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# Sensing and responding to diverse extracellular signals: an updated analysis of the sensor kinases and response regulators of *Streptomyces* species

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**Abstract**

*Streptomyces venezuelae* is a Gram-positive, filamentous actinomycete with a complex developmental life cycle. Genomic analysis revealed that *S. venezuelae* encodes a large number of two-component systems (TCSs): these consist of a membrane-bound sensor kinase (SK) and a cognate response regulator (RR). These proteins act together to detect and respond to diverse extracellular signals. Some of these systems have been shown to regulate antimicrobial biosynthesis in *Streptomyces* species, making them very attractive to researchers. The ability of *S. venezuelae* to sporulate in both liquid and solid cultures has made it an increasingly popular model organism in which to study these industrially and medically important bacteria. Bioinformatic analysis identified 58 TCS operons in *S. venezuelae* with an additional 27 orphan SK and 18 orphan RR genes. A broader approach identified 15 of the 58 encoded TCSs to be highly conserved in 93 *Streptomyces* species for which high-quality and complete genome sequences are available. This review attempts to unify the current work on TCS in the streptomycetes, with an emphasis on *S. venezuelae*.

## INTRODUCTION

A response to a stimulus or changes in the environment is one of the seven criteria of life [1]. Bacteria sense their environment mainly via two-component systems (TCSs) and the number of TCSs varies greatly depending on the environmental niche the bacterium occupies. Intracellular pathogens living in a homeostatic environment encode very few TCSs whereas bacteria living in competitive environments such as soil contain many more [2]. Soil-dwelling *Streptomyces* bacteria have some of the largest bacterial genomes and the highest numbers of TCSs found in bacteria, perhaps reflecting an adaptation to their highly variable environmental niche [3].

TCSs were first described in the late 1980s [4] and since then they have been found in all Domains of life [5]. The absence of TCSs in humans or animals makes them an ideal target for antibiotics, particularly because some TCSs are essential [6, 7] and others regulate virulence and antibiotic resistance [8]. There are three different classes of TCSs: the classical TCS has a transmembrane sensor kinase (SK) that senses the signal and transfers a phosphate group to its cognate response regulator (RR); the hybrid TCS has an SK and RR fused together, and thus the whole TCS complex is membrane bound; finally the phosphorelay TCS includes phosphotransferases that transfer the phosphoryl group from the SK to the ultimate target via multiple phosphotransfer steps [9]. In general, SKs receive a stimulus and autophosphorylate at a conserved histidine residue using ATP. The phosphate group is then transferred to a conserved aspartate in the cognate RR. SKs contain two domains: a variable sensor region and a highly conserved kinase core [10]. The kinase core contains a dimerization domain and a catalytic domain which contains the ATP binding site [11]. RRs also consist of two domains: a conserved N-terminal receiver domain containing the conserved aspartate and a variable output domain. The receiver domain contains the phosphorylation site and the

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**Abbreviations:** ACT, actinorhodin; BGC, biosynthetic gene cluster; BLI, biolayer interferometry; CA, clavulanic acid; CDA, calcium-dependent antibiotic; ChIP, chromatin immunoprecipitation; DBD, DNA-binding domain; DRU, direct repeat unit; EMSA, electrophoreticmobility shift assay; GBL, γ-butyrolactone; GCPD, glycerophosphodiesterphosphodiesterase; HGT, horizontal gene transfer; HTH, helix–turn–helix; LSE, lineage-specific expansion; MRSA, methicillin-resistant *Staphylococcus aureus*; RED, undecylprodigiosin; ROS, reactive oxygen species; RR, response regulator; SK, sensor kinase; TCS, two-component system; VRE, vancomycin-resistant *Enterococcus*.

