Depression of streptomycin production by Streptomyces griseus at elevated growth temperature: studies using gene fusions

V. Jayne Deeble, Helen K. Lindley, Mohammad Reza Fazeli, Jonathan H. Cove and Simon Baumberg

Streptomyces griseus ATCC 12475 fails to produce streptomycin when grown at 34 °C or above, although growth is appreciable up to at least 37 °C. This depression of streptomycin production at elevated growth temperature is manifest equally in liquid and on solid, and with complex and minimal, media. We report studies with gene fusions of the reporter genes aph or xylE to restriction fragments containing the streptomycin biosynthesis promoter PstrB1. aph constructs were in high, and xylE constructs in low, copy number vectors. Two strB1 promoter fragments were used, one requiring activation by the pathway-specific activator StrR of S. griseus, the other reportedly activator independent. PstrB1 expression in the aph constructs in S. griseus and in S. lividans was significantly reduced at 37 °C compared to 30 °C. Some of this reduction could be explained by lower plasmid copy number at the higher temperature, but strR-dependent expression was clearly temperature controlled. Using the xylE reporter system, the temperature dependence of PstrB1 expression was confirmed but, surprisingly, the strR dependence of the two promoter fragments differed from that observed in the multicopy aph constructs. These data identify a temperature-dependent promoter which may contribute to the depressive effect of elevated growth temperature on streptomycin production.

Keywords: antibiotic production, antibiotics, regulation of secondary metabolism, Streptomyces, streptomycin

INTRODUCTION

Secondary metabolite production in streptomycetes is subject to complex physiological and environmental controls (Martin & Demain, 1980). A frequent finding is that growth at elevated temperature leads to a marked depression in production or none at all (Votruba & Vanek, 1989). Three hypotheses accounting for this effect postulate at the elevated growth temperature: (i) lowered expression—which may involve reduction in transcription, translation initiation, or mRNA stability, or any combination of these—of one or more genes whose products are needed for secondary metabolite synthesis; (ii) in vivo heat lability of one or more proteins involved in secondary metabolite production; and/or (iii) diminished availability of precursors due, in some way, to the altered physiological state of the culture. In principle, hypothesis (i) can be distinguished from (ii)/(iii) by its testable prediction of diminished gene expression at the elevated temperature.

One of the best-characterized secondary metabolite systems in terms of regulation is streptomycin biosynthesis and resistance in Streptomyces griseus (Retzlaff et al., 1993; Piepersberg, 1994). str genes, encoding enzymes of streptomycin synthesis, are activated by the presumably pathway-wide activator StrR (Distler et al., 1987b, 1992). Current models suggest that physiological and environmental parameters determine, through a regulatory cascade, the expression of strR and thus the internal level of StrR, which in turn controls the extent of activation of the biosynthetic genes (Vujaklija et al., 1993; Piepersberg, 1994). We have studied regulation at the promoter PstrB1, which directs expression of the strB1 gene; the gene order

Abbreviations: APH, aminoglycoside 3' phosphotransferase; CO, catechol oxygenase.

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in this region of the *S. griseus* chromosome is \( strD - strR - apbD = strA \) - PstrB1 - strB1, where \( apbD \) encodes the major streptomycin resistance enzyme, the 6-phosphotransferase (Retzlaff et al., 1993). We report here the use of fusions of PstrB1 to the reporter genes \( apb \) and \( xylE \) in distinguishing between the above hypotheses (i) and (ii)/(iii) as to the nature of the effect of elevated growth temperature on streptomycin production.

**METHODS**

**Bacterial strains and plasmids.** The bacterial strains used were *S. griseus* ATCC 12475 and *S. lividans* TK54 (Hopwood et al., 1983), a *his leu Spe"* derivative of 1326. The plasmids used are listed in Table 1, and the structures of the gene fusions employed are shown in Fig. 1.

**Media and growth conditions.** Complex media R5 and YEME, and minimal media MM and NMMP, were as in Hopwood et al. (1985); modified YEME was as in Fazeli et al. (1995). Spore suspensions were prepared according to Hopwood et al. (1985).

