Regulatory Elements in Tetracycline-Encoding Gene Clusters: the otcG Gene Positively Regulates the Production of Oxytetracycline in *Streptomyces rimosus*

**Urška Lešnik**¹, **Amelie Gormand**², **Vasilka Magdevska**¹, **Štefan Fujs**³, **Peter Raspor**¹*, **Iain Hunter**² and **Hrvoje Petković**¹,³

¹University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

²University of Strathclyde, Strathclyde Institute of Pharmacy and Biomedical Sciences, The John Arbuthnott Building, 27 Taylor Street, Glasgow G4 0NR, Scotland, UK

³Acies Bio Ltd., Železna cesta 18, SI-1000 Ljubljana, Slovenia

Received: January 8, 2009
February 16, 2009

**Summary**

The expression of bacterial polyketide synthase gene clusters is often controlled by a number of different families of regulatory proteins that can have either a pathway-specific or a pleiotropic mode of action, e.g. the SARP family (*Streptomyces* antibiotic regulatory proteins), ribosome-associated ppGpp synthetase, γ-butyrolactone-binding regulatory proteins, and two-component regulatory proteins. The molecular genetics of such regulatory mechanisms that govern the biosynthesis of tetracyclines is poorly understood. In this work, a comparative bioinformatic analysis of regulatory genes present in three tetracycline antibiotic gene clusters, namely oxytetracycline (OTC), chlortetracycline and recently cloned chelocardin gene clusters of *S. rimosus*, *S. aureofaciens* and *Amycolatopsis sulphurea* has been performed. A SARP family regulatory protein is located in the chlortetracycline gene cluster, but is not detected in the gene cluster encoding OTC biosynthesis. Interestingly, the only regulatory element identified in chelocardin gene cluster was chdA, an otrR and ctcR homologue from the TetR family of regulators that regulates the expression of the otrB and ctc05 exporter genes in the oxytetracycline gene cluster. In the oxytetracycline gene cluster, a new LAL (LuxR) family regulatory gene homologue, otcG, was identified. This homologue is also present in the ctc gene cluster. By gene disruption and overexpression experiments, a ‘conditionally positive’ role of otcG in OTC biosynthesis has been demonstrated. The observation, the bioinformatics data and the previous work on phosphate regulation suggest the presence of a more complex, fine tuning role of the otcG gene product in overall expression of genes for OTC biosynthesis.

**Key words**: type II polyketide synthase, oxytetracycline biosynthesis, *Streptomyces rimosus*, regulatory gene, LAL (LuxR) family regulator, gene cluster, chelocardin, *Amycolatopsis sulphurea*

**Introduction**

Chlortetracycline (CTC) and oxytetracycline (OTC) produced by *Streptomyces aureofaciens* and *S. rimosus*, respectively, were the first members of the tetracycline antibiotic family to reach the clinic. Historically, several research groups have focused on different aspects of the biosynthesis of tetracyclines (1–5). For the extensive ref-
Chelocardin (CHD) on the other hand, produced by Amycolatopsis sulphurea, is also a powerful tetracycline antibiotic with an unknown mode of action, but it did not reach the clinic (7,8). We have recently cloned the entire gene cluster encoding CHD biosynthesis from A. sulphurea (unpublished results).

The expression of polyketide synthase (PKS) gene cluster elements is often controlled by a number of different families of regulatory proteins that can have either a pathway-specific or a pleiotropic mode of action (9). The SARP family (Streptomyces antibiotic regulatory proteins) of positive regulatory proteins encoding genes for pathway-specific transcriptional regulation are the most common positive regulators of type II PKS gene clusters (10). These proteins tend to interact with promoters of genes (gene clusters) involved in antibiotic biosynthesis and are generally found in a number of PKS clusters including the type II PKS gene clusters of actinorhodin and daunorubicin (11,12). Some examples of the more general pleiotropic signals affecting biosynthesis of type II PKSs include the ribosome-associated ppGpp synthetase (RelA) (9), γ-butyrolactone-binding regulatory proteins (13), and two-component regulators (14). Excluding the publication on phosphate regulation by McDowall et al. (15), any other information on the molecular genetics of the regulatory mechanisms governing the biosynthesis of OTC in S. rimosus has not been found.