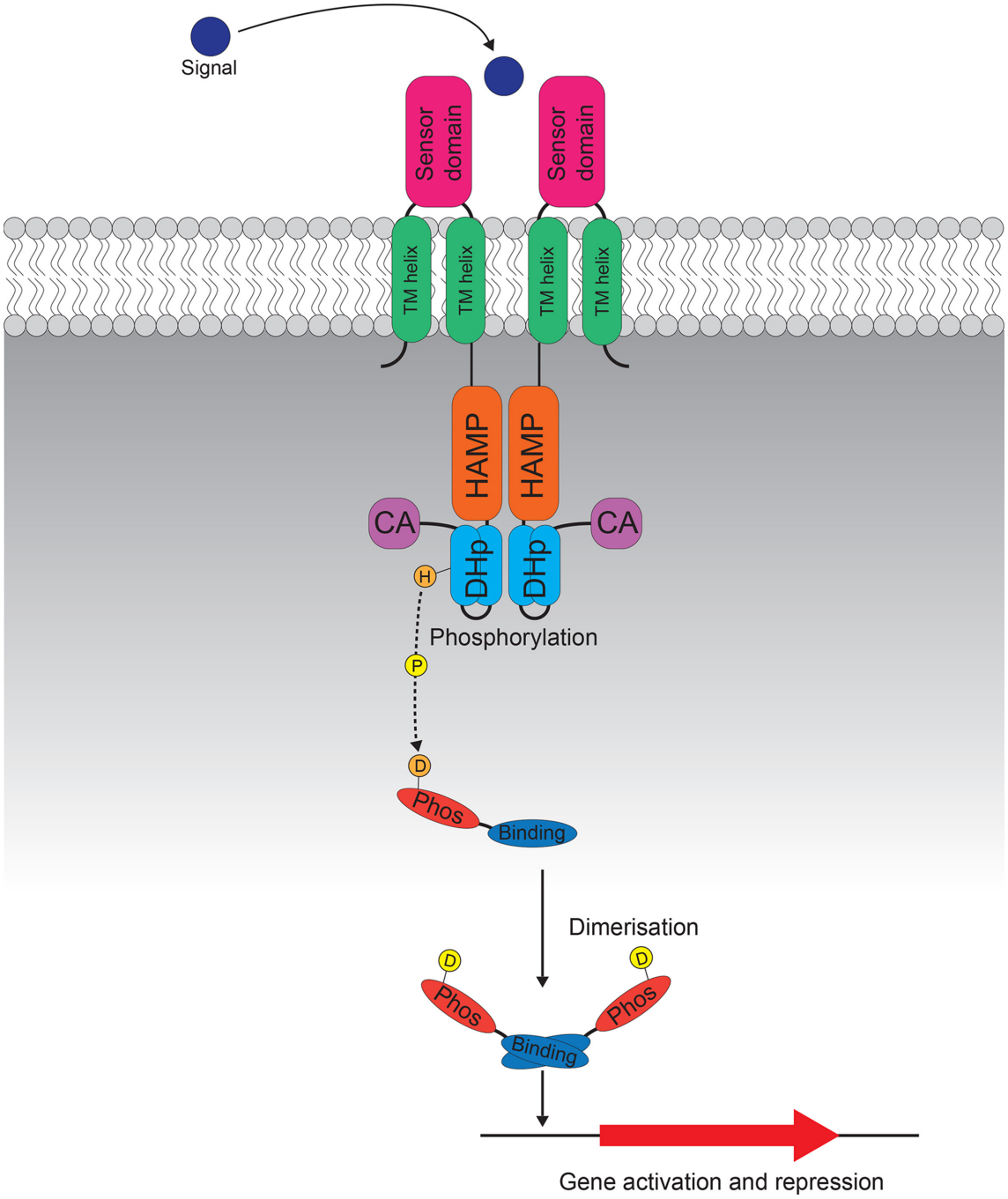


**Fig. 1.** A generalized diagram of a two-component system. The sensor kinase can be found in the cytoplasmic membrane, often as a dimer. Autophosphorylation occurs at the conserved histidine. Following activation by a signal the histidine kinase phosphorylates the partner response regulator at the target aspartate residue. Following phosphorylation, the response regulator dimerizes in order to bind target DNA sequences and effect gene activation and/or repression.

output domain is usually a helix–turn–helix (HTH) DNA binding domain, but can also be an RNA binding, protein binding or an enzymatic domain [2]. A schematic diagram is provided in Fig. 1.

In this review we compare the conservation of TCSs in 93 *Streptomyces* complete genome sequences and review all TCSs in the model organism *S. venezuelae* NRRL B-65442 to give an update on the known function of TCSs in the genus *Streptomyces*.

## TCSs IN *STREPTOMYCES* SPECIES

Despite the vast number of *Streptomyces* strains isolated so far, much of our knowledge of this genus has been obtained

from two model organisms: *Streptomyces coelicolor* and *S. venezuelae*. *S. coelicolor* was the first model organism to be adopted due to the pioneering work of Sir David Hopwood and colleagues [12]. *S. coelicolor* produces five antibiotics under laboratory conditions: the blue actinorhodin (ACT) [13], the red undecylprodigiosin (RED) [14], the colourless calcium-dependent antibiotic (CDA) [15], the yellow-pigmented coelimycin P1 (yCPK) [16, 17] and the unusual, plasmid encoded cyclopentanone antibiotic methylenomycin [18]. Most of the developmental regulatory genes were first identified and studied in *S. coelicolor* and it was the first streptomycete to have its genome sequenced [3]. In recent years, *S. venezuelae* has been adopted as a model to study the regulation of development and antibiotic production. *S. venezuelae* is unusual for the genus because it sporulates in liquid culture and is known to make three antibiotics under laboratory conditions: chloramphenicol, jadomycin and watasemycin [19–21].

### Identification and classification of TCSs

We searched the genome of *S. venezuelae* NRRL B-65442 [22] and *S. coelicolor* A3(2) [3] for TCS, orphan RR, unpaired SK and other regulatory genes with the online tool P2RP [23]. Within this software, regulatory proteins are identified by using RPSBLAST (reverse PSI-BLAST) which searches a query sequence against the SMART and Pfam databases [23]. The program uses an E-value cut-off of 0.01 [24] and the secondary structure of regulatory proteins is computed using the PSIPRED method with the presence or absence of transmembrane segments predicted using the HMMTOP predictor. This method identifies more regulatory proteins than previous studies [3, 25] due to improved protein domain identification and classification (Table 1).

Analysis of the *S. venezuelae* NRRL B-65442 genome showed that there are more than twice the number of one-component systems as there are TCSs. One-component systems are transcription factors containing known or predicted input and output domains but lack the histidine kinase and receiver domains. This is consistent with the work of Ulrich *et al.* [26] which argued that TCSs are evolutionary derivatives of one-component systems. In total, 58 TCSs were identified in *S. venezuelae*. The SK/RR pair *vnz_32020*/*vnz_32020* is judged to be a 'false positive' TCS because the genes are convergent and not in an operon. We therefore classified the SK and RR as orphans and identified a further 26 unpaired SKs and 17 orphan RRs (Tables 2 and 3). Whilst many of these SKs were listed as potentially incomplete in the analysis due to lack of an identifiable histidine residue for phosphorylation, some of them may still be able to pair with some of the orphan RRs or RRs of paired TCSs, as exemplified by Spo0F [27].