The following growth regimes were used with liquid media. For the estimation of streptomycin production in liquid media, *S. griseus* spores were inoculated either directly into the final modified YEME or MM medium, or used to provide a vegetative preculture as follows. Spores were inoculated into modified YEME and grown at 30 °C to provide a first preculture, which was inoculated either into the final modified YEME or to MM to provide a second preculture, the latter being inoculated into the final MM. The modified YEME or MM cultures were incubated at the appropriate temperature, the mycelium being sampled at intervals for estimation of dry weight and the supernatant medium assayed for streptomycin. (Further details are in Fazeli et al., 1995.) For the estimation of streptomycin yield at different temperatures, incubation was continued for up to 8 d; the streptomycin level recorded was either the maximum or that attained at the end of incubation. For the growth of cultures to be assayed for APH (aminoglycoside 3'-phosphotransferase, encoded by \( apb \) from Tn5), inocula consisted of frozen aliquots of YEME precultures. These were inoculated into NMMP medium containing glucose and thiostrepton. Cultures were incubated at 30 °C or 37 °C for 72 h (*S. lividans*) or 48 h (*S. griseus*); preliminary experiments showed that these time points gave the maximum enzyme activities, up to 30% above those of samples 24 h on either side. Mycelia were harvested, washed with phosphate buffer, resuspended in Tris/Mg\(^2+\)/NH\(_4\)^\(^+\) buffer, and disrupted ultrasonically, cell debris being removed by centrifugation to provide protein extracts. (Further details are in Lindley et al., 1995.) All liquid cultures in flasks were grown with coiled stainless steel springs.

The following growth regimes were used with solid media. For the estimation of streptomycin production on solid media, \( 10^7 \) *S. griseus* spores were inoculated on to 9 cm diameter sterile dialysis membranes (Gallenkamp) on the surface of modified YEME or MM agar plates. The membranes were removed at appropriate times, the mycelium being collected for estimation of cell dry weight (carried out as for liquid cultures) and the underlying agar assayed for streptomycin (see below). For cultures to be assayed for CO (catechol oxygenase, the product of the \( xylE \) gene), spores were inoculated on to dialysis membranes on the surface of R5 plates supplemented with appropriate antibiotics. In this case, one membrane for each combination of plasmid pairing and temperature was removed daily; the mycelium was resuspended, disrupted ultrasonically, and freed of cell debris by centrifugation to provide protein extracts. (Further details are in Lindley et al., 1995.)

**Estimation of streptomycin in culture media.** For liquid cultures, this was performed by bioassay as in Vallins &

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**Table 1. Plasmids used in this work**

For pIJ702, pIJ486 and pIJ2839, see Hopwood et al. (1985), Ward et al. (1986), and Ingram et al. (1989), respectively. All the other plasmids are described in Lindley et al. (1995), and their construction is depicted in Fig. 1.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Plasmid vector/replicon in Streptomyces</th>
<th>Plasmid vector/replicon in E. coli</th>
<th>Inserted fragment bearing the PstrB1 promoter</th>
<th>Reporter gene</th>
<th>Inserted fragment bearing an strR allele</th>
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<tbody>
<tr>
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<td>pIJ486</td>
<td>pBR322</td>
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<td>( apb )</td>
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</tr>
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<td>pHL714</td>
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<td>pBR322</td>
<td>‘Short’ fragment</td>
<td>( xylE )</td>
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<td>( xylE )</td>
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<td>None</td>
<td>strR</td>
</tr>
<tr>
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<td>pIJ702</td>
<td>pACYC184</td>
<td>None</td>
<td>None</td>
<td>strR</td>
</tr>
<tr>
<td>pES2strRA7</td>
<td>pIJ702</td>
<td>pACYC184</td>
<td>None</td>
<td>None</td>
<td>strRA7</td>
</tr>
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</table>
Temperature effects on streptomycin production

Streptomycin production by *S. griseus* at elevated growth temperature in liquid media

*S. griseus* ATCC 12475 was grown in liquid complex or minimal media at temperatures from 20°C to 37°C. A typical time course demonstrating the kinetics of growth and streptomycin production is shown in Fig. 2(a); in complex medium growth was rapid but streptomycin only began to appear as growth slowed, while in minimal medium growth was much slower but was accompanied by streptomycin formation. A plot of the highest streptomycin levels reached at each temperature is shown in Fig. 2(b). Whatever the medium, streptomycin production dropped to virtually zero at 37°C, although at this temperature growth was still about 50% of that at 30°C. This strain of *S. griseus* thus clearly shows depression of streptomycin production at elevated growth temperatures in liquid media.

