part of inverted repeats (GCAAG-2 and 12 bp-CTTGC), while another inverted repeat (TCTTGC-11 bp-GCAAGA) occurs just upstream of the putative −35 region (Fig. 2). There is also a small inverted repeat (CGTCG-2 bp-CGACG) that overlaps the *malE* transcription start site. Any or all of these sequences may be binding sites for proteins involved in the regulation of *malE* transcription. Interestingly, the 5′ direct repeat and the inverted repeat located just upstream of the −35 region are also found in the promoter region of the α-amylase gene (*aml*) of *S. limosus* (although they are not located in precisely the same positions with respect to the transcription start sites of *malE* and *aml*), while the direct repeat appears to play a role in induction of *aml* transcription, the inverted repeat has been implicated in both substrate induction and glucose repression (Virolle and Gagnat 1994). Direct repeats have also been implicated in induction and glucose repression of chitinase gene expression in *S. lividans* (Delić et al. 1992).

In conclusion, our data suggest that MalEFG are crucial components of the maltose transport system of *S. coelicolor* that are essential for maltose utilization.

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References

Bedouelle H, Hofnung M (1982) A DNA sequence containing the control regions of the *malEFG* and *malK-lamB* operons in *Escherichia coli* K12. Mol Gen Genet 185:82–87

Boos W, Lucht JM (1996) Periplasmic binding protein-dependent ABC transporters. In: Neidhardt FC (ed) *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology. ASM Press, Washington DC, pp 1175–1209

Buttner MJ, Brown NL (1985) RNA polymerase-DNA interactions in *Streptomyces*. In vitro studies of a *S. lividans* plasmid promoter with *S. coelicolor* RNA polymerase. J Mol Biol 185:177–188

Delić I, Robins P, Westpheling J (1992) Direct repeat sequences are implicated in the regulation of two *Streptomyces* chitinase promoters that are subject to carbon catabolite control. Proc Natl Acad Sci USA 89:1885–1889

Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12:387–395

Di Guan C, Li P, Riggs PD, Inouye H (1988) Vectors that facilitate the expression and purification of foreign peptides in *Escherichia coli* by fusion to maltose-binding protein. Gene 67:21–30

Duplay P, Bedouelle H, Fowler A, Zabin I, Saurin W, Hofnung M (1984) Sequences of the *malE* gene and of its product, the maltose-binding protein of *Escherichia coli* K12. J Biol Chem 259:10606–10613

Floriano B, Bibb MJ (1996) *afsR* is a pleiotropic but conditionally required regulatory gene for antibiotic production in *Streptomyces coelicolor* A3(2). Mol Microbiol 21:385–396

Froshauer S, Green GN, Boyd D, McGovern K, Beckwith J (1988) Genetic analysis of the membrane insertion and topology of MalF, a cytoplasmic membrane protein of *Escherichia coli*. J Mol Biol 200:501–511

Higgins CF (1992) ABC transporters – from microorganisms to man. Annu Rev Cell Biol 8:67–113

Hodgson DA (1982) Glucose repression of carbon source uptake and metabolism in *Streptomyces coelicolor* A3(2) and its perturbation in mutants resistant to 2-deoxyglucose. J Gen Microbiol 128:2417–2430

Hoffman K, Stoffel W (1993) TMbase – a database of membrane spanning protein segments. Biol Chem 347:166

Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM, Schrempf H (1985) Genetic manipulation of *Streptomyces*: a laboratory manual. John Innes Foundation, Norwich

Hor L-I, Shuman HA (1993) Genetic analysis of periplasmic binding protein-dependent transport in *Escherichia coli*. Each lobe of maltose-binding protein interacts with a different subunit of the $MalFGK_2$ membrane transport complex. J Mol Biol 233:659–670

Hurtubise Y, Shareck F, Kluepfel D, Morosoli R (1995) A cellulase/xylanase-negative mutant of *Streptomyces lividans* 1326 defective in cellobiose and xylobiose uptake is mutated in a gene encoding a protein homologous to ATP-binding proteins. Mol Microbiol 17:367–377

MacNeil DJ (1988) Characterization of a unique methyl-specific restriction system in *Streptomyces avermitilis*. J Bacteriol 170:5607–5612

MacNeil DJ, Gewain KM, Ruby CL, Dezeny G, Gibbons PH, MacNeil T (1992) Analysis of *Streptomyces avermitilis* genes required for avermectin biosynthesis utilising a novel integration vector. Gene 111:1–68

Nikaido H (1994) Maltose transport system of *Escherichia coli*: an ABC-type transporter. FEBS Lett 346:55–58

Prentki P, Krisch HM (1984) In vitro insertional mutagenesis with a selectable DNA fragment. Gene 29:303–313

Puyet A, Espinosa M (1993) Structure of the maltodextrin-uptake locus of *Streptococcus pneumoniae*. J Mol Biol 230:800–811

Redenbach M, Kieser HM, Denapaite D, Eichner A, Cullum J, Kinashi H, Hopwood DA (1996) A set of ordered cosmids and a detailed genetic and physical map for the 8 Mb *Streptomyces coelicolor* A3(2) chromosome. Mol Microbiol 21:77–96

Richardson JP, Greenblatt J (1996) Control of RNA chain elongation and termination. In: Neidhardt FC (ed) *Escherichia coli* and *Salmonella typhimurium*. Cellular and molecular biology. ASM Press, Washington DC, pp 822–848

Russell RRB, Aduse-Opoku J, Sutcliffe IC, Tao L, Ferretti JJ (1992) A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. J Biol Chem 267:4631–4637

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual (2nd edn). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Strauch E, Takano E, Baylis HA, Bibb MJ (1991) The stringent response in *Streptomyces coelicolor* A3(2). Mol Microbiol 5:289–298

Strohl WR (1992) Compilation and analysis of DNA sequences associated with apparent *Streptomyces* promoters. Nucleic Acids Res 20:961–974

van Wezel GP, Vijgenboom E, Bosch L (1991) A comparative study of the ribosomal RNA operons of *Streptomyces coelicolor* A3(2) and sequence analysis of *rrnA*. Nucleic Acids Res 19:4399–4403

van Wezel GP, White J, Young P, Postma PW, Bibb MJ (1997) Substrate induction and glucose repression of maltose utilization by *Streptomyces coelicolor* A3(2) is controlled by *malR*, a member of the *lacI-galR* family of regulatory genes. Mol Microbiol 23:537–549

Virolle M-J, Gagnat J (1994) Sequences involved in growth-phase-dependent expression and glucose repression of a *Streptomyces* α-amylase gene. Microbiol 140:1059–1067

Virolle M-J, Long CM, Chang S, Bibb MJ (1988) Cloning, characterization and regulation of an α-amylase gene from *Streptomyces venezuelae*. Gene 74:321–334