**Table 1.** Comparison of signalling proteins of *S. venezuelae* NRRL B-65442 and *S. coelicolor* M145 with the online tool P2RP and with our previous review (Hutchings *et al.* [25] and the first sequencing of *S. coelicolor* (Bentley *et al.* [3])

| | ***S. venezuelae*** **P2RP** | ***S. coelicolor*** **P2RP** | ***S. coelicolor*** **Hutchings *et al.* [25]** | ***S. coelicolor*** **Bentley *et al.* [3]** |
|---|---|---|---|---|
| Sensor kinase | 85 | 109 | 84 | 85 |
| Response regulator | 76 | 87 | 80 | 79 |
| Two-component system | 58 | 69 | 67 | 53 |
| Orphan RR | 18 | 21 | 13 | N/A |
| Unpaired SK | 27 | 39 | 17 | N/A |
| Phosphotransfer protein | 1 | 0 | N/A | N/A |
| Transcriptional regulators | 418 | 483 | N/A | 117 |
| One-component systems | 132 | 146 | N/A | N/A |
| Sigma factors | 55 | 64 | N/A | 65 |
| Other DNA binding proteins | 39 | 36 | N/A | 25 |

## EVOLUTION AND CONSERVATION OF TCSs

In addition to the P2RP analysis we investigated the pan genome of 93 fully sequenced streptomycetes using the BPGA tool [42] identifying the conservation of TCSs throughout *Streptomyces* species. Fifteen TCSs are highly conserved in these 93 genomes and 12 are of known function or predicted by homology to TCSs in other genera. Three highly conserved TCSs have no known function. The TCS *vnz_24545/50* consists of a classic SK and an OmpR RR and is not only conserved throughout *Streptomyces* species but also in closely related genera and the suborders *Corynebacterineae*, *Micrococcineae* and *Bifidobacteriales* [43]. The TCSs *vnz_07060/65* and *vnz_08930/35* both consist of a classical SK and an NarL RR and seem to be conserved only within *Streptomyces* and closely related genera. OmpR and NarL RRs both contain a DNA binding HTH domain at the output domain and bind to DNA, controlling gene expression [5].

Analyses of total TCSs in genomes have shown that typically the number of TCSs roughly correlates with the square of the genome size [44, 45]. The gain of a new TCS has been attributed to two avenues: (1) lineage-specific expansion (LSE), where there is a duplication event or reshuffling, and (2) horizontal gene transfer (HGT), where genes are transferred from one species to another [46]. The gain of new TCSs may be evenly spread between LSE and HGT as is seen in *Bradyrhizobium japonicum* or may be biased towards one of these methods such as for *Pseudomonas aeruginosa,* which has gained the majority of its new TCSs from HGT. This is in contrast to *S. coelicolor,* which has evolved nearly all of its new TCSs through LSE [46]. This is not surprising because *Streptomyces* species are known for their tendency for gene duplication [3]. Thus, it is likely that acquiring TCSs through LSE may not be a common theme in *Streptomyces* species as

**Table 2.** Unpaired sensor kinases (SK) predicted with the P2RP software All SKs are classic except OsaD (*vnz_01755*) which is a hybrid SK with no transmembrane domains. Domain descriptions are obtained from the Pfam database. The transmembrane helices (TM) are predicted by the HMMTOP software

