Appl Microbiol Biotechnol (2011) 90:11–21
DOI 10.1007/s00253-011-3128-3

MINI-REVIEW

Mupirocin: biosynthesis, special features and applications of an antibiotic from a Gram-negative bacterium

Rachel Gurney · Christopher M. Thomas

Received: 21 October 2010 / Revised: 12 January 2011 / Accepted: 12 January 2011 / Published online: 20 February 2011

Abstract Mupirocin is a polyketide antibiotic produced by *Pseudomonas fluorescens*. The biosynthetic cluster encodes 6 type I polyketide synthase multifunctional proteins and 29 single function proteins. The biosynthetic pathway belongs to the *trans*-AT group in which acyltransferase activity is provided by a separate polypeptide rather than in-*cis* as found in the original type I polyketide synthases. Special features of this group are in-*cis* methyltransferase domains and a *trans*-acting HMG-CoA synthase-cassette which insert α- and β- methyl groups respectively while enoyl reductase domains are absent from the condensing modules. In addition, for the mupirocin system, there is no obvious loading mechanism for initiation of the polyketide chain and many aspects of the pathway remain to be elucidated. Mupirocin inhibits isoleucyl-tRNA synthetase and has been used since 1985 to help prevent infection by methicillin-resistant *Staphylococcus aureus*, particularly within hospitals. Resistance to mupirocin was first detected in 1987 and high-level resistance in *S. aureus* is due to a plasmid-encoded second isoleucyl-tRNA synthetase, a more eukaryotic-like enzyme. Recent analysis of the biosynthetic pathway for thiomarinols from marine bacteria opens up possibilities to modify mupirocin so as to overcome this resistance.

Keywords Mupirocin · Polyketide · Antibiotic · *Trans*-AT · *Staphylococcus aureus* · *Pseudomonas fluorescens*

R. Gurney (✉) · C. M. Thomas
School of Biosciences, University of Birmingham,
Edgbaston,
Birmingham B15 2TT, UK
e-mail: Rsg893@bham.ac.uk

C. M. Thomas
e-mail: c.m.thomas@bham.ac.uk

Introduction

Mupirocin is a polyketide antibiotic produced by the soil bacterium *Pseudomonas fluorescens* NCIMB 10586, isolated from Hampstead Heath in London (LGC Standards, 2009). *Pseudomonas* spp. are Gram-negative rods found in soil and water as well as on surfaces of plant roots and leaves. *Pseudomonas fluorescens* produces pyoverdine during iron starvation, a green pigment that chelates iron and fluoresces under ultraviolet light. *Pseudomonas fluorescens* can grow at 4 °C and degrades lipids and proteins and has been implicated in the spoilage of refrigerated milk, meat, eggs and seafood (Prescott et al. 2005). It uses oxygen as electron acceptor when available, but nitrate can be used under anaerobic conditions. Different strains produce a variety of chemicals that interfere with neighbouring organisms inhabiting the rhizosphere. Production of secondary metabolites is activated by signal molecules in a phenomenon known as quorum sensing (Lugtenberg and Bloemberg 2004).

Polyketides are secondary metabolites produced by bacteria, fungi and some plants that display a wide range of biological activities that are increasingly exploited as therapeutic tools. Type I and II polyketide synthases (PKSs) are closely related to fatty acid synthases (FASs) and are classified according to their protein architecture (Ridley et al. 2008). Type I PKSs consist of large multifunctional enzymes with domains joined covalently, for example the 6-deoxyerythronolide B synthase (DEBS) that makes the core of erythromycin (Cane 2010; Hill and Staunton 2010). Type II PKSs consist of a multifunctional PKS complex built from separate monofunctional proteins, for example the *Streptomyces coelicolor* actinorhodin synthase (Tang et al. 2004a) and type II FAS have a similar structure (Schujman and Mendoza 2008). For bacteria, *Escherichia coli* has become a model organism, providing valuable

information about the processes that take place during fatty acid synthesis (Magnuson et al. 1993). The FAS II of *E. coli* consists of seven core polypeptides (encoded by the *fab* genes) that work together to catalyse fatty acid synthesis (Maier et al. 2008) and the manipulation of this pathway can lead to increased production of biodiesel (Liu et al. 2010b). However, there is great interest in the diversity of PKS systems since this provides a growing source of genetic building blocks for synthetic biology which aims to generate novel biologically active molecules.

Mupirocin biosynthesis

The first reported study of mupirocin was in 1971 (Fuller et al. 1971). Subsequent investigations revealed a mixture of four pseudomonic acids (A–D), collectively named mupirocin, with extensive antibacterial activity. The structure of mupirocin, shown in Fig. 1, comprises a monic acid (a heptaketide) containing a pyran ring, attached to 9-hydroxynonanoic acid (9-HN) via an ester linkage (Fuller et al. 1971; Chain and Mellows 1974, 1977; Alexander et al. 1978; Whatling et al. 1995). Mupirocin targets bacterial isoleucyl-tRNA synthase (IleS) competitively inhibiting the formation of Ile tRNA, ultimately blocking protein synthesis (Hughes and Mellows 1978).

The *mup* cluster

A >65-kb region of the chromosome involved in mupirocin biosynthesis was identified by transposon mutagenesis (Whatling et al. 1995) and subsequently analysed by DNA sequencing, gene knockouts and complementation studies (El-Sayed et al. 2003). The cluster can be conveniently split into two sections—the first mainly encoding three large multifunctional PKS proteins as well as the multifunctional protein with acyltransferase (AT) domains, and the other encoding two smaller multifunctional PKS genes as well as 29 individual "tailoring" genes. Table 1 shows all of the genes involved in mupirocin biosynthesis and their putative functions. The cluster is unusual in that the order of the genes does not match the order of biosynthetic steps. The multifunctional genes *mmpD* and *mmpA* together encode the first four and last two elongating modules respectively and one putative transfer/non-elongating/processing module. They comprise appropriate ketosynthase (KS), acyl carrier protein (ACP), ketoreductase (KR), dehydratase (DH) and methyltransferase (MT) functions for monic acid backbone synthesis while *mmpC* encodes two AT domains and a putative enoyl reductase (ER) domain. The *mmpB* gene encoding single KS, KR and DH domains and triple ACP domains is thought to be responsible for synthesis of the 9-HN moiety, but also encodes the only thioesterase (TE) suggesting that it controls the final steps of the pathway and release of products. Resistance to mupirocin is encoded within the cluster by MupM which shows significant similarity to other mupirocin-resistant IleS proteins (El-Sayed et al. 2003).

Monic acid biosynthesis

The heptaketide chain that comprises the backbone of monic acid is synthesised by six condensation reactions of acetate-derived units catalysed by MmpD (modules 1–4) and MmpA (modules 5–6) as shown in Fig. 2a (Martin and Simpson 1989; El-Sayed et al. 2003). Synthesis could begin with one of the *trans*-acting ATs transferring an activated starter unit (acetyl-coenzyme A intermediate) to the 4′-phosphopantetheine arm