Based on a comparative bioinformatics study of the gene clusters encoding OTC and CTC biosynthesis, we have identified a new LAL (LuxR) family regulatory gene in the otc gene cluster. Interestingly, numerous gene clusters encoding type I PKSs contain the so-called LAL (LuxR) family of transcriptional regulators, such as positive regulators RapG from the rapamycin gene cluster, PimM from the pimaricin gene cluster, and PikD from the pikromycin gene cluster (16–18), which are usually not found in type II PKS gene clusters.

In this work a detailed analysis of the regulatory elements potentially regulating OTC biosynthesis in S. rimosus has been carried out. Putative regulatory elements present in the chd gene cluster from A. sulphurea have also been analysed. Apart from SARP and tetR-like regulatory protein, a new LAL (LuxR) gene homologue, otcG, has been identified in the otc gene cluster. Its 'conditionally positive' role in the biosynthesis of OTC has been demonstrated.

Materials and Methods

Bacterial strains and culture conditions

S. rimosus M4018 (19) was used as the donor of DNA and as the host for gene disruption and overexpression experiments. Electroporation of S. rimosus was performed as described previously (20). For sporation and selection of morphological variants and resistance phenotypes, S. rimosus was grown on soya manitol medium (21). The S. rimosus ATCC10970 wild type strain (4), and the industrial high-producing strain S. rimosus AB04 (Acies Bio Ltd., Ljubljana, Slovenia) were used for DNA isolation and otcG sequencing. S. rimosus strains were maintained and grown in fermentation medium as described previously (22). Thiostrepton (30 μg/mL in solid medium and 5 μg/mL in liquid medium) and erythromycin (50 μg/mL) were added to the media as required. A. sulphurea NRRL 2822 strain (8) was used as a source of genomic DNA for cloning the chd gene cluster. Escherichia coli DH10β (23) was used for cloning and E. coli ET12567 (24) for obtaining unmethylated DNA for transformation into S. rimosus. E. coli cultures were grown on 2TY medium at 37 °C, supplemented with ampicillin (100 μg/mL) and apramycin (50 μg/mL), as required. Plasmids and Streptomyces rimosus strains used in this study are described in Table 1.

Table 1. Plasmids and Streptomyces rimosus strains used in this study

<table>
<thead>
<tr>
<th>Plasmid and strain</th>
<th>Key characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SuperCos1</td>
<td>contains cos sites for construction of cosmid library</td>
<td>Stratage Inc.</td>
</tr>
<tr>
<td>pGEM-T easy</td>
<td>cloning vector Amp&lt;sup&gt;+&lt;/sup&gt; f1-ori lacZ</td>
<td>Promega Inc.</td>
</tr>
<tr>
<td>pTS55</td>
<td>pSAM2-based integrative plasmid</td>
<td>28</td>
</tr>
<tr>
<td>pIJ4026</td>
<td>pUC18 carrying erm&lt;sup&gt;E&lt;/sup&gt; gene from Saccharopolyspora erythraea</td>
<td>21</td>
</tr>
<tr>
<td>pSGset2</td>
<td>pSET152-derived with an erm&lt;sup&gt;E&lt;/sup&gt; promoter</td>
<td>21</td>
</tr>
<tr>
<td>pTS55erm&lt;sup&gt;E&lt;/sup&gt;-otcG</td>
<td>pTS55 with a 1.6-kb HindIII fragment containing erm&lt;sup&gt;E&lt;/sup&gt;-otcG construct</td>
<td>this study</td>
</tr>
<tr>
<td>pAotG</td>
<td>pIJ4026 with a truncated (469 nt) otcG cloned in a single EcoRI site</td>
<td>this study</td>
</tr>
<tr>
<td>S. rimosus</td>
<td>the natural wild type progenitor strain from which most of industrial strains were derived</td>
<td>4</td>
</tr>
<tr>
<td>ATCC10970</td>
<td>the genetically most characterised strain, the oxytetracycline-producing Pfizer strain</td>
<td>19</td>
</tr>
<tr>
<td>M4018</td>
<td>industrial high-producing strain</td>
<td>Acies Bio Ltd.</td>
</tr>
</tbody>
</table>