| *vnz* gene no. | Name | TM | Annotation | Function | SCO homologue | References |
|---|---|---|---|---|---|---|
| *vnz_00475* | CvnA6 | 0 | 1 NIT, 1 HAMP, 1 HATPase_c | Conservon | *SCO6069* | |
| *vnz_01755* | OsaD | 0 | 6 HAMP, 1 GAF, 1 HisKA, 1 HATPase_c, 1 Response_reg | Possibly important in osmoprotection | *SCO7327* | Bishop *et al.* [28] |
| *vnz_02660* | CvnA9, RarA | 2 | Probably incomplete; 1 HATPase_c | Conservon regulating *bld* genes in response to high glucose | *SCO1630* | Komatsu *et al.* [29] |
| *vnz_04805* | CvnA4 | 2 | 1 NIT, 1 HAMP, 1 HATPase_c | Conservon | *SCO1402* | |
| *vnz_05825* | OhkA | 0 | 1 PAS_4, 1 HisKA, 1 HATPase_c | Development and antibiotic production | *SCO1596* | Pokhrel *et al.* [30] |
| *vnz_07065* | | 0 | 1 GAF, 1 HisKA_3, 1 HATPase_c | | *SCO1802* | |
| *vnz_07490* | | 1 | 1 PAS, 1 GAF, 1 SpoIIE, 1 HATPase_c | | *SCO0676* | |
| *vnz_10900* | | 0 | Probably incomplete; 2 GAF, 1 HisKA_3 | | *SCO0213* | |
| *vnz_14475* | | 5 | 1 HisKA_3, 1 HATPase_c | | *SCO0676* | |
| *vnz_17535* | | 0 | Probably incomplete; 1 GAF, 1 SpoIIE, 1 HATPase_c | | *SCO3796* | |
| *vnz_18260* | | 0 | 2 GAF, 1 HisKA_3, 1 HATPase_c | | *SCO3948* | |
| *vnz_18495* | | 0 | Probably incomplete; 2 PAS, 1 HisKA, 1 REC | | *SCO4009* | |
| *vnz_19115* | | 0 | Probably incomplete; 1 PAS_4, 1 SpoIIE, 1 HATPase_c | | *SCO4120* | |
| *vnz_23260* | | 0 | Probably incomplete; 1 GAF, 1 SpoIIE, 1 HATPase_c | | *SCO5040* | |
| *vnz_23470* | | 0 | Probably incomplete; 2 PAS_4, 1 GAF, 1 SpoIIE, 1 HATPase_c | | *SCO5104* | |
| *vnz_24250* | | 0 | 1 PAS_4, 1 HisKA_2, 1 HATPase_c | | *SCO5239* | |
| *vnz_24625* | CvnA5 | 0 | Probably incomplete; 1 NIT, 1 HAMP, 1 HATPase_c | Conservon | *SCO5289* | |
| *vnz_24695* | | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *SCO5304* | |
| *vnz_25860* | CvnA2 | 2 | Probably incomplete; 1 NIT, 1 HAMP, 1 HATPase_c | Conservon | *SCO5540* | |
| *vnz_25880* | CvnA1 | 2 | Probably incomplete; 1 NIT, 1 HAMP, 1 HATPase_c | Conservon regulating aerial hyphae formation and antibiotic production | *SCO5544* | Takano *et al.* [31] |
| *vnz_31910* | CvnA7 | 2 | Probably incomplete; 1 HATPase_c | Conservon | *SCO6794* | |
| *vnz_32020* | | 0 | 1 PAS_2, 1 GAF, 1 PHY, 1 HisKA, 1 HATPase_c | | *SCO871* | |
| *vnz_33360* | | 0 | Probably incomplete; 1 GAF, 1 SpoIIE, 1 HATPase_c | | *SCO0767* | |
| *vnz_33580* | | 0 | Probably incomplete; 1 PAS_3, 1 SpoIIE, 1 HATPase_c | | *SCO0946* | |
| *vnz_33610* | | 1 | 1 His_kinase, 1 HATPase_c | | *SCO1217* | |
| *vnz_35820* | CvnA3 | 0 | Probably incomplete; 1 NIT, 1 HATPase_c | Conservon | *SCO1160* | |
| *vnz_36410* | | 5 | 1 HisKA_3, 1 HATPase_c | | – | |

**Table 3.** Orphan response regulator (RR) predicted by P2PR Annotations are based on the Pfam database. An RR is typical when all conserved residues (DD, D54, S/T82 and K105) are present.

| vnz gene number | Name | Conserved residues | | | | | Type | Annotation | Function | SCO homologue | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DD | D54 | S/T82 | K105 | | | | | | |
| *vnz_06090* | | y | y | y | y | Typical | NarL | 1 Response_reg, 1 HTH_LUXR | Upregulated in response to pH shock in *S. coelicolor* | *SCO1654* | Yague *et al.* [32] |
| *vnz_06620* | | n | y | y | y | Atypical | CheY | 1 Response_reg | | – | |
| *vnz_08050* | | 1/2 | y | y | y | Typical | AmiR_NasR | 1 Response_reg, 1 ANTAR | Development | *SCO2013* | Zhao *et al.* [33] |
| *vnz_08820* | | n | n | n | n | Atypical | unclassified | 1 Response_reg | | *SCO2137* | |
| *vnz_10435* | | n | n | n | n | Atypical | unclassified | 1 Response_reg | | *SCO1993* | |
| *vnz_13500* | | y | y | y | y | Typical | NarL | 1 Response_reg, 1 HTH_LUXR | RED production in *S. coelicolor* | *SCO3008* | Xu *et al.* [34] |
| *vnz_14550* | | y | y | n | y | Atypical | NarL | 1 Response_reg, 1 GerE | Downregulated by glucose | *SCO3134* | Romero-Rodríguez *et al.* [35] |
| *vnz_14570* | | y | n | n | n | Atypical | NarL | 1 Response_reg, 1 HTH_LUXR | | *SCO3144* | |
| *vnz_17650* | | y | y | y | y | Typical | NarL | 1 Response_reg, 1 HTH_LUXR | Phosphorylated by SK SCO0203 which is not present in *S. venezuelae* | *SCO3818* | Wang *et al.* [36] |
| *vnz_19510* | | 1/2 | y | n | n | Atypical | RsbU | 1 Response_reg, 1 SpoIIE | Downregulated in response to ciprofloxacin | *SCO4201* | Patkari and Mehra [37] |
| *vnz_22005* | BldM | y | y | y | y | Pseudo | NarL | 1 Response_reg, 1 HTH_LUXR | Development | *SCO4768* | Molle and Buttner [38] |
| *vnz_23115* | MinD | n | n | y | n | Atypical | CheY | 1 Response_reg | minD-like | *SCO5006* | Nguyen *et al.* [39] |
| *vnz_24730* | | y | y | y | y | Typical | CheY | 1 Response_reg | Secondary metabolites and development | *SCO5351* | Lu *et al.* [40] |
| *vnz_25330* | | n | n | n | y | Atypical | NarL | 1 Response_reg, 1 HTH_LUXR | Thiopeptide BGC | – | |
| *vnz_28820* | WhiI | n | y | y | n | Atypical | NarL | 1 Response_reg, 1 HTH_LUXR | | *SCO6029* | Aínsa *et al.* [41] |
| *vnz_29530* | JadR1 | n | y | y | y | Atypical | OmpR | 1 Response_reg, 1 Trans_reg_C | Jadomycin BGC | – | Wang *et al.*[36] |
| *vnz_33595* | | y | y | n | y | Atypical | LytTR | 1 Response_reg, 1 LytTR | | *SCO1220* | |
| *vnz_32025* | | 1/2 | y | y | y | Typical | CheY | 1 Response_reg | | – | |