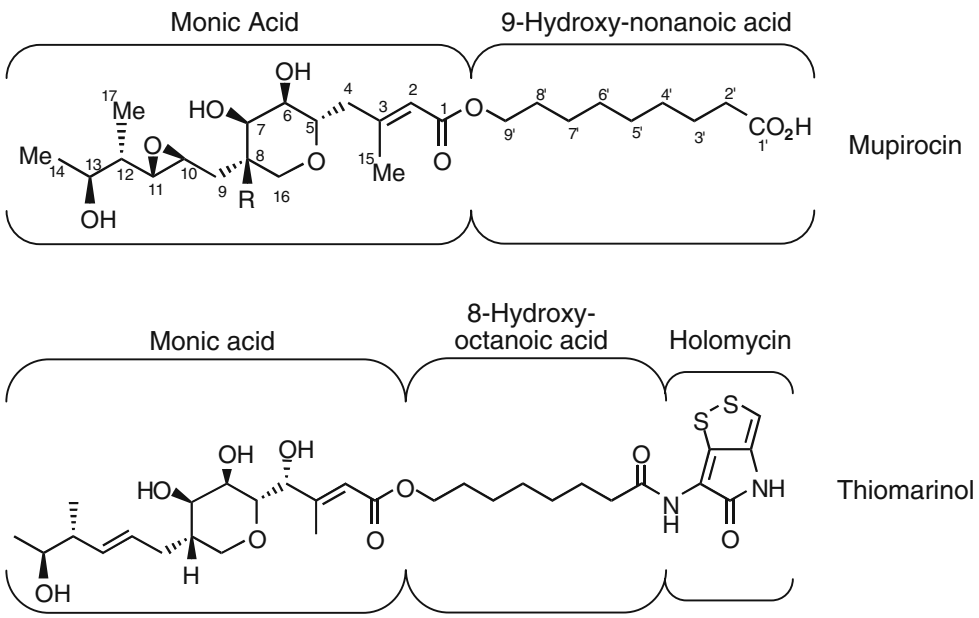

Fig. 1 *Top* the structure of mupirocin, showing the monic acid and 9-hydroxynonanoic acid moieties. Mupirocin is a mixture of four pseudomonic acids: PA-A (about 90%) R=H; PA-B (about 8%) R=OH; PA-C (<2%) R=H and C10/11 *E*-alkene; PA-D (<2%) R=H, C4′/5′ *E*-alkene. *Bottom* structure of thiomarinol A, showing the marinolic acid (equivalent to pseudomonic acid/mupirocin) and holomycin moieties

Table 1 Gene functions of the mupirocin biosynthetic cluster

ORF	Proposed function
mupA	Reduced flavin mononucleotide ($FMNH_2$) oxygenase
mmpA	PKS: load/transfer KS, ACP; module 5 KS, KR, ACP; module 6 KS, ACP, ACP
mupB	3-Oxo-ACP synthase
mmpB	PKS: KS, DH, KR, ACP, ACP, ACP, TE
mmpC	Dual AT and putative ER
mmpD	PKS: module 1 KS, DH, KR, MT, ACP; module 2 KS, DH, KR, ACP; module 3 KS, DH, KR, MT, ACP; module 4 KS, KR, ACP
mupC	Dienoyl CoA reductase
mAcpA	ACP
mupD	3-Oxo-ACP synthase
mupE	ER
mAcpB	ACP
mupF	KR
mAcpC	ACP
mupG	3-Oxo-ACP synthase
mupH	β-Hydroxyl-β-methyl glutarate (HMG) CoA synthase
mupJ	Enoyl CoA hydratase
mupK	Enoyl CoA hydratase
mmpE	PKS: KS, hydroxylase
mupL	Hydrolase
mupM	Isoleucyl-tRNA synthetase
mupN	Phosphopantetheinyl transferase
mupO	Cytochrome P450
mupP	Unknown
mupQ	Acyl CoA synthase
mupS	3-Oxo-ACP reductase
mAcpD	ACP
mmpF	PKS: KS
mAcpE	ACP
mupT	Ferrodoxin dioxygenase
mupU	Acyl CoA synthase
mupV	Oxidoreductase
mupW	Dioxygenase
mupR	Transcriptional activator
mupX	Amidase
mupI	N-acyl homoserine lactone synthase

Abbreviations: *mmp* mupirocin multifunctional polypeptide gene, *PKS* polyketide synthase, *KS* ketosynthase, *DH* dehydratase, *KR* ketoreductase, *ACP* acyl carrier protein, *TE* thioesterase, *AT* acyltransferase, *ER* enoyl reductase, *MT* methyltransferase, *mAcp* mupirocin acyl carrier protein, *ORF* open reading frame (El-Sayed et al. 2003; Hothersall et al. 2007)

of ACP-D1 and then to the thiol group of the active cysteine of KS-D1. An activated extender unit (malonyl-CoA) could then be transferred by one of the ATs to the vacant ACP-D1, prior to decarboxylative (Claisen) condensation catalysed by KS-D1. Alternatively, an activated starter unit could be transferred directly to the KS-D1 (see below). The first module then carries out ketoreduction and α-methylation to create the structure that mimics the isoleucine side chain. Synthesis continues through three further rounds of condensation and modification on successive modules of MmpD (KS/ACP-D2, D3 and D4), including a second α-methylation by the MT in module 3, before the acyl intermediate is passed to MmpA. The first module of MmpA (module 5 in the pathway) is likely to act as a transfer or processing (non-elongating) module due to the combination of atypical KS (KS^0) and ACP domains. Deletion (as well as mutation of active site residues) of these domains resulted in a loss of antibiotic production, indicating an important role in mupirocin production (El-Sayed et al. 2003). Two further elongation modules on MmpA extend the chain to give the C_{17} precursor to monic acid.

The mupirocin cluster contains many genes that are needed to modify ("tailor") the PKS-bound intermediate before completion of the final pseudomonic acid structure. The modifications involve incorporation of a methyl group at C15, hydroxylation at C6, epoxidation at C10,11 and formation of the pyran ring (Fig. 2b) (Cooper et al. 2005a; Hothersall et al. 2007). Bioinformatics can often predict both biochemical function and partner genes which work together (based on being found together in other genomes). These include the hydroxymethylglutaryl-CoA synthase (HCS) cassette genes *mAcpC*, *mupG*, *mupH*, *mupJ*, *mupK* (for function see below) as well as two other blocks *mAcpD*, *mupS*, *mupQ*, *mmpF* (functional currently unknown) and *mupD*, *mupE* (an enoyl reductase). However, gene knockouts and product analysis are essential since other gene sets such as *mupW*, *mupT* (pyran ring formation) and *mAcpE*, *mupO*, *mupU*, *mupV*, *mupC* and *mupF* (further reduction around the pyran ring) seem to be functions currently unique to this cluster. While it is clear at what stage in the pathway some of them work, for example the HCS cassette that functions at module A3, for others it is either flexible or still unclear. Gene functions relating to C6-hydroxylation and C-10,-11 epoxidation are currently under investigation.

Gene knockouts have been particularly instrumental in indicating a possible pathway for pyran ring formation and pseudomonic acid A synthesis (Fig. 2b). In this scheme, MupW and MupT catalyse the epoxidation of the C-8,-16 double bond (essential for formation of the tetrahydropyran ring) which makes the C-16 more receptive to attack by the C-5 hydroxyl group. Esterification with 9-HN and C-10,-11 epoxidation, which may occur before or after this, result in pseudomonic acid B. Mutation of *mupW* and *mupT* resulted in accumulation of a novel metabolite, mupirocin W, lacking the tetrahydropyran ring but having the attached 9-HN confirming the role of MupW/MupT in mupirocin biosynthesis but not defining when it normally occurs (Cooper et al. 2005b). To produce pseudomonic acid A, MupU is proposed to mediate the transfer to mAcpE before MupO, a cytochrome P_{450} may catalyse oxidation of the C-7