DNA manipulations

Standard methods for isolation and manipulation of DNA were performed as described by Sambrook and Russell (25) and Kieser et al. (21). Southern hybridization was undertaken using the non-radioactive DIG DNA Labelling and Detection Kit from Roche (Mannheim, Germany), according to the manufacturer’s instructions. For PCR amplification of the complete otcG gene (714 bp) the primers otcGF: 5′-GGGATATGGGATAACAAAAGTC-ATC-3′ and otcGR: 5′-TCAGGTGCCGGCGCTGTAC-3′ were used. An Ndel restriction site was included in one primer to facilitate subcloning and is indicated by underscoring. For the truncated sequence of the otcG gene, the primers partF: 5′-AAGTGCTACCGTGCTCGTC-3′ and partR: 5′-CGGCCAGTTCTCCATTATCT-3′ were used, amplifying nucleotides 11 to 480.
Cloning of the chd gene cluster

A SuperCos1-based genomic library of A. sulphurea NRRL 2822 genomic DNA was constructed by using the GigapackIII Gold Packaging Kit (Stratagene). The library was screened by colony hybridization for the presence of a type II PKS gene cluster using a ketosynthase (KSc) gene from the OTC gene cluster as a probe. The selected clone was sequenced (Macrogen, Korea) and putative open reading frames (ORFs) were determined using the FramePlot beta 4.0 software (26). The putative gene functions were determined using the BLASTx algorithm at the NCBI (27).

Plasmids

The PCR products of the complete (714 bp) and truncated (470 bp) otcG putative regulator gene were excised with Ndel and cloned into the Ndel site of pSGset2 (21) downstream of the ermE promoter. The HindIII fragment carrying the ermE-otcG construct was cloned into the HindIII site of the pTS55 integrative vector (28), resulting in the pTS55ermE-otcG plasmid. For the disruption of otcG gene, a suicide vector pΔotcG was constructed by cloning the EcoRI fragment with the truncated otcG sequence from the pGEM-T easy plasmid into the EcoRI site of the pJL4026 suicide plasmid (21).

Gene inactivation and overexpression

For the inactivation of the otcG gene, a suicide pΔotcG vector was used. The pΔotcG vector contained a 470-bp internal fragment of otcG. Single-crossover recombinants were selected by their resistance to erythromycin. For the overexpression of otcG, the pTS55ermE-otcG plasmid, a pTS55-based integrative vector with an intact otcG gene downstream of the ermE promoter, was used. The transformants were selected using thiostrepton. Since the OTC-producing strains of S. rimosus often display a phenotype were selected. Fermentations using the selected colonies (transformants) of all mutants were undertaken in parallel to ensure reproducibility. Genetic instability, often accompanied with gene deletion and/or DNA amplification (29,30) of the otc gene cluster did not occur, as confirmed by DNA restriction analysis and gel electrophoresis, and finally by Southern hybridization. The OTC production of 18 S. rimosus M4018 colonies with a disrupted copy of otcG (pΔotcG) and 42 colonies with an additional copy under the ermE promoter (pTS55ermE-otcG) were compared to 12 colonies of S. rimosus M4018 with pTS55 as a control. The yields were analyzed using the SAS/STAT program and expressed as the least square means with standard errors of the means.

OTC analytical method

Accumulation of oxytetracycline during fermentation was measured by reversed-phase HPLC according to European Pharmacopoeia 5.5 (31). The 714-bp sequence for the oxytetracycline regulatory gene, otcG, reported here has been deposited in GenBank under accession number FJ503048.