TCS expansion in *S. coelicolor* has largely occurred though lineage-specific means. This may explain why streptomycete RR effector domains appear to be largely restricted to transcriptional regulation [2]. The gain of new TCSs through HGT is thought to be more likely to preserve the positioning of the genes in their adjacent position or closely within a regulon; however, those gained through duplication events are more likely to cause separations [46]. Most investigated TCSs in *Streptomyces* are involved in secondary metabolite production (Table 4) either directly binding to cluster situated regulators (e.g. AfsQ1) or as global regulators (e.g. PhoP). However, biosynthetic gene clusters (BGCs) do not consistently contain a TCS that autoregulates the biosynthesis of the respective secondary metabolite.

After duplication events, the build-up of mutations and reshuffling events can change the function and recognition of TCSs to generate new TCSs. In addition to reshuffling and

**Table 4.** Two component systems (TCSs) in *Streptomyces venezuelae* NRRL B-65442 predicted with P2RP software Annotations are based on the Pfam database. The transmembrane helices (TM) are predicted by HMMTOP software

| vnz gene number | Name | Conserved | Component | Type | TM | Annotation | Function | SCO homologue | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *vnz_00735* | | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | Under control of the SARP CSR CcaR in the cephamycin C-clavulanic acid BGC | – | Álvarez-Álvarez *et al.* [47] |
| *vnz_00740* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_02225* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | – | |
| *vnz_02230* | | | SK | Classic | 1 | 1 HAMP, 1 HisKA, 1 HATPase_c | | – | |
| *vnz_02755* | | | RR | TrxB | | 1 RR-N_rec, 2 Pyr_redox, Thioredoxin reductase at C terminus | Upregulated in response to pH shock in *S. coelicolor* | *7298* | Kim *et al.* [48] |
| *vnz_02760* | | | SK | Classic | 0 | Possible incomplete, 1 cNMP_binding, 1 HATPase_c | | *7297* | |
| *vnz_03370* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *1070* | |
| *vnz_03375* | | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | *1071* | |
| *vnz_07060* | | yes | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | upregulated in liquid culture in *S. coelicolor* | *1801* | Yague *et al.* [49] |
| *vnz_07065* | | | SK | Classic | 0 | 1 GAF, 1 HisKA_3, 1 HATPase_c | | *1802* | ¨ |
| *vnz_07460* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_07465* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *1260* | |
| *vnz_08725* | MacR | yes | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Activation of actinorhin and repression of aerial hyphae formation in *S. coelicolor* | *2120* | Liu *et al.* 2019[50] |
| *vnz_08730* | MacS | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | | *2121* | |
| *vnz_08930* | | yes | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Not involved in antibiotic production in *S. coelicolor* | *2165* | Yepes *et al.* [51] |
| *vnz_08935* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *2166* | |
| *vnz_09645* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_09650* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *1259* | |
| *vnz_09810* | | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | | *2307* | |
| *vnz_09815* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *2308* | |
| *vnz_10715* | | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | | *3757* | |

Continued

**Table 4.** Continued

| vnz gene number | Name | Conserved | Component | Type | TM | Annotation | Function | SCO homologue | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *vnz_10720* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *3756* | |
| *vnz_10755* | | | SK | Classic | 0 | 1 HAMP, 1 HisKA, 1 HATPase_c | Upregulated in response to pH shock in *S. coelicolor* | *7231* | Kim *et al.* [48] |
| *vnz_10760* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *7232* | |
| *vnz_11720* | | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_11725* | | | SK | Classic | 0 | 1 PAS_4, 1 HisKA, 1 HATPase_c | | – | |
| *vnz_11730* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | – | |
| *vnz_12685* | | | SK | Classic | 3 | 1 HAMP, 1 HisKA,1 HATPase_c | | *2800* | |
| *vnz_12690* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *2801* | |
| *vnz_12785* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Upregulated in response to pH shock in *S. coelicolor* | *4668* | Kim *et al.* [48] |
| *vnz_12790* | | | SK | Classic | 3 | 1 HisKA_3, 1 HATPase_c | | *4667* | |
| *vnz_13520* | MtrB | yes | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | Life cycle and antibiotics | *3012* | Som *et al.* [52] |
| *vnz_13525* | MtrA | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *3013* | |
| *vnz_13755* | DraK | yes | SK | Classic | 2 | 1 HisKA, 1 HATPase_c | Represses and activates secondary metabolites in response to pH stress | *3062* | Yu *et al.* [53], Yeo *et al.* [54] |
| *vnz_13760* | DraR | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *3063* | |
| *vnz_15850* | CseB | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | Envelope stress | *3358* | Paget *et al.* [55] |
| *vnz_15855* | CseC | | SK | Classic | 3 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *3359* | |
| *vnz_15960* | TunR | yes | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Tunicamycin resistance | *3389* | Wyszynski *et al.* [56] |
| *vnz_15965* | TunS | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *3390* | |
| *vnz_16275* | | | SK | Classic | 2 | 1 HisKA_3, 1 HATPase_c | | *3750* | |
| *vnz_16280* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_16750* | | | RR | NarL | 0 | 1 RR-N_rec, 1 HTH_LUXR | | *3638* | |
| *vnz_16755* | | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | | *3639* | |