Streptomycin production by *S. griseus* at standard and elevated growth temperature on solid media

Streptomycetes in their natural environment, the soil, grow on the surfaces of particles; growth in liquid suspension is therefore in a sense unnatural. We therefore wished to examine whether the kinetics of streptomycin production, and its variation with growth temperature, differ as between cultures on solid and in liquid media. Cultures were grown (see Methods) on dialysis membranes placed on solid complex or minimal media. Growth was estimated by removing the membranes, washing, and estimating dry weight, while the streptomycin accumulated in the agar was determined by bioassay. The properties of the mycelia grown on solid media, such as heterogeneity due to diffusion limitation, partial anoxia, and degree of branching, might well be quite different as between solid- and liquid-grown cultures on media of the same composition. The kinetics of growth and strep-

Baumberg (1985) with the modifications of Fazeli *et al.* (1995). For cultures on solid media, 7 mm plugs of agar were removed from the medium underlying a mycelial mat on a dialysis membrane, and transferred to similarly sized wells in bioassay plates. The bioassay was carried out as in Fazeli *et al.* (1995).

Enzyme assays. APH, CO and protein were assayed as in Lindley *et al.* (1995). As described in that reference, CO activity was barely detectable in liquid-grown cultures, and for its estimation mycelia had to be grown by inoculation of spores on to a set of dialysis membranes placed on R5 agar appropriately supplemented with antibiotic(s), as indicated above. Activities shown in Table 3 were the maximum recorded with a particular set of membranes, usually in samples harvested at 3–4 d. Experiments (results not shown) showed that neither APH nor CO activities were reduced by holding protein extracts at 37°C for up to 2 h, far longer than the length of any incubation employed in enzyme assays in this work. A further control was performed to check whether CO activity fell in vivo on incubation of mycelia at 37°C. Cultures known to show high levels of CO (*S. lividans* carrying pHL308 and pHL314 together with pES2strR – see below) were incubated on solid media, in the usual way for CO assay, for 3 d at 30°C and then shifted to 37°C. No consistent decrease in CO activities was found. Statistical tests on enzyme specific activities (standard deviation of means, *t*-test) were carried out according to Parker (1973).

RESULTS

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Streptomycin yield on complex and minimal agar at 30 °C as a function of time are shown in Fig. 3(a). On complex medium, streptomycin production lagged behind growth, whereas on minimal medium, they accompanied each other. These results are qualitatively very similar to those for liquid-grown cultures in the corresponding media shown in Fig. 2(a). Fig. 3(b) shows the variation of maximum streptomycin level with growth temperature on complex and minimal media. Though growth at 34 °C was appreciable, streptomycin production was undetectable at this temperature. Again, behaviour on solid media is strikingly similar to that in the corresponding liquid media.

Expression at 37 °C of the aph reporter gene under the direction of PstrB1 in high copy number plasmid constructs