Results

Regulatory elements present in the ctc, otc and chd gene clusters

The gene cluster encoding OTC biosynthesis from the S. rimosus strain M4018 was defined as a 34-kb EcoRI-fragment that conferred OTC production on the naive host, S. lividans (32). Parts of that otc gene cluster and the entire gene cluster derived from low-producing strain ATCC 10970 have been sequenced (4). Gene organization of both otc gene clusters derived from M15883 and ATCC10970 S. rimosus strains is identical. On the other hand, comparative analysis of the gene organization between otc and ctc gene clusters revealed significant differences, including the regulatory genes (Fig. 1).

When analysing the ORFs of the ctc gene cluster (33), regulatory elements can be identified at both extremities of the gene cluster. On the right extremity of the ctc gene cluster (Fig. 1a), a tetR-family homologue of the repressor regulatory element ctcR can be identified, adjacent to the exporter gene ctc05. Analogously to the ctc gene cluster, the counterparts of the ctc05/ctcR, the otrB/otrR homologues from the otc (2), are located at the right extremity of the otc gene cluster (Fig. 1b), albeit in the opposite orientation with respect to the biosynthetic genes.

The tetR-like repressor from the otc gene cluster encodes the MarR (multiple antibiotic resistance regulator) protein, which was originally characterized as the repressor of the multiple antibiotic resistance operon marRAB in Escherichia (34). The TetA system of the transposon Tn10, which is a paradigm of this class of resistance, has been studied extensively (35). The TetR homologue in OTC biosynthesis regulates the expression of the exporter (36). However, it is not clear whether the otrR gene product is solely involved in the regulation of otrB or also in the regulation of other genes in the cluster. Two ORFs encoding transcriptional regulators can be identified on the left extremity of the ctc gene cluster in S. aureofaciens, just adjacent to the ribosomal tetracycline resistance gene ctc14, as reported earlier (Fig. 1a; 33).

Both gene homologues belong to the putative positive regulatory genes according to BLAST homology searches. The ctcII gene homologue is a typical SARP family, positive pathway specific transcriptional regulator, such as the acII-ORF4 in the actinorhodin gene cluster in S. coelicolor (37). The ORF ctc13, which is located at the extreme left fringe of the ctc cluster, adjacent to the SARP family homologue and transcribed in the opposite direction of the ctcII gene, is a homologue of two component transcriptional regulators of the LAL (LuxR) family (Fig. 1a). We have identified a new ORF designated as otcG in the otc cluster, with a high homology to the ctc13 gene in the ctc gene cluster, designated according to Hunter and Hill (2). The otcG gene lies adjacent to the otrA gene (encoding ribosomal resistance) and it is expressed in the opposite orientation. Surprisingly, no ctcII gene homologue encoding a SARP pathway specific regulator could be identified in the OTC gene cluster (Fig. 1b). Interestingly, only chdA and chdR, the otrR and otrB homologues from oxytetracycline gene cluster, can be identified in the chelocardin gene cluster (Fig. 1c).
Database comparisons revealed that the otcG gene product (237 aa) has amino acid sequence homology with the LAL (LuxR) family of bacterial regulatory proteins, a putative two-component system response transcriptional regulatory protein. The characteristic architecture of the LAL (LuxR) family of DNA binding proteins, containing a nucleotide triphosphate (NTP) binding motif at the N-terminus and a C-terminally-located helix-turn-helix (HTH) motif, can be clearly assigned to the otcG gene product (Fig. 2).

Interestingly, there is a rare TTA codon within the coding region of the otcG gene (Fig. 2), which is characteristic of many SARP genes and can have a profound influence on secondary metabolism and morphological differentiation, as demonstrated in _S. coelicolor_ (9) where the appearance of the tRNA associated with the TTA codon is developmentally-regulated. Considering the interesting features of the otcG gene and its putative role in the regulation of OTC biosynthesis in _S. rimosus_, we have also sequenced the otcG gene homologue in the two independent strains, namely _S. rimosus_ ATCC10970 wild type strain (4), and the industrial high-producing strain _S. rimosus_ AB04 (DNA provided by Acies Bio Ltd., Slovenia). Sequencing of the otcG gene from all three strains revealed 100 % identity at the DNA level (data not shown); therefore, no mutation was introduced in the otcG gene during the intensive strain improvement processes carried out over the years.