Continued

**Table 4.** Continued

| vnz gene number | Name | Conserved | Component | Type | TM | Annotation | Function | SCO homologue | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *vnz_16940* | EcrE2 | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Regulation of RED production in *S. coelicolor* | *6422* | Wang *et al.* [57] |
| *vnz_16945* | EcrE1 | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *6421* | |
| *vnz_17085* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Unique in *S. venezuelae* NRRL B-65442 | – | |
| *vnz_17090* | | | SK | Classic | 3 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_17480* | | | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | – | |
| *vnz_17485* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | – | |
| *vnz_17825* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_17830* | | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_18555* | CagR | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | Global regulator of clavulanic acid and primary metabolism in *S. clavuligerus* | *4020* | Song *et al.* [58] |
| *vnz_18560* | CagS | | SK | Classic | 1 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *4021* | Fu *et al.*[59] |
| *vnz_18845* | RagR | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Regulation of development | *4072* | San Paolo *et al.* [60] |
| *vnz_18850* | RagK | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | | *4073* | |
| *vnz_19150* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *4123* | |
| *vnz_19155* | | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | *4124* | |
| *vnz_19330* | CssS | yes | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | Misfolded protein stress | *4155* | Gullón *et al.* [61] |
| *vnz_19335* | CssR | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *4156* | |
| *vnz_19580* | PhoR | yes | SK | Classic | 1 | 1 HisKA, 1 HATPase_c | Phosphate limitation | *4229* | |
| *vnz_19585* | PhoP | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *4230* | |
| *vnz_19880* | SenR | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *4276* | |
| *vnz_19885* | SenS | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | *4275* | |
| *vnz_21250* | AbrC3 | yes | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Antibiotics | *4596* | Rodríguez *et al.* [62] |
| *vnz_21255* | AbrC2 | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *4597* | |
| *vnz_21260* | AbrC1 | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *4598* | |

Continued

**Table 4.** Continued

| vnz gene number | Name | Conserved | Component | Type | TM | Annotation | Function | SCO homologue | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *vnz_22090* | EsrS | yes | SK | Classic | 6 | 1 PspC, 1 HATPase_c | Envelope stress | *4791* | |
| *vnz_22095* | EsrR | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *4792* | |
| *vnz_22600* | AfsQ2 | yes | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | Nitrogen limitation | *4906* | |
| *vnz_22605* | AfsQ1 | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *4907* | |
| *vnz_24545* | | yes | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *5282* | |
| *vnz_24550* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *5283* | |
| *vnz_24860* | ChiR | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Chitin utilisation | *5377* | Tsujibo *et al.* [63] |
| *vnz_24865* | ChiS | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | *5378* | |
| *vnz_25130* | | | RR | CheY | | 1 RR-N_rec | | *5434* | |
| *vnz_25135* | | | SK | Classic | 0 | Probable incomplete, 1 PAS, 1 HATPase_c | | *5435* | |
| *vnz_25235* | | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | Butyrolactone BGC | *7711* | |
| *vnz_25240* | AbsA2 | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *3226* | Brian *et al.* [64] |
| *vnz_26330* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *6667* | |
| *vnz_26335* | | | SK | Classic | 6 | 1 HisKA_3, 1 HATPase_c | Upregulated in response to pH shock in *S. coelicolor* | *6668* | Yague *et al.* [49] |
| *vnz_26705* | OsaC | yes | SK | Hybrid | 0 | 1 HATPase_c_2, 1 PAS, 2 GAF, 1 SpoIIE | Osmotic stress | *5747* | |
| *vnz_26710* | OsaA | | SK | Hybrid | 1 | 11 HAMP, 1 GAF, 1 HisKA, 1 HATPase_c | | *5748* | |
| *vnz_26715* | OsaB | | RR | CheY | | 1 RR-N_rec | | *5749* | |
| *vnz_26805* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Siderophore BGC | – | |
| *vnz_26810* | | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_26890* | GluR | yes | RR | OmpR | | RR-N_rec, 1 Trans_reg_C | Glutamate uptake | *5778* | Li *et al.* [65] |
| *vnz_26895* | GluK | | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *5779* | |
| *vnz_27015* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *2358* | |