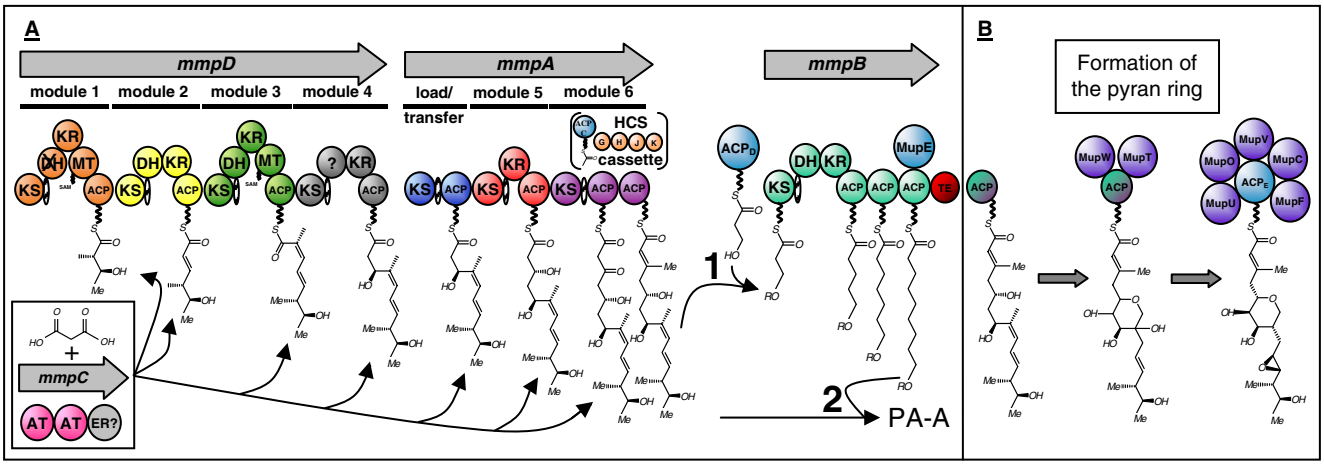

Fig. 2 Proposed scheme for biosynthesis of mupirocin. **a** The monic acid (MA) backbone is synthesised by condensation of extender units and further modifications by the six modules of MmpD and MmpA. 9-HN is synthesised by the iterative condensations and further ketoreduction and dehydration catalysed by MmpB on a proposed 3-hydroxypropionate starter unit. There are two possible pathways for the addition of 9-HN to the MA backbone: continued extension onto MA that is already esterified to 3-HP (pathway 1, R=monic acid); or, separate synthesis followed by esterification (pathway 2, R=H). **b** The MA structure is modified by tailoring enzymes, such as MupW, MupT, MupO, MupU, MupV, MupC and MupF, which function to form the pyran ring and the epoxide group at C-10,-11. Whether this happens before or after joining to 9-HN is not known. The ACP holding the intermediate in panel **b** before it is transferred to mAcpE is shown as *green/purple* to indicate that we do not know whether this normally happens on ACP5/6/7 (*green*) or ACP3/4 (*purple*). Key: all functional domains are labeled except for "docking domains" (*hatched black and white oval*) that are remnants of in-*cis* AT domains that may be required for function. Introduction of new nomenclature for the mupirocin gene cluster means that the modules and domains of multifunctional proteins are now named according to their position within each protein. For example, module 3 of MmpA is termed "A3" and the ACPs that form a didomain on MmpA are now termed ACP-A3a and ACP-A3b (formerly ACPs 3 and 4)

hydroxyl to the ketone, while subsequent dehydration by MupV generates a C-8,-9 enoyl bond. MupC is proposed to reduce the C-8,-9 bond, before MupF catalyses ketoreduction at C-7 and the resultant product is released as pseudomonic acid A (Cooper et al. 2005a, b; Hothersall et al. 2007; Wu et al. 2007).

The HCS cassette, comprised of MupG, MupH, MupJ, MupK and mAcpC, is responsible for the incorporation of the β-methyl group at C-15: MupG catalyses the decarboxylation of acetate from a malonate bound to mAcpC; MupH catalyses the condensation to produce a gluconate intermediate; dehydration, catalysed by MupJ is finally followed by decarboxylation mediated by MupK to produce the β-methylthioester (Wu et al. 2007, 2008). The functions of related HCS cassettes have been proposed by a number of groups and experimental evidence supporting these hypotheses have been provided from studies on myxovirescin (Simunovic et al. 2006), jamaicamide (Edwards et al. 2004), leinamycin (Tang et al. 2004b), bacillaene (Butcher et al. 2007) and curacin A (Chang et al. 2004).

9-Hydroxynonanoic acid biosynthesis

Since there are strong indications for the role of MmpD and MmpA in monic acid (MA) synthesis it seems logical that MmpB, the third type I PKS, is responsible for 9-HN although as yet there is no direct evidence for this. However, 9-HN is proposed to be derived from a 3-hydroxypropionate (3-HP) starter unit with MmpB catalysing three rounds of condensation with malonate as the extender unit (Fig. 2a). MmpB does not contain an ER domain but an in-frame deletion of MupE results in a 6′–7′ enoyl bond suggesting that it, possibly in conjunction with MupD, is responsible for at least part of the required ER activity (Hothersall et al. 2007; Macioszek, PhD thesis, University of Birmingham, 2009; Hothersall and Wu, unpublished data). Since mutagenesis of *mupE* did not result in the formation of a completely unsaturated fatty acid chain, an additional enzyme must be responsible for reduction of the fatty acid chain to give 9-HN. There is a third domain in MmpC which has predicted ER activity, that could function during the formation of 9-HN. Recent work by Bumpus et al. (2008) demonstrated that PksE, an enzyme from the dihydrobacillaene PKS consisting of AT and ER domains, provided ER activity in-*trans*. The terminal TE domain of MmpB could then either catalyse the release of the saturated 9-HN or provide a means of esterification with monic acid (El-Sayed et al. 2003; Hothersall et al. 2007). The order in which events occur is still under investigation. It is possible either that 9-HN and monic acid are synthesised separately and then joined together to complete the mupirocin structure, or that 9-HN is elongated on a starter unit (3-HP) esterified with the product of MmpD/MmpA (J Hothersall, J Wu and A Murphy et al. unpublished data).

Mupirocin production is regulated by quorum sensing

A quorum sensing mechanism controls expression of the mupirocin biosynthetic genes. This involves the constitutive production of diffusible signal molecules (autoinducers, in this case N-acyl homoserine lactones) that accumulate in the environment of the bacteria and, when the population reaches a critical density (quorum), switch on target gene transcription via an activator protein. Based on sequence alignments with the *Vibrio harveyi lux* system, the genes *mupR* and *mupI* were predicted to mediate quorum-regulated expression of the mupirocin cluster (El-Sayed et al. 2001). MupI is required to produce the diffusible substance that activates the *mup* promoter, while MupR is the activator that responds to it (El-Sayed et al. 2001). Thus, the MupR–MupI system activates transcription of the *mup* operon upon binding to the *lux* box promoter regions of *mupA*, *mAcpC* and *mupF*, while surprisingly *mupI* does not appear to have a *lux* box region (Fuqua et al. 1994; El-Sayed et al. 2001). At present, it is unknown whether *mupR* requires transcription right through the mupirocin cluster for expression or whether *mupI* gene expression is boosted when the quorum switch is turned on.

Special features of the mupirocin cluster

The mupirocin cluster contains various features that distinguish it from typical PKSs such as the DEBS system and whose activities may be useful in the generation of novel pathways.

The mupirocin cluster depends on in-*trans* ATs

The mupirocin PKSs differ from those of the well studied erythromycin biosynthetic system from *Saccharopolyspora erythraea* (Lal et al. 2000) in that the AT domains are absent from each module and are encoded by a separate gene, *mmpC* (El-Sayed et al. 2003). They thus belong to the group of PKS systems termed in-*trans* AT PKSs, of which a growing number have been described and analysed including those that produce myxovirescin, virginiamycin, leinamycin, lankacidin, pederin, rhizoxin, bryostatin, kirromycin, mycosubtilin, bacillaene, difficidin, macrolactin, chivosazol, disorazol and thiomarinol (Piel 2002, 2010; Cheng et al. 2003; Chen et al. 2006; Kopp et al. 2005; Mochizuki et al. 2003; Perlova et al. 2006; Simunovic et al. 2006; Aron et al. 2007; Partida-Martinez and Hertweck 2007; Pulsawat et al. 2007; Schneider et al. 2007; Sudek et al. 2007; Weber et al. 2008). There are several key active site amino acids thought to be critical in determining AT specificity for its substrate, particularly the YASH and YAFH motifs, defining methylmalonyl-CoA and malonyl-CoA specificity respectively (DelVecchio et al. 2003). Both of the mupirocin ATs contain partial YAFH motifs, particularly the crucial FH residues, indicating malonyl-CoA specificity. Phylogenetic analysis of in-*trans* ATs from different systems as shown in Fig. 3 indicates two evolutionary pathways with the mupirocin ATs falling in separate clades. Sequence analysis of these clades based on methods described by Yadav et al. (2003) shows the AT2 clade to be specific for malonyl-CoA, while the AT1 clade displays far more active site residue diversity indicating a broader range of substrate specificity. It is possible that the AT2-like enzymes represent the "main" ATs within clusters, while the AT1-like enzymes provide increased turnover, or more specialized functions.