When a putative gene cluster encoding CHD was compared to the _ctc_ and _otc_ gene clusters (Fig. 1), no SARP or LAL (LuxR) family of bacterial regulatory proteins were detected in the chd gene cluster. Adjacent to the chdR exporter gene homologue, we have identified the chdA gene homologue, a putative tetR-like repressor gene, analogously to the _otc_ and _ctc_ gene clusters (Fig. 1). Although chdA gene homologue is clearly a putative tetR gene homologue (34), it shows little similarity to the _otc_ and _ctc_ gene cluster counterparts otrR and _ctcR_, which share 46 % identity and 57 % similarity (Fig. 3). Unexpectedly, the chdA gene shows the highest similarity to the tetR gene homologues from Gram-negative bacteria such as *Klebsiella pneumoniae*, *Burkholderia* spp. and *E. coli* (38,39).

**Inactivation of the otcG gene**

Gene inactivation and overexpression was adopted as the fastest way to examine a possible role of otcG gene product in the regulation of OTC biosynthesis in _S. rimosus_. As _otcG_ is transcribed convergently with the _otrA_ ribosomal resistance gene, a simple and straightforward single-cross over, suicide-disruption experiment could be undertaken using a plasmid carrying a truncated version of the _otcG_ gene without influencing the expression of the surrounding ORFs, particularly the _otrA_ gene (Fig. 1b). For this purpose, the truncated DNA fragment of the _otcG_ gene was inserted into the suicide vector pIJ4026 (21).

The OTC-producing _S. rimosus_ strains are well known for their morphological instability (40). This instability is promoted even further by gene manipulation, resulting
in a great proportion of the colonies having a range of morphological deformations, most often causing a significant decrease of the OTC yield (29,30). Therefore, only morphologically stable, well-sporulated colonies were selected for further testing in order to reduce the variability in OTC production caused by morphological instability. Eighteen independent transformants carrying the disrupted \( otcG \) gene were then tested in industrial production medium, as described in Materials and Methods. The inactivation of the \( otcG \) gene did not abolish the production of OTC. However, the production of OTC in the wt: \( D \) \( otcG \) mutants was statistically significantly reduced by more than 40 % in comparison with the wt:pTS55 as demonstrated by the p-value of 0.0034 (Fig. 4). Inactivation of the \( otcG \) gene carried out under the conditions applied during the experiments presented in this work did not affect any of the morphological characteristics of the 4018 strain.

**Overexpression of the \( otcG \) gene**

The entire \( otcG \) gene was amplified by PCR and subcloned into the pTS55-based integrative vector (28) to generate pTS55ermE: \( otcG \). Expression of the \( otcG \) gene was under the control of the \( ermE \) promoter (21), proven to be functional in the \( S. rimosus \) 4018 strain. Forty-two
independent well-sporulated transformants from SM agar medium were then tested for the production of OTC, as described in the Materials and Methods. The introduction of a second copy of otcG, expressed under the constitutive promoter ermE, did not yield any statistically significant change in OTC production (p=0.1602) compared to the S. rimosus 4018 strain containing pTS55 plasmid alone (Fig. 4).

Discussion

The aim of this work was to gain a fresh look into the putative regulatory elements that may govern the biosynthesis of OTC, through a comparative bioinformatics study of the entire ctc, otc and ctd gene clusters from S. aureofaciens, S. rimosus and A. sulphurea, respectively. The DNA sequence of part of the gene cluster encoding OTC biosynthesis in S. rimosus ATCC10970 was recently published by Zhang et al. (4), thus allowing us to compare the entire gene clusters encoding CTC and OTC biosynthesis. Importantly, gene clusters encoding OTC and CTC biosynthesis were expressed heterologously in S. lividans, confirming that all the necessary genes for the biosynthesis of CTC and OTC were encoded by the published DNA sequences (32,33).