Continued

**Table 4.** Continued

| vnz gene number | Name | Conserved | Component | Type | TM | Annotation | Function | SCO homologue | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *vnz_27020* | | | SK | Classic | 2 | 1 HisKA_3, 1 HATPase_c | | *2359* | |
| *vnz_27230* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *5824* | |
| *vnz_27235* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *5825* | |
| *vnz_27250* | | | RR | unclassified | | 1 RR-N_rec, 1 HTH_11 | | *5828* | |
| *vnz_27255* | | | SK | Classic | 1 | Probable incomplete; 1 HATPase_c | | *5829* | |
| *vnz_27390* | CutR | yes | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | Antibiotic production | *5862* | Chang *et al.* [66] |
| *vnz_27395* | CutS | | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *5863* | |
| *vnz_27435* | KdpD | | SK | Classic | 4 | 1 HisKA, 1 HATPase_c | Potassium transport | *5871* | |
| *vnz_27440* | KdpE | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | *5872* | |
| *vnz_28060* | DegS | | SK | Classic | 2 | 1 HAMP, 1 HisKA_3, 1 HATPase_c | Antibiotics and secretion of proteins | *5784* | Rozas *et al.* [61] |
| *vnz_28065* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | | |
| *vnz_28455* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_28460* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_28510* | DegU | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *5785* | |
| *vnz_28515* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_30150* | HrrA | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | Haem-dependent homologue0 of *Corynebacterium diphtheriae* | *1370* | Bibb *et al.* [67] |
| *vnz_30155* | HrrS | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | *1369* | |
| *vnz_30210* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | Homologues in Polyenoyltetramic Acid α-Lipomycin BGC in *S. aureofaciens* Tü117 | *6253* | Bihlmaier *et al.* [68] |
| *vnz_30215* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | *6254* | |
| *vnz_30700* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_30705* | | | SK | Classic | 4 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_32125* | ResD | | RR | OmpR | | RR-N | Homologues from *B. subtilis* possible involvement in anaerobic stress | *3741* | van Keulen *et al.* [69] |
| *vnz_32130* | ResE | | SK | Classic | 3 | 1 HisKA, 1 HATPase_c | | *3740* | |

Continued

**Table 4.** Continued

| vnz gene number | Name | Conserved | Component | Type | TM | Annotation | Function | SCO homologue | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *vnz_33685* | VanR | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | Vancomycin resistance but no *vanJ*, *vanK* or *vanHAX* genes in *S. venezuelae* | *3590* | Hong *et al.* [70] |
| *vnz_33690* | VanS | | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | *3589* | |
| *vnz_35360* | | | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | – | |
| *vnz_35365* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | – | |
| *vnz_36030* | | | SK | Classic | 5 | 1 HisKA_3, 1 HATPase_c | | – | |
| *vnz_36035* | | | RR | NarL | | 1 RR-N_rec, 1 HTH_LUXR | | – | |
| *vnz_36355* | | | RR | OmpR | | 1 RR-N_rec, 1 Trans_reg_C | | – | |
| *vnz_36360* | | | SK | Classic | 2 | 1 HAMP, 1 HisKA, 1 HATPase_c | | – | |

duplication, hybridization can also occur, resulting in hybrid TCSs. This can occur at stop codons or independently of this but both reshuffling and hybridization are very rare, especially in *Streptomyces* [45]. In the process of mutation build-up, the two components may gain or lose functions and pseudogenes may be formed or it may result in multiple RRs being activated by a single SK or multiple SKs regulating a single RR (branched pathways). The regulatory circuit provided by a TCS enables the coupling of the target regulon genes to each system via the RR. The eco-evolutionary processes that determine how these regulons differ in closely related species depends upon the presence of orthologous genes and may reflect horizontal gene transfer events or the occupation of different environmental niches. This is an area of research that will develop given the availability of genetic tools to dissect gene function in these organisms [71].

## FUNCTION AND PHENOTYPES OF *STREPTOMYCES* TCSs

### The conserved TCSs

#### MacRS: vnz_08725/vnz_08730

The function of the novel TCS MacRS (SCO2120/21) was discovered by transposon mutagenesis in *S. coelicolor* [34]. Further studies showed that deletion of *macRS* blocked production of ACT and enhanced formation of aerial mycelium. The NarL-type RR MacR regulates the expression of eight target genes encoding the lipoproteins SCO0607 and SCO7460 and the integral membrane proteins SCO1700, SCO4011 (MmpC), SCO4225, SCO4924 (MmpB), SCO6728 (MmpA) and SCO7613. The effect of MacRS on ACT production is probably indirect because MacR did not bind to the ACT BGC in the reported chromatin immunoprecipitation and sequencing (ChIP-seq) experiment. Interestingly, the deletion of *macRS* caused rapid aerial mycelium formation and the authors speculated that this phenotype could be due to the involvement of the MacRS target genes in membrane integrity and/or other membrane-associated activities [50]. MacRS homologues from *S. venezuelae* and *S. avermitilis* complemented the deletion mutant, which indicates that MacRS function is conserved throughout *Streptomyces*. The signal sensed by MacS is unknown.

#### MtrAB: vnz_13525/vnz_13520

MtrAB is highly conserved throughout the phylum *Actinobacteria* and has been characterised in *Mycobacterium* species, *Corynebacterium glutamicum*, *S. coelicolor* and *S. venezuelae*. It is the only essential TCS in *Mycobacterium tuberculosis* but is not essential in other tested mycobacteria or in *C. glutamicum* [72–74]. MtrA (*M. tuberculosis* regulator A) is the RR in this system and MtrB the SK. $MtrA_{MT}$ (from *M. tuberculosis*) regulates expression of *dnaA* and *dnaN*, encoding the DNA replication initiator and DNA polymerase III stabiliing protein, respectively, and binds to the *oriC* region between these two genes, sequestering the chromosome origin to prevent further DNA replication. Direct interaction of MtrA with the DnaA protein was also demonstrated and may facilitate the binding of MtrA-P (phosphorylated MtrA) to *ori*C. This occurs in the post-replication period of the cell [75]. MtrA is thought to stop further DNA replication and move the cycle towards cell division. $MtrB_{MT}$ localizes to the cell membrane in an FtsZ (cell division initiator)-dependent but phosphorylation-independent manner and has also been shown to interact with Wag31 (DivIVA), FtsI and PknA/PknB (protein kinase A and B; Ser/Thr kinases), which are all involved in controlling cell division and shape [76–78]. There is a third component, the genetically linked lipoprotein, LpqB, which has been shown to interact with the sensor domain of *M. smegmatis* MtrB