A 200 bp leader region separates PstrB1 from the beginning of strB1. Because this shows a complex pattern of repeats and for other reasons, it was initially proposed that StrR might act as an anti-terminator to antagonize transcription termination within this region. This now seems unlikely, and StrR probably functions by binding to DNA in the vicinity of PstrB1 and stimulating the initiation of transcription there (Retzlaff et al., 1993). As shown in Fig. 1(a), two PstrB1-containing restriction fragments were used to construct fusions to reporter genes: a 'short' fragment which lacks most of the inverted repeats within the PstrB1-strB1 leader region, and a 'long' fragment which includes the entire leader region and the beginning of strB1. The DNA sequence of these fragments is given in Distler et al. (1987a, b). These fragments were cloned (Lindley et al., 1995) upstream of aph first in the pBR322-pIJ486 hybrid pHL623 to give plasmids pHL708 and 614 (Fig. 1b), carrying the 'short' and 'long' fragments respectively. Two contiguous PstI fragments carrying the promoterless aph alone or aph fused to the 'short' or 'long' fragments were then cloned into pIJ702 to give pHL723, 728 and 714 respectively (Fig. 1c). We showed (Lindley et al., 1995) that the 'short' fragment fused to aph in pHL708 and pHL728 directs StrR-independent APH expression. Results are shown in Table 2(a) for pHL723, 728 and 714 in S. lividans grown at
30 °C or 37 °C, APH activity in extracts of cultures carrying pHL723, lacking the PstrB7 promoter, was low and almost unchanged as between 30 °C and 37 °C. Where the cultures carried pHL728, with the 'short' promoter fragment capable of directing aph expression in the absence of StrR, APH activity at 30 °C was 27-fold higher than with pHL723, and fell by a factor of about 3 at 37 °C. With pHL714, with the 'long' fragment unable to direct aph expression in the absence of StrR, APH activity at 30 °C was not significantly different (by t-test) from that with pHL723. However, it fell at 37 °C by an even larger factor, about 6, than with pHL728; the activity at 37 °C was not significantly different from that with pHL723 (0:05 < P < 0-1 by t-test). As we did not possess a plasmid compatible with pHL708 that carried strR, the effect of growth at 37 °C on StrR-activated expression of PstrB7 had to be carried out in S. griseus. Table 2(b) shows the APH activities in S. griseus at 30 °C and 37 °C directed by the same three plasmids. Activities with pHL723 were much as in S. lividans. Activity with pHL728 at 30 °C was identical to that in S. lividans, and at 37 °C was not significantly different (again by t-test). With pHL714, at 30 °C the APH activity in S. griseus was only 42% of that with pHL728, implying that insufficient StrR was present for the high copy number plasmid. At 37 °C, APH activity was not significantly different from that with pHL723 (0-05 < P < 0-1 by t-test).

Changes in copy number between 30 °C and 37 °C may affect the expression of cloned genes, especially for high copy number vectors. We have estimated pIJ702 copy number in S. griseus by subjecting mycelial total DNA extracts to agarose gel electrophoresis, the photographic trace after ethidium bromide staining then being scanned densitometrically (H. K. Lindley, unpublished results). The results suggest that at 37 °C plasmid copy number is one-third to one-half that at 30 °C (mean copy number approximately 100 and 250 respectively).

**Expression at 37 °C of the xylE reporter gene under the direction of PstrB7 in low copy number plasmid constructs**

The availability of low copy number promoter-probe vectors such as pIJ2839 (Ingram et al., 1989; Clayton & Bibb, 1990; Fig. 1 d) based on SCP2*, and of strR cloned in the pACYC184-pIJ702 hybrid pES2 (Fig. 1 e; Lindley et al., 1995) permitted testing in S. lividans of the effect of growth at 37 °C on PstrB7-directed expression of xylE in the presence of strR. The effect of the cloned strR could be compared with that of the deletion variant strRA7 (O'Neill, 1992; Lindley et al., 1995). Results for the nine possible combinations of promoter-bearing fragment (none, 'short' in pHL308, or 'long' in pHL314; Fig. 1 D) and strR allele cloned in pES2 (none, wild-type strR, or strRA7) are shown in Table 3. At 30 °C, as reported by Lindley et al. (1995), CO activities showed no increase with the 'short' fragment in the absence of StrR (pHL308/pES2) over those where no promoter-bearing fragment was present (pIJ2839 with any of the three pIJ702 derivatives). The presence of the cloned strR in the companion plasmid gave an approximately 20-fold increase in CO activity with both the 'short' and 'long' promoter-bearing fragments. strRA7 unexpectedly appeared to give appreciable stimulation of xylE expression with pHL308 – yielding a CO activity about 25% of that with strR – but not with pHL314 (the difference between mean activities with pHL314 and pIJ2839 is not significant by t-test). At 37 °C, no combination of promoter and strR allele gave activity significantly above background, implying a lowering in xylE expression from that at 30 °C by a factor of at least ~ 30.

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**Table 2. APH specific activities in S. lividans and S. griseus carrying aph gene fusions in high copy number**