Although the structures of the otc and ctc gene clusters do differ significantly, the expected DNA homologues with high identity/similarity can be identified in both gene clusters (Figs. 1a and b). The annotation of the otc gene clusters, which was carried out in both strains of S. rimosus, the ATCC10970 and the M15883 strains, presumed that all the necessary genes encoding OTC biosynthesis were flanked by the otrA (ribosomal) resistance gene at one end, and the otrB (exporter) gene on the other side of the gene cluster (2). However, led by the annotation of the putative ORFs from the ctc cluster, we have identified an additional ORF in the otc gene cluster from a putative LAL (LuxR) family of transcriptional regulator, otcG (Fig. 1b), with a characteristic nucleotide triphosphate (NTP) binding motif at the N-terminus and a helix-turn-helix (HTH) motif at its C-terminal side (Fig. 2). This gene lies within the EcoRI fragment used by Binnie et al. (32) for the heterologous production of OTC in S. lividans. Another objective of this work was to determine whether the otcG gene product is actually involved in OTC biosynthesis. To our knowledge, the LAL (LuxR) family of regulatory proteins has not been found previously in type II PKS gene clusters. Little is understood of the overall role of these proteins. Given the location of the otcG gene, flanking externally the otrA gene (Fig. 1b), it was not unreasonable to question whether this protein is actually involved in the regulation of OTC biosynthesis. Simple and straightforward gene disruption and overexpression experiments were carried out to answer this question.

Inactivation of the otcG gene did not entirely abolish, but instead significantly reduced OTC biosynthesis. Overexpression of the otcG gene, through the ectopic expression of a second copy of the otcG gene did not influence the final OTC yield, thus demonstrating an only 'conditionally-positive' regulatory involvement of the otcG gene product.

We have identified a rare TTA codon within the coding region of the otcG gene, which is characteristic of many SARP genes (9). In the context of actinorhodin biosynthesis, it has been demonstrated that the expression of an actII-ORF4 carrying a mutated TTA codon leads to bldA-independent actinorhodin production (41). However, the inactivation of the otcG gene did not lead to any obvious morphological changes. Interestingly, the ctc gene cluster counterpart of otcG, ctc13, does not contain the rare leucine TTA codon, nor does the SARP homologue from the ctc cluster contain the rare TTA codon. The OTC biosynthesis in S. rimosus is not regulated by the SARP family of regulatory elements located in the otc gene cluster. A SARP family regulator is clearly identified in the ctc gene cluster in S. aureofaciens. The regulatory activity of the SARP-like regulatory protein located outside the otc gene cluster, which positively regulates OTC biosynthesis, is also a feasible option, as demonstrated in S. coelicolor. It has been shown that the actII-ORF4 gene product of the SARP family, which is located in the actinorhodin gene cluster also influences the expression of the spore pigment-encoding whiE locus and the gene clusters encoding the biosynthesis of coelichenin and calcium-dependent (CDA) antibiotics, and the genes governing the biosynthesis of undecylprodigiosin (9). McDowall et al. (15) predicted the existence of a regulatory element involved in OTC biosynthesis, when studying the phosphate-mediated control of OTC production by S. rimosus. In this study they showed that the transcription of the OTC biosynthetic genes otcC and otcX is triggered by phosphate starvation, indicating that the synthesis of OTC is controlled, at least in part, at the level of transcription. Interestingly, promoters of OTC biosynthesis overlap by tandem repeats, whose spacing resembles that of the activation sites of the OmpR family of transcriptional regulators (18). Based on these findings, McDowall et al. (15) suggested that the activator of the otc genes may be a member of the OmpR family, most likely a member of the SARP group. However, no such type of regulatory element has been identified in the gene cluster encoding OTC biosynthesis (2). On the other hand, the otcG gene product belongs to the LAL (LuxR) family of transcriptional regulators, which is classified as a member of the bigger group of the
OmpR family of transcriptional regulators with a typical conserved gene architecture consisting of a helix-turn-helix DNA binding motif, a signal receiver domain (Fig. 2). The otcG gene product may likely interfere with the promoters of the genes involved in OTC biosynthesis, a regulatory element which was predicted by McDowall et al. (15). Interestingly, the putative phosphorylation site in the ctc3 gene in *S. aureofaciens* seems to be mutated from aspartic acid to glycine residue; thus, ctc13 might even be inactive in the ctc gene cluster (Fig. 2).