AT "docking domains" have been identified in several in-*trans* AT PKSs, including mupirocin and thiomarinol, and are located immediately downstream of the KS domains on the multifunctional proteins. Tang et al. (2004b) proposed that these domains were remnants of once-functional AT domains, and may have a role to play in interactions between ATs and domains within multifunctional proteins. By contrast, Aron et al. (2007) proposed that it was more likely that they played a role in regulating acyl-group transfer. Further investigation is required to determine the functions of these domains and whether they do indeed play a role in metabolite biosynthesis.

The mupirocin cluster does not contain integrated ER domains

The *trans*-AT systems either completely lack ER domains or have them in unusual positions. For example, for the myxovirescin biosynthetic cluster, it has been proposed that the ER domain of TaO is shared between modules 7 and 8, encoded on Ta-1 and TaO, respectively (Simunovic et al. 2006). ER domains are also thought to be encoded by modules on discrete proteins such as LnmG and MmpC (Cheng et al. 2003; El-Sayed et al. 2003). Figure 3 includes the domain architecture of the AT-encoding genes. There are four distinct architectures: AT didomains with an ER domain, AT didomains without an ER domain, single AT domains with an ER domain, and finally stand alone ATs. These architectures are distributed among the two evolutionary pathways. Their functionality and relevance is yet to be determined.

MT domains and a HCS cassette are responsible for incorporation of methyl groups

In type I systems with in-*cis* AT domains each module can specify the nature of the extender unit, for example choosing methyl-malonate rather than malonate. When all modules share one or two in-*trans* ATs incorporation of α-methyl groups can be specified by MT domains in a module. These occur in the *mup* cluster and all other in-*trans* AT PKSs mentioned previously, with the exceptions of macrolatin and mycosubtilin. However, this is not obligatory because some

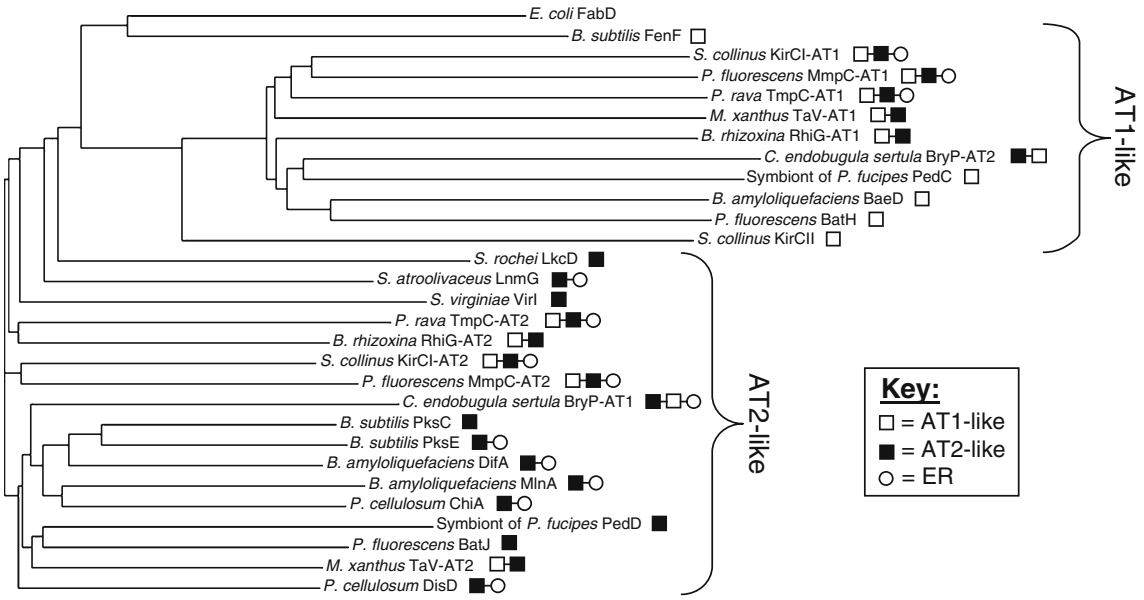

Fig. 3 Phylogenetic tree showing a selection of acyltransferases that operate in-*trans* throughout their respective PKS systems. *Escherichia coli* FabD is included as the out-group for reference. Each AT is also accompanied by connected symbols that show the polypeptide context (architecture) in which that AT is found—alone or joined to a second AT or to an ER domain. Accession numbers: *Bacillus subtilis* ATCC 6633 (AAF08794), *Streptomyces collinus* Tu365 (AM746336), *P. fluorescens* NCIMB 10586 (AAM12912), *Pseudoalteromonas rava* SANK 73390 (FN689524), *Myxococcus xanthus* DK 1622 (YP_632122), *Burkholderia rhizoxina* (CAL69887), *Candidatus endobugula sertula* (ABK51299), symbiont of *Paederus fuscipes* (AAS47559 and AAS47563), *Bacillus amyloliquefaciens* FZB42 (YP_001421286, YP_001421027, and CAG23974), *P. fluorescens* BCCM_ID9359 (GU479979), *Streptomyces rochei* (AB088224), *Streptomyces atroolivaceus* (AAN85520), *Streptomyces virginiae* (BAF50719), *B. subtilis 168* (NP_389591 and CAB13584), and *Polyangium cellulosum* (DQ065771 and AJ874112)

systems employ a mixture of in-*trans* and in-*cis* ATs. In mupirocin, the MT domains in modules 1 and 3 are responsible for the methyl groups C-17 and C-16 in the final structure, which are incorporated from S-adenosyl methionine (El-Sayed et al. 2003; Feline et al. 1977; Wu et al. 2008). In addition, many of the in-*trans* AT group of PKS contain β-branches to the polyketide backbone that are incorporated under the actions of an HCS cassette (Chen et al. 2006; Cheng et al. 2003; Partida-Martinez and Hertweck 2007; Pulsawat et al. 2007; Simunovic et al. 2006; Sudek et al. 2007). As detailed in the previous section, the mupirocin HCS cassette catalyses the incorporation of the methyl group at C-15. Studies on mupirocin have also provided evidence of the functions of the HCS cassette—mutation of *mupH* produced a new metabolite, mupirocin H, which appeared to be a truncated version of monic acid incorporating a 3-hydroxy-γ-lactone ring (Wu et al. 2007).

The mupirocin cluster has tandemly duplicated acyl carrier proteins

The multifunctional proteins MmpA and MmpB contain tandem doublet and triplet ACPs respectively (El-Sayed et al. 2003). There are several other *trans*-AT PKSs with similar unusual domain architecture: leinamycin, lankacidin, bacillaene, difficidin, chivosazol, virginiamycin and macrolactin (Chen et al. 2006; Cheng et al. 2003; Mochizuki et al. 2003; Perlova et al. 2006; Pulsawat et al. 2007; Schneider et al. 2007). The ACPs of the doublet (ACPs-A3a and -A3b—formerly ACPs 3 and 4) and triplet (ACPs-Ba, -Bb and -Bc—formerly ACPs 5, 6 and 7) are more closely related to each other than any other ACPs within the cluster, indicating they may have arisen from gene duplication events. Another unusual feature of the tandem ACPs is the unusually short linker regions between the individual domains—the spacers between domains on the Mmps are usually approximately 100 amino acids in length, but ACPs-A3a and -A3b are separated by just 12 amino acids and ACPs-Ba, -Bb and -Bc by only 3. Rahman et al. (2005) produced various mutants to determine the roles of these tandem ACPs: mutants of ACPs-A3a and -A3b, and ACPs-Ba, -Bb and -Bc resulted in loss of mupirocin production when analysed by bioassay and HPLC. Pairwise mutants of ACPs-Ba/Bb, -Bb/Bc and -Ba/Bc reduced mupirocin production to less than 20% of wild type, while individual mutants of ACPs-A3a and -A3b, and ACPs-Ba, -Bb and -Bc resulted in approximately 60% and 25–36% of production when compared to wild type, respectively. The authors concluded that while any one ACP from the cognate doublet and triplet clusters is sufficient for mupirocin biosynthesis, production was significantly improved by an increase in numbers, indicating that the doublet and triplet set of ACPs are functionally redundant (Rahman et al. 2005).