Cultures of S. lividans or S. griseus carrying the plasmids indicated were grown (see Methods) at the temperature shown, mycelium harvested, and its protein extracted and assayed for APH, as described in Methods. For the definitions of 'short' and 'long' promoter-bearing fragments, see Fig. 1. The number in parentheses following the mean is the number of determinations. Specific activities are in (units mg⁻¹) x 10³.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Promoter-bearing fragment</th>
<th>Mean APH activity ± SD at 30 °C</th>
<th>Mean APH activity ± SD at 37 °C</th>
<th>Ratio of mean at 30 °C to mean at 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHL723</td>
<td>None</td>
<td>4·1 (4) ±1·0</td>
<td>5·0 (3) ±1·6</td>
<td>0·8</td>
</tr>
<tr>
<td>pHL728</td>
<td>'Short'</td>
<td>110 (5) ±12</td>
<td>9·8 (3) ±1·9</td>
<td>0·8</td>
</tr>
<tr>
<td>pHL714</td>
<td>'Long'</td>
<td>8·3 (3) ±1·9</td>
<td>1·3 (3) ±0·22</td>
<td>6·2</td>
</tr>
<tr>
<td>pHL723</td>
<td>None</td>
<td>3·5 (4) ±1·1</td>
<td>4·3 (4) ±0·71</td>
<td>0·8</td>
</tr>
<tr>
<td>pHL728</td>
<td>'Short'</td>
<td>110 (6) ±6·5</td>
<td>51 (5) ±2·7</td>
<td>2·2</td>
</tr>
<tr>
<td>pHL714</td>
<td>'Long'</td>
<td>47 (7) ±3·7</td>
<td>6·9 (6) ±1·1</td>
<td>6·8</td>
</tr>
</tbody>
</table>

† Data from Lindley et al. (1995).
Table 3. CO specific activities in S. lividans carrying xylE gene fusions in low copy number

<table>
<thead>
<tr>
<th>SCP2*-derived plasmid carrying xylE (Fig. 1d)</th>
<th>Promoter-bearing fragment</th>
<th>plIJ702 derivatives with strR or strRA7 (Fig. 1e)</th>
<th>Mean CO activity ± SD at 30 °C†</th>
<th>Mean CO activity ± SD at 37 °C</th>
<th>Ratio of mean at 30 °C to mean at 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pIJ2839</td>
<td>None</td>
<td>pES2</td>
<td>4.6 (6) ± 1.1</td>
<td>3.3 (6) ± 0.39</td>
<td>1.4</td>
</tr>
<tr>
<td>pIJ2839</td>
<td>None</td>
<td>pES2strR</td>
<td>2.4 (5) ± 0.49</td>
<td>4.3 (6) ± 1.3</td>
<td>0.6</td>
</tr>
<tr>
<td>pHL308</td>
<td>‘Short’</td>
<td>pES2</td>
<td>6.7 (6) ± 1.1</td>
<td>4.7 (6) ± 0.66</td>
<td>1.4</td>
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<td>pHL308</td>
<td>‘Short’</td>
<td>pES2strRA7</td>
<td>36.6 (6) ± 7.0</td>
<td>4.9 (6) ± 0.65</td>
<td>7.3</td>
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<tr>
<td>pHL314</td>
<td>‘Long’</td>
<td>pES2</td>
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<td>1.1</td>
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<td>6.1 (6) ± 0.70</td>
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<td>9.2 (6) ± 2.3</td>
<td>6.5 (6) ± 0.82</td>
<td>1.4</td>
</tr>
</tbody>
</table>

† Data from Lindley et al. (1995), expressed in the units defined in this paper.

DISCUSSION

We have shown above that in S. griseus ATCC 12475, streptomycin production is depressed at elevated growth temperatures. This applies in both complex and minimal media, and both in liquid and on solid media.

Lindley et al. (1995) and others (Distel et al., 1987b, 1992; Vujaklija et al., 1991) have shown that the ‘short’ BamHI fragment, when fused to apb in high copy number pIJ101-based constructs, directs (at 30 °C) substantial levels of APH in S. lividans in the absence of StrR; on the other hand, as reported by Lindley et al. (1995), this fragment when fused to xylE, whether in low copy number SCP2*-based constructs or in high copy number pIJ101-based constructs, failed in the same situation to direct levels of CO above background with no promoter-bearing fragment present. The reason for this reporter gene dependent difference is unknown. The promoter activity of the ‘short’ fragment in S. lividans permitted the estimation of the effect of growth at 37 °C on APH in the absence of StrR. apb expression directed by pHL728, carrying the ‘short’ PstrB1-bearing fragment fused to apb within pIJ702, fell threefold in S. lividans and twofold in S. griseus. It was pertinent to consider whether this reduction might be accounted for by a change in plasmid copy number. If the level of gene product were proportional to gene dosage - which is not always the case - the fall in StrR-independent APH activity directed by the ‘short’ fragment at 37 °C in both S. lividans and S. griseus could be due solely to change in plasmid copy number. However, the activities directed by pHL714, with the ‘long’ fragment plus apb within pIJ702, determined solely in S. griseus due to the requirement for StrR activation, showed a considerably greater reduction, the activity at 37 °C not being significantly different from the pHL723 control lacking a promoter fragment. This could be due to: (i) a greater drop in copy number for pHL714 than for pIJ702 and presumably for pHL728, which seems unlikely (because the replicon and most of the insert are the same in all cases) but cannot be ruled out; or (ii) a genuine reduction in StrR-activated PstrB1 expression. Trivial explanations based on enzyme instability for the reduction in APH specific activity in extracts from cultures grown at 37 °C seem unlikely: as noted in Methods, no in vitro instability could be detected, while the similarity in activities conferred by the control plasmid pHL723 as between 30 °C and 37 °C in both S. lividans and S. griseus makes it unlikely that the higher growth temperature causes the appearance of an APH-inactivating proteolytic activity.