Finally, no regulatory genes were identified in the chelocardin gene cluster, with the exception of chdA gene, a putative tetR gene homologues located beside the chdR exporter gene homologue (Fig. 1c). It is reasonable to believe that the chdR exporter gene is the only resistance determinant present in the chd gene cluster. We could not identify the otrA ribosomal resistance gene homologue in the chd cluster. However, this is not surprising, considering that the chelocardine does not target the ribosome (42). However, it was very surprising that the putative tetR homologue chdA shows very high similarity to the tetR gene homologues present in the Gram-negative bacteria, considering that *A. sulphurea* taxonomically belongs to the order of Actinomycetales, i.e. a Gram-positive and the DNA GC-rich bacteria.

**Conclusion**

Regulatory elements from three gene clusters encoding tetracycline biosynthesis have been compared. Significant overall differences have been identified in the regulatory elements that are likely governing the corresponding biosynthetic pathways. When comparing the tetR homologue (chdA) from the chelocardin-producing strain *A. sulphurea* to the otc and ctc counterparts, unexpected evolutionary distance was identified. In this work, a new regulatory element has been identified, not previously identified in the otc gene cluster, and clearly demonstrated that the otcG gene, a member of the LAL (LuxR) family of regulatory proteins, exerts a positive role in the regulation of tetR gene homologues present in the chd gene cluster. We could not identify the otrA ribosomal resistance gene homologue in the chd cluster. However, this is not surprising, considering that the chelocardine does not target the ribosome (42). However, it was very surprising that the putative tetR homologue chdA shows very high similarity to the tetR gene homologues present in the Gram-negative bacteria, considering that *A. sulphurea* taxonomically belongs to the order of Actinomycetales, i.e. a Gram-positive and the DNA GC-rich bacteria.

**Acknowledgements**

This research was supported in part by grants from the Slovenian Research Agency (7-576-1/2004), the Slovene Human Resources and Scholarship Fund (Ad Futura Programmes 2006/523-17), the Socrates/Erasmus Programme (2006/07-420) and the British Council (Partnership in Science Award RNP 7/2008).

**References**


2. I.S. Hunter, R.A. Hill: *Biotechnology of Antibiotics – Tetra-
cyclines*, Marcel Dekker, New York, USA (1997).


9. M.J. Bibb, Regulation of secondary metabolism in strepto-

10. A. Wietzorrek, M. Bibb, A novel family of proteins that regu-

11. P.J. Sheldon, S.B. Busarow, C.R. Hutchinson, Mapping the DNA-binding domain and target sequences of the *Strep-
tomyces peucetius* daunorubicin biosynthesis regulatory pro-

12. P. Arias, M.A. Fernández-Moreno, F. Malpartida, Characteri-
gization of the pathway-specific positive transcriptional regu-
lator for actinorhodin biosynthesis in *Streptomyces coeli-


14. A. Sola-Landa, R.S. Moura, J.F. Martin, The two-compo-
nent PhoR-PhoP system controls both primary metabolism and secondary metabolite biosynthesis in *Streptomyces li-

15. K.J. McDowall, A. Thamchaipenet, I.S. Hunter, Phosphate control of oxytetracycline production by *Streptomyces rimo-
sus* is at the level of transcription from promoters overlapped by tandem repeats similar to those of the DNA-binding sites of the OmpR family, *J. Bacteriol.* 181 (1999) 3025–3032.


17. N. Antón, J. Santos-Aberturas, M.V. Mendes, S.M. Guerra, J.F. Martin, J.F. Aparicio, PimM, a PAS domain positive regu-