Reducing the tandem ACPs to a single ACP would be rate limiting. The results indicated that the doublet ACPs (A3a/A3b) work in parallel, while ACP-Ba physically blocks access of other ACPs to some part of the machinery if it is inactivated by a point mutation (Rahman et al. 2005).

The mupirocin cluster does not have a loading module for system initiation

The lack of an obvious loading module within the mupirocin cluster is unusual. Many PKS systems, of both *cis*- and *trans*-AT architecture, have loading modules specifically designed to accept the starter unit for initiation of metabolite production (Hertweck 2009). The model DEBS system contains a loading domain consisting of an AT and an ACP, thought to provide the propionyl-CoA starter unit to the first module (Hill and Staunton 2010). The loading modules of *trans*-AT PKSs vary in the domains that are present, from the non-ribosomal peptide synthase of leinamycin to the minimal ACP of chivosazol and virginiamycin and to the more intricate loading module of bryostatin which contains four domains (Cheng et al. 2003; Perlova et al. 2006; Pulsawat et al. 2007; Sudek et al. 2007). Some such as the disorazol and macrolactin systems are similar to the mupirocin system in not having obvious loading modules. In these systems it has been proposed that a malonate residue is loaded to the first KS domain and then decarboxylated to provide the required starter acetate unit (Kopp et al. 2005; Schneider et al. 2007). Such a mechanism might occur in the mupirocin system, with an as yet unidentified protein providing the decarboxylative function to generate the first acetate for module 1 of MmpD. Alternatively, KS-D1 may decarboxylate malonate to acetate before the first condensation. It is also possible that one of the uncharacterised type II ACPs accepts malonate and catalyses the decarboxylation before transfer to KS-D1. Determining the substrate specificities of the mupirocin ATs may shed some light on how the whole system is initiated. In-frame deletion of AT1 only reduced mupirocin production while a similar mutation in AT2 abolished it (El-Sayed et al. 2003; Hothersall, unpublished data). Current work has shown that AT2 exclusively prefers malonyl-CoA as a substrate over acetyl-, propionyl-, and methylmalonyl-CoA (Gurney et al. unpublished data). As yet it has not been possible to produce soluble AT1 and achieving this is essential to allow direct comparison of the biochemical properties of AT1 and AT2.

Clinical significance and applications

Mupirocin competitively inhibits IleS, blocking the formation of Ile tRNA and thus inhibiting protein synthesis (Hughes and Mellows 1978). IleS catalyses the transfer of isoleucine onto its tRNA via the formation of aminoacyl-adenylate (aa-AMP). The C-14 to C-11 terminus of monic acid resembles the side-chain structure of isoleucine and interacts with the isoleucine-specific binding pocket of IleS (Yanagisawa et al. 1994). The pyran ring interacts with the ATP binding site of IleS, and it is thought the 9-HN moiety may stabilise the binding by its affinity for a hydrophobic groove (Nakama et al. 2001). Mupirocin has a remarkably broad spectrum of activity; it is active against both Gram-positive and Gram-negative organisms, and particularly effective against those staphylococcal and streptococcal species most commonly responsible for infections of the skin (Sutherland et al. 1985). Mupirocin cannot be used systemically due to its high affinity for serum protein and rapid metabolism, but has been successfully used topically for many years (Basker et al. 1980; Sutherland et al. 1985).

Pseudomonic acid was tentatively used as a potential therapeutic agent for skin infections and nasal carriage of antibiotic-resistant strains of *Staphylococcus aureus* in 1983 (Wuite et al. 1983; Dacre et al. 1983). In 1985, it was introduced for the treatment of bacterial skin infections, and in 1988 for nasal carriage of *Staphylococci*, including methicillin-resistant *S. aureus* (MRSA) (Cookson et al. 1990). MRSA colonising the skin and nose can easily be transferred to other areas of the body or wounds, thus causing particular concern during surgical procedures and when cuts or burns are open (Neu 1992). Marketed globally, mupirocin is now used worldwide for topical treatment of impetigo, infected skin lesions and for decolonisation of patients with nasal carriage of *Staphylococci*, including MRSA (GlaxoSmithKlein 2010; Medimetriks 2008; TEVA 2003). Decolonisation can reduce the risk of MRSA infection in patients and decrease transmission to other patients (Gilpin et al. 2010).

Resistance

The initial widespread use of mupirocin to treat MRSA lead to resistance first being recorded in 1987 (Rahman et al. 1987). Resistance to mupirocin has two distinct levels: high-level resistance >500 μg/ml, and the more common low level between 8–256 μg/ml (Ramsey et al. 1996). Low level resistance normally arises from spontaneous mutations in the chromosomally encoded IleS which reduce affinity for mupirocin, but are non-transferable and generally of little clinical significance (Eltringham 1997; Slocombe and Perry 1991). High-level resistance has a more substantial effect on clinical treatments and involves a plasmid-encoded gene, *mupA*, responsible for the production of a novel IleS (Dyke et al. 1991; Eltringham 1997; Farmer et al. 1992; Gilbart et al. 1993; Hodgson et al. 1994; Rahman et al. 1987). The *mupA* gene is associated with transposable elements as part of different plasmids, often self-transmissible, that also

confer resistance to other antibiotics, such as gentamicin, tetracycline and trimethoprim (Patel et al. 2009; Perez-Roth et al. 2010). A recent study to assess the situation in China, where mupirocin is used for treating skin infections, determined that 6.6% of isolates displayed high-level mupirocin resistance and carried the *mupA* gene. Of these isolates, over 80% were also resistant to gentamicin, erythromycin and clindamycin, in addition to observations of resistance to trimethoprim, rifampicin and levofloxacin (Liu et al. 2010a).

Comparison of sequences surrounding *mupA* genes from self-transmissible plasmids in *S. aureus* indicates apparently multiple gene capture events with varying amounts of the same flanking sequences as if the gene comes from the chromosome of an as yet unidentified organism (Perez-Roth et al. 2010). Phylogenetic analysis of IleS proteins put MupA as well as MupM and TmlM proteins (that confer high-level resistance to mupirocin and thiomarinol on the strains that produce these antibiotics) in a group of IleS proteins that have eukaryote-like sequence motifs in their active sites, presumably explaining their resistance (Yanagisawa and Kawakami 2003). Further analysis of the growing wealth of bacterial genome sequences also reveals that many bacteria carry two IleS proteins—the second (IleS2) belonging to this eukaryote-like mupirocin resistance type correlating with a mupirocin-resistant phenotype. The reasons for carriage of a second IleS are at present unclear, but it seems quite likely that it is one such IleS2 gene in an as yet unidentified bacterium that is the source of *mupA* in *S. aureus*.