With the SCP2*-based low copy number constructs with xylE as reporter gene, there is virtually no detectable increase in expression of the reporter gene at 37 °C over the background without a promoter-bearing fragment. As the copy number of SCP2* is usually estimated as 1–2 per chromosome (Hopwood et al., 1985), there seems to be little scope for attributing the difference at elevated growth temperature to change in copy number. Regarding explanations in terms of differential loss of enzyme activity at the higher temperature, there is again no indication of in vitro instability; and the appearance of a CO-inactivating proteolytic activity at the higher growth temperature is rendered improbable by the finding (V. J. Deeb, unpublished results) that when S. lividans TK54(pHL314(pES2strR) is grown at 30 °C and the temperature then shifted to 37 °C for 1 or 2 d, CO activity in extracts does not consistently diminish by comparison with that for a control whose incubation is continued at 30 °C.

We suggest that the findings with xylE confirm the more likely explanation in the case of the high copy number constructs with apb, namely that the effectiveness of the StrR-activated PstrB1 promoter virtually disappears at
37 °C. StrR-independent expression of PstrB1 with the ‘short’ fragment construct pHL728 seems to be less affected – possibly not at all – by growth at elevated temperature. The nature of this expression, shown by Lindley et al. (1995) to occur only when the reporter gene is aph and not when it is xylE, is still unclear; but this result may indicate that elevated temperature has its effect through the StrR activation mechanism. Many reasons for this – not mutually exclusive – are possible. The StrR protein itself may be especially heat-labile, or its mode of action may be affected by the higher temperature. Also, strR expression is thought to result from a regulatory cascade involving several other regulatory proteins acting at the DNA level; StrR level may therefore be affected by similar heat lability in one or more of these regulatory proteins or temperature sensitivity of their interaction with DNA. Further, at least the first of these regulatory steps involves a low molecular mass ligand, A-factor (Miyake et al., 1990; Vujaklija et al., 1993), and it may be that the availability of this compound is limited at elevated growth temperature. There is already an intriguing connection between A-factor and elevated temperature, in that growth of S. griseus at 37 °C results in the accumulation of non-A-factor-producing variants (Hara & Beppu, 1982). Finally, it has been suggested to us (T. Kieser, personal communication) that the apparent temperature effect may be due to the lower solubility of oxygen at higher temperature; this possibility will be worth pursuing.

Granted that an effect of growth at elevated temperature is to reduce StrR-activated PstrB1 function – by whatever mechanism – to what extent does this explain the apparently complete loss of streptomycin production? It seems a priori likely that PstrB1 is not the only streptomycin biosynthesis promoter to show the effect. Reduced str gene expression could therefore be a major, or even the sole, cause of diminished streptomycin production even if the effect on PstrB1 and other promoters is less than 100%, since: (a) there may be one enzyme in the streptomycin pathway whose activity is rate-determining, and expression of the gene for that enzyme may be especially sensitive to the temperature effect; or (b) even if all the str genes show only moderate effects, the sum total of these may be complete disappearance of flux through the pathway, a possibility predicted by metabolic control theory (Kell et al., 1989). In conclusion, we suggest that at least part of the reason for depression in streptomycin production at elevated growth temperatures is a failure in expression of genes encoding enzymes of streptomycin biosynthesis.

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REFERENCES


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