Resistance has been reported globally at varying degrees of severity. A study in Kuwait which sampled 53 MRSA isolates (39 of which expressed high-level mupirocin resistance) reported a significant increase in the number of high-level mupirocin-resistant MRSA isolates between 1993 and 1995; over the total study period (1990–1995), 42% of the isolates demonstrated high-level resistance (Udo et al. 1999; Vasquez et al. 2000). Between 1994 and 1995 at two closely situated hospitals in Brazil, resistance to mupirocin was >50% in one and approximately 6% in the other—the difference being that mupirocin was used far more frequently in the first hospital (Orrett 2008). In areas where mupirocin is readily available, the occurrence of resistance is high: in New Zealand, mupirocin became available over the counter in 1991, and by 1999, up to 28% of *S. aureus* isolates were mupirocin resistant (Upton et al. 2003). After increased mupirocin use in Western Australia, high-level mupirocin resistance reached 15% but subsequent government-issued guidance on limiting use reduced these levels to 0.3% after 4 years (Torvaldsen et al. 1999). A recent study showed that high-level mupirocin resistance was detected in 17% of patients involved, and this lead to decolonisation failure (Gilpin et al. 2010). Among these studies, a recurring conclusion is evident—prudent use of mupirocin can reduce the levels of resistance.

Future perspectives

While much has been learned about the mupirocin biosynthetic cluster, particularly over the last ten years, there are still aspects of the pathway to be fully worked out. Understanding these aspects in conjunction with what we already know may allow these genes to be used in combinatorial genetics, as in the case of erythromycin (Reeves et al. 2001), rapamycin (Gregory et al. 2005), daptomycin (Nguyen et al. 2006), lovastatin (Xie et al. 2006) and pikromycin (Gupta et al. 2008). The thiomarinols have a chemical structure which is essentially a combination of two independent antibiotics, pseudomonic acid and holomycin highlight the potential of making hybrid antibiotics based on mupirocin (Fig. 1). With a broad range of activity against both Gram-positive and -negative bacteria, thiomarinol A displays far more potency than mupirocin with activity approximately 20-fold higher against organisms such as MRSA and *E. coli* (Shiozawa et al. 1993). In addition to increasing potency, genetic manipulation may also be used to overcome the high affinity of mupirocin for serum protein—its rapid metabolism means that it cannot be used systemically (Basker et al. 1980; Sutherland et al. 1985). While chemical modifications have been explored to try to resolve these issues, genetic modifications have not (Basker et al. 1980). It is possible that analogues of mupirocin and thiomarinols could generate compounds that are both more metabolically stable and more active (El-Sayed et al. 2001; Marion et al. 2009).

Since the discovery of penicillin by Alexander Fleming in 1928, the development of antibiotics has revolutionised the treatment of infectious diseases. Resistance to penicillin was observed a mere 3 years after clinical development by Pfizer in 1944, despite the warnings of Fleming himself (Fleming 1945). Between the 1940s and 1970s, many clinically important antibiotics were discovered and developed, but there have been few in recent years (Bush and Macielag 2000; Critchley and Ochsner 2008; Larkin 2003) so novel compounds and strategies are needed. Despite resistance to mupirocin continuing to be an issue (Caffrey et al. 2010; Fawley et al. 2006; Gilpin et al. 2010), the potential to produce novel derivatives may mean that it will continue to play a role in the future treatment of infectious diseases.

Acknowledgments The work on mupirocin and thiomarinol being carried out in the authors' lab is funded by UK Research Councils BBSRC and EPSRC under grant number E021611. RG is funded by a BBSRC Doctoral Training Centre Studentship. We are grateful to Joanne Hothersall for critical reading of the manuscript.

References

Alexander RG, Clayton JP, Luk K, Rogers NH, King TJ (1978) The chemistry of pseudomonic acid. Part 1. The absolute configuration of pseudomonic acid A. J Chem Soc Perkin Trans I:561–565

Aron ZD, Fortin PD, Calderone CT, Walsh CT (2007) FenF: servicing the Mycosubtilin synthetase assembly line *in trans*. Chembiochem 8:613–616

Basker MJ, Comber KR, Clayton PJ, Hannan PT, Mizen LW, Rogers NH, Slocombe B, Sutherland R (1980) Ethylmonate A: a semisynthetic antibiotic derived from pseudomonic acid A. In: Nelson JD, Grassi C (eds) Current chemotherapy and infectious disease, vol 1. American Society for Microbiology, Washington, pp 471–473

Bumpus SB, Magarvey NA, Kelleher NL, Walsh CT, Calderone CT (2008) Polyunsaturated fatty-acid-like trans-enoyl reductases utilized in polyketide biosynthesis. J Americal Chem Soc 130:11614–11616

Bush K, Macielag M (2000) New approaches in the treatment of bacterial infections. Curr Opin Chem Biol 4:433–439

Butcher RA, Schroeder FC, Fischbach MA, Straight PD, Kolter R, Walsh CT, Clardy J (2007) The identification of bacillaene, the product of the PksX megacomplex in *Bacillus subtilis*. Proc Natl Acad Sci USA 104(5):1506–1509

Caffrey AR, Quilliam BJ, LaPlante KL (2010) Risk factors associated with mupirocin resistance in meticillin-resistant *Staphylococcus aureus*. J Hosp Infect. doi:10.1016/j.jhin.2010.06.023

Cane DE (2010) Programming of erythromycin biosynthesis by a modular polyketide synthase. J Biol Chem 285(36):27515–27523

Chain EB, Mellows G (1974) Structure of pseudomonic acid, an antibiotic from *Pseudomonas fluorescens*. Journal of the Chemical Society, Chemical Communications 1974:847–848

Chain EB, Mellows G (1977) Pseudomonic acid. Part 1. The structure of pseudomonic acid A, a novel antibiotic produced by *Pseudomonas fluorescens*. J Chem Soc Perkin Trans I:294–309

Chang Z, Sitachitta N, Rossi JV, Roberts MA, Flatt PM, Jia J, Sherman DH, Gerwick WH (2004) Biosynthetic pathway and gene cluster analysis of curacin A, an antitubulin natural product from the tropical marine cyanobacterium *Lyngbya majuscula*. J Nat Prod 67:1356–1367

Chen X-H, Vater J, Piel J, Franke P, Scholz R, Schneider K, Koumoutsi A, Hitzeroth G, Grammel N, Strittmatter AW, Gottschalk G, Süssmuth RD, Borriss R (2006) Structural and functional characterization of three polyketide synthase gene clusters in *Bacillus amyloliquefaciens* FZB 42. J Bacteriol 188 (11):4024–4036

Cheng Y-Q, Tang G-L, Shen B (2003) Type I polyketide synthase requiring a discrete acyltransferase for polyketide biosynthesis. Proc Natl Acad Sci USA 100(6):3149–3154

Cookson BD, Lacey RW, Noble WC, Reeves DS, Wise R, Redhead RJ (1990) Mupirocin-resistant *Staphylococcus aureus*. Lancet 335:1095–1096

Cooper SM, Cox RJ, Crosby J, Crump MP, Hothersall J, Laosripaiboon W, Simpson TJ, Thomas CM (2005a) Mupirocin W, a novel pseudomonic acid produced by targeted mutation of the mupirocin biosynthetic gene cluster. The Royal Society of Chemistry Chemical Communications 2005:1179–1181

Cooper SM, Laosripaiboon W, Rahman AS, Hothersall J, El-Sayed AK, Winfield C, Crosby J, Cox RJ, Simpson TJ, Thomas CM (2005b) Shift to pseudomonic acid B production in *P. fluorescens* NCIMB10586 by mutation of mupirocin tailoring genes *mupO*, *mupU*, *mupV*, and *macpE*. Chem Biol 12:825–833

Critchley IA, Ochsner UA (2008) Recent advances in the preclinical evaluation of the topical antibacterial agent REP8839. Curr Opin Chem Biol 12:409–417

Dacre JE, Emmerson AM, Jenner EA (1983) Nasal carriage of gentamicin and methicillin resistant *Staphylococcus aureus* treated with topical pseudomonic acid. Lancet 322(8357):1036

DelVecchio F, Petkovic H, Kendrew SG, Low L, Wilkinson B, Lill R, Cortés J, Rudd BAM, Staunton J, Leadlay PF (2003) Active-site residue, domain and module swaps in modular polyketide synthases. J Ind Microbiol Biotechnol 30:489–494

Dyke KGH, Curnock SP, Golding M, Noble WC (1991) Cloning of the gene conferring resistance to mupirocin in *Staphylococcus aureus*. FEMS Microbiol Lett 77:195–198

Edwards DJ, Marquez BL, Nogle LM, McPhail K, Goeger DE, Roberts MA, Gerwick WH (2004) Structure and biosynthesis of the jamaicamides, new mixed polyketide-peptide neurotoxins from the marine cyanobacterium *Lyngbya majuscula*. Chem Biol 11:817–833

El-Sayed AK, Hothersall J, Thomas CM (2001) Quorum-sensing-dependent regulation of biosynthesis of the polyketide antibiotic mupirocin in *Pseudomonas fluorescens* NCIMB 10586. Microbiology 147:2127–2139

El-Sayed AK, Hothersall J, Cooper SM, Stephens E, Simpson TJ, Thomas CM (2003) Characterization of the mupirocin biosynthesis gene cluster from *Pseudomonas fluorescens* NCIMB 10586. Chem Biol 10:419–430

Eltringham I (1997) Mupirocin resistance and methicillin-resistant *Staphylococcus aureus* (MRSA). J Hosp Infect 35:1–8

Farmer TH, Gilbart J, Elson SW (1992) Biochemical basis of mupirocin resistance in strains of *Staphylococcus aureus*. J Antimicrob Chemother 30:587–596

Fawley WN, Parnell P, Hall J, Wilcox MH (2006) Surveillance for mupirocin resistance following introduction of routine peri-operative prophylaxis with nasal mupirocin. J Hosp Infect 62:327–332

Feline TC, Jones RB, Mellows G, Phillips L (1977) Pseudomonic acid. Part 2. Biosynthesis of pseudomonic acid A. J Chem Soc Perkin Trans I:309–318

Fleming A (1945) Penicillin. Nobel lecture

Fuller AT, Mellows G, Woolford M, Banks GT, Barrow KD, Chain EB (1971) Pseudomonic acid: an antibiotic produced by *Pseudomonas fluorescens*. Nature 234:416–417

Fuqua WC, Winans SC, Greenberg EP (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J Bacteriol 176(2):269–275

Gilbart J, Perry CR, Slocombe B (1993) High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl-tRNA synthetases. Antimicrob Agents Chemother 37 (1):32–38

Gilpin DF, Small S, Bakkshi S, Kearney MP, Cardwell C, Tunney MM (2010) Efficacy of a standard meticillin-resistant *Staphylococcus aureus* decolonisation protocol in routine clinical practice. J Hosp Infect 75:93–98

GlaxoSmithKlein (2010) Bactroban. http://public.gsk.co.uk/products/bactroban/. Accessed 9 August 2010

Gregory MA, Petkovic H, Lill RE, Moss SJ, Wilkinson B, Gaisser S, Leadlay PF, Sheridan RM (2005) Mutasynthesis of rapamycin analogues through the manipulation of a gene governing starter unit biosynthesis. Angew Chem Int Ed 44:4757–4760

Gupta S, Lakshmanan V, Kim BS, Fecik R, Reynolds KA (2008) Generation of novel pikromycin antibiotic products through mutasynthesis. Chembiochem 9:1609–1616

Hertweck C (2009) The biosynthetic logic of polyketide diversity. Angew Chem Int Ed 48:4688–4716

Hill AM, Staunton J (2010) Type I modular PKS. In: Mander L, Liu HW (eds) Comprehensive natural products II: chemistry and biology, vol 1. Oxford, Elsevier, pp 385–452

Hodgson JE, Curnock SP, Dyke KGH, Morris R, Sylvester DR, Gross MS (1994) Molecular characterization of the gene encoding high-level

mupirocin resistance in *Staphylococcus aureus* J2870. Antimicrob Agents Chemother 38(5):1205–1208

Hothersall J, Wu JE, Rahman AS, Shields JA, Haddock J, Johnson N, Cooper SM, Stephens ER, Cox RJ, Crosby J, Willis CL, Simpson TJ, Thomas CM (2007) Mutational analysis reveals that all tailoring region genes are required for production of polyketide antibiotic mupirocin by *Pseudomonas fluorescens*. J Biol Chem 282(21):15451–15461

Hughes J, Mellows G (1978) Inhibition of isoleucyl-transfer ribonucleic acid synthetase in *Escherichia coli* by pseudomonic acid. Biochem J 179:305–318

Kopp M, Irschik H, Pradella S, Müller R (2005) Production of the tubulin destabilizer disorazol in *Sorangium cellulosum*: biosynthetic machinery and regulatory genes. Chembiochem 6:1277–1286

Lal R, Kumari R, Kaur H, Khanna R, Dhingra N, Tuteja D (2000) Regulation and manipulation of the gene clusters encoding type-I PKSs. Trends Biotechnol 18:264–274

Larkin M (2003) Daptomycin approved for skin and skin-structure infections. Lancet 3:677

Liu Q-Z, Wu Q, Zhang Y-B, Liu M-N, Hu F-P, Xu X-G, Zhu D-M, Ni Y-X (2010a) Prevalence of clinical meticillin-resistant *Staphylococcus aureus* (MRSA) with high-level mupirocin resistance in Shanghai and Wenzhou, China. Int J Antimicrob Agents 35:114–118

Liu TG, Vora H, Khosla C (2010b) Quantitative analysis and engineering of fatty acid biosynthesis in *E. coli*. Metab Eng 12:378–386

Lugtenberg BJJ, Bloemberg GV (2004) Life in the rhizospere. In: Ramos J-L (ed) Pseudomonas, vol 1. Kluwer Academic/Plenum Publishers, New York, pp 403–430

Magnuson K, Jackowski S, Rock C, John E, Cronan J (1993) Regulation of fatty acid biosynthesis in *Escherichia coli*. Microbiol Rev 57(3):522–542

Maier T, Leibundgut M, Ban N (2008) The crystal structure of a mammalian fatty acid synthase. Science 321:1315–1322

Marion O, Gao X, Marcus S, Hall DG (2009) Synthesis and preliminary antibacterial evaluation of simplified thiomarinol analogs. Bioorg Med Chem 17:1006–1017

Martin FM, Simpson TJ (1989) Biosynthetic studies on pseudomonic acid (mupirocin), a novel antibiotic metabolite of *Pseudomonas fluorescens*. J Chem Soc Perkin Trans I:207–209

Medimetriks (2008) Products. http://medimetriks.com/content/blogsection/6/36/. Accessed 9 August 2010

Mochizuki S, Hiratsu K, Suwa M, Ishii T, Sugino F, Yamada K, Kinashi H (2003) The large linear plasmid pSLA2-L of *Streptomyces rochei* has an unusually condensed gene organization for secondary metabolism. Mol Microbiol 48(6):1501–1510

Nakama T, Nureki O, Yokoyama S (2001) Structural basis for the recognition of isoleucyl-adenylate and an antibiotic, mupirocin, by isoleucyl-tRNA synthetase. J Biol Chem 276(50):47387–47393

Neu HC (1992) The crisis in antibiotic resistance. Science 257:1064–1073

Nguyen KT, Ritz D, Gu J-Q, Alexander D, Chu M, Miao V, Brian P, Baltz RH (2006) Combinatorial biosynthesis of novel antibiotics related to daptomycin. Proc Natl Acad Sci USA 103(46):17462–17467

Orrett FA (2008) The emergence of mupirocin resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in Trinidad: a first report. Jpn J Infect Dis 61:107–110

Partida-Martinez LP, Hertweck C (2007) A gene cluster encoding rhizoxin biosynthesis in "*Burkholderia rhizoxina*", the bacterial endosymbiont of the fungus *Rhizopus microsporus*. Chembiochem 8:41–45

Patel JB, Gorwitz R, Jernigan JA (2009) Mupirocin resistance. Clin Infect Dis 49:935–941

Perez-Roth E, Kwong SM, Alcoba-Florez J, Firth N, Mendez-Alvarez S (2010) Complete nucleotide sequence and comparative analysis of pPR9, a 41.7-kilobase conjugative staphylococcal multiresistance plasmid conferring high-level mupirocin resistance. Antimicrob Agents Chemother 54(5):2252–2257

Perlova O, Gerth K, Kaiser O, Hans A, Müller R (2006) Identification and analysis of the chivosazol biosynthetic gene cluster from the myxobacterial model strain *Sorangium cellulosum* So ce56. J Biotechnol 121:147–191

Piel J (2002) A polyketide synthase-peptide synthetase gene cluster from an uncultured bacterial symbiont of *Paederus* beetles. Proc Natl Acad Sci USA 99(22):14002–14007

Piel J (2010) Biosynthesis of polyketides by trans-AT polyketide synthases. Nat Prod Rep 27:996–1047

Prescott LM, Harley JP, Klein DA (2005) Microbiology, 6th edn. McGraw-Hill, New York

Pulsawat N, Kitani S, Nihira T (2007) Characterization of biosynthetic gene cluster for the production of virginiamycin M, a streptogramin type A antibiotic, in *Streptomyces virginiae*. Gene 393:31–42

Rahman M, Noble WC, Cookson B (1987) Mupirocin-resistant *Staphylococcus aureus*. Lancet 330(8555):377–378

Rahman AS, Hothersall J, Crosby J, Simpson TJ, Thomas CM (2005) Tandemly duplicated acyl carrier proteins, which increase polyketide antibiotic production, can apparently function either in parallel or in series. J Biol Chem 280(8):6399–6408

Ramsey MA, Bradley SF, Kauffman CA, Morton TM (1996) Identification of chromosomal location of mupA gene, encoding low-level mupirocin resistance in staphylococcal isolates. Antimicrob Agents Chemother 40(12):2820–2823

Reeves CD, Murli S, Ashley GW, Piagentini M, Hutchinson CR, McDaniel R (2001) Alteration of the substrate specificity of a modular polyketide synthase acyltransferase domain through site-specific mutations. Biochemistry 40(51):15464–15470

Ridley CP, Lee HY, Khosla C (2008) Evolution of polyketide synthases in bacteria. PNAS 105(12):4595–4600

Schneider K, Chen X-H, Vater J, Franke P, Nicholson G, Borriss R, Süssmuth RD (2007) Macrolactin is the polyketide biosynthesis product of the pks2 cluster of *Bacillus amyloliquefaciens* FZB42. J Nat Prod 70:1417–1423

Schujman GE, Dd M (2008) Regulation of type II fatty acid synthase in Gram-positive bacteria. Curr Opin Microbiol 11:148–152

Shiozawa H, Kagasaki T, Kinoshita T, Haruyama H, Domon H, Utsui Y, Kodama K, Takahashi S (1993) Thiomarinol, a new hybrid antimicrobial antibiotic produced by a marine bacterium. J Of Antibiot 46(12):1834–1842

Simunovic V, Zapp J, Rachid S, Krug D, Meiser P, Müller R (2006) Myxovirescin A biosynthesis is directed by hybrid polyketide synthases/nonribosomal peptide synthetase, 3-hydroxy-3-methylglutaryl–CoA synthases, and trans-acting acyltransferases. Chembiochem 7:1206–1220

Slocombe B, Perry C (1991) The antimicrobial activity of mupirocin—an update on resistance. J Hosp Infect 19(Supp2):19–25

Sudek S, Lopanik NB, Waggoner LE, Hildebrand M, Anderson C, Liu H, Patel A, Sherman DH, Haygood MG (2007) Identification of the putative bryostatin polyketide synthase gene cluster from "*Candidatus* Endobugula sertula", the uncultivated microbial symbiont of the marine bryozoan *Bugula neritina*. J Nat Prod 70 (1):67–74

Sutherland R, Boon RJ, Griffin KE, Masters PJ, Slocombe B, White AR (1985) Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. Antimicrob Agents Chemother 27(4):495–498

Tang G-L, Cheng Y-Q, Shen B (2004a) Leinamycin biosynthesis revealing unprecedented architectural complexity for a hybrid polyketide synthase and nonribosomal peptide synthetase. Chem Biol 11:33–45

Tang Y, Lee TS, Lee HY, Khosla C (2004b) Exploring the biosynthetic potential of bimodular aromatic polyketide synthases. Tetrahedron 60:7659–7671

TEVA (2003) Press Release: Teva Announces Approval Of Mupirocin Ointment http://www.tevapharm.com/pr/2003/pr_421.asp. Accessed 9 August 2010

Torvaldsen S, Roberts C, Riley TV (1999) The continuing evolution of methicillin-resistant *Staphylococcus aureus* in Western Australia. Infect Control Hosp Epidemiol 20(2):133–135

Udo EE, Farook VS, Mokadas EM, Jacob LE, Sanyal SC (1999) Molecular fingerprinting of mupirocin-resistant methicillin-resistant *Staphylococcus aureus* from a burn unit. Int J Infect Dis 3(2):82–87

Upton A, Lang S, Heffernan H (2003) Mupirocin and *Staphylococcus aureus*: a recent paradigm of emerging antibiotic resistance. J Antimicrob Chemother 51:613–617

Vasquez JE, Walker ES, Franzus BW, Overbay BK, Reagan DR, Sarubbi FA (2000) The epidemiology of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* at a Veterans' Affairs Hospital. Infect Control Hosp Epidemiol 21(7):459–464

Weber T, Laiple KJ, Pross EK, Textor A, Grond S, Welzel K, Pelzer S, Vente A, Wohlleben W (2008) Molecular analysis of the kirromycin biosynthetic gene cluster revealed beta-alanine as precursor of the pyridone moiety. Chem Biol 15:175–188

Whatling C, Hodgson JE, Burnham MKR, Clarke NJ, Franklin CH, Thomas CM (1995) Identification of a 60 kb region of the chromosome of *Pseudomonas fluorescens* NCIB 10586 required for the biosynthesis of pseudomonic acid (mupirocin). Microbiology 141:973–982

Wu J, Cooper SM, Cox RJ, Crosby J, Crump MP, Hothersall J, Simpson TJ, Thomas CM, Willis CL (2007) Mupirocin H, a novel metabolite resulting from mutation of the HMG-CoA synthase analogue, mupH in *Pseudomonas fluorescens*. Chem Commun 2007:2040–2042

Wu J, Hothersall J, Mazzetti C, O'Connell Y, Shields JA, Rahman AS, Cox RJ, Crosby J, Simpson TJ, Thomas CM, Willis CL (2008) In vivo mutational analysis of the mupirocin gene cluster reveals labile points in the biosynthetic pathway: the "leaky hosepipe" mechanism. Chembiochem 9:1500–1508

Wuite J, Davies BI, Go M, Lambers J, Jackson D, Mellows G (1983) Pseudomonic acid: a new topical antimicrobial agent. Lancet 322 (8346):394

Xie X, Watanabe K, Wojcicki WA, Wang CCC, Tang Y (2006) Biosynthesis of lovastatin analogs with a broadly specific acyltransferase. Chem Biol 13:1161–1169

Yadav G, Gokhale RS, Mohanty D (2003) Computational approach for prediction of domain organisation and substrate specificity of modular polyketide synthases. J Mol Biol 328:335–363

Yanagisawa T, Kawakami M (2003) How does *Pseudomonas fluorescens* avoid suicide from its antibiotic pseudomonic acid? J Biol Chem 278(28):25887–25894

Yanagisawa T, Lee JT, Wu HC, Kawakami M (1994) Relationship of protein structure of isoleucyl-tRNA synthetase with pseudomonic acid resistance of *Escherichia coli*. J Biol Chem 269(39):24304–24309