to modulate signalling [79]. An *M. smegmatis ΔlpqB* mutant demonstrated multidrug sensitivity and *Streptomyces*-like filamentous growth with polyploidy. A similar phenotype was also exhibited by an *mtrA* transposon insertion inactive mutant in *M. smegmatis* [79]. The lipoprotein *lpqB* is highly conserved as the third gene in the *mtrAB-lpqB* operon and s described as a signature protein for the phylum *Actinobacteria* [80]. This may indicate its importance for the effective functioning of MtrAB TCS signalling.

More recently, MtrA has been studied in *Streptomyces* species and has been found to be a regulator of antibiotic production. Deletion of *mtrB* induced a >30-fold increase in chloramphenicol production in *S. venezuelae* and MtrA binds directly within the chloramphenicol cluster [52]. Further analysis of MtrA gene targets in *S. coelicolor* using ChIP-seq shows that it binds to sites in BCGs including upstream of *actII-orf4* and *redZ* [52], suggesting that MtrA may also modulate ACT and RED production. Consistent with this, an *S. coelicolor ΔmtrB* mutant over-produced both antibiotics. The fact that MtrA is essential in the pathogen *M. tuberculosis* and regulates antibiotic production in *Streptomyces* makes it a very attractive TCS for further study, whether as a target for antibiotic therapy or to activate antibiotic production. It is particularly interesting that $MtrA_{TB}$ was able to switch on chloramphenicol production in *S. venezuelae* [52].

In addition to the regulation of antibiotics in *Streptomyces* species, MtrAB has been found to play a critical role in the regulation of development. Deletion of *mtrA* resulted in a conditional bald phenotype in *S. coelicolor*, *S. lividans* and *S. venezuelae* that was dependent on the growth medium, in a similar manner to deletion mutants of *sapB*, or chaplins or *bld* genes [81]. Moreover in *S. coelicolor*, MtrA is involved in nutrient uptake, regulating the Mce sterol uptake system which is important for the colonization of plant roots [82].

#### DraRK: vnz_13760/vnz_13755

The function of this highly conserved TCS was investigated in *S. coelicolor* [53]. Deletion of *draRK* led to the conditional repression of ACT biosynthesis but increased RED production on minimal medium supplemented with a high concentration of a nitrogen source. The same mutant overproduced the yellow-pigmented type-I polyketide Cpk on minimal medium supplemented with glutamate. The phosphorylated RR DraR binds to the promoter of *actII-ORF4* and *kasO* and probably directly regulates the production of these antibiotics. However, DraR does not bind to *redZ* and therefore the effect of DraRK on increased production of RED is likely to be indirect. Additionally, DraRK represses the growth of *S. coelicolor* under high glutamine and glutamate conditions, probably by regulating the transcription of genes involved in primary metabolism *[SCO6748* a putative enoyl-CoA hydratase (ECH), *SCO2014*, encoding a pyruvate kinase and *gltB*, encoding a glutamate synthase] [53].

Consistent with our analysis, Yu *et al.* [53] stated that DraRK is highly conserved throughout the genus *Streptomyces*. Furthermore, the DNA binding domain is nearly identical in *S. coelicolor*, *S. griseus*, *S. venezuelae*, *S. clavuligerus*, *S. scabies* and *S. avermitilis*. The functional conservation was demonstrated by deleting *draKR* in *S. avermitilis*. This strain showed increased production of avermectin B1a and slightly reduced oligomycin A production. Electrophoretic mobility shift assays (EMSAs) using the regulatory gene promoters of the avermectin and oligomycin cluster indicated that DraR directly regulates production of oligomycin A but the repression of avermectin biosynthesis is indirect [53].

#### TunRS: vnz_15960/vnz_15965

The role of this highly conserved TCS remains elusive but it is always genetically linked to a *tmrB*-like gene (*vnz_15955*). The membrane-associated ATP-binding protein TmrB is responsible for resistance to the antibiotic tunicamycin in *Bacillus subtilis* either by blocking passive diffusion or by acting as a tunicamycin efflux pump [83]. Tunicamycins are fatty acyl nucleoside antibiotics originally isolated in 1971 from *Streptomyces lysosuperificus* and inhibit bacterial cell wall biosynthesis by preventing the formation of undecaprenyl-pyrophosphoryl-*N*-acetylmuramoyl pentapeptide, a key peptidoglycan precursor, via MraY. Not all species of *Streptomyces* are capable of producing tunicamycin and the *tmrB* homologue is not located within the tunicamycin BGC. Thus, it is possible that it has another role beyond protection from self-made antibiotics [56]. The *tmrB* homologue *SCO3388* shares 38.9 % identity with TmrB and is likely to be under the control of the TunRS TCS with a possible operon promoter region detected by differential RNA-seq [84]. A *SCO3388::tsr* mutant was defective in growth and sporulation on R4 agar and is suggested to be a spore cell-wall-remodelling factor with TmrB-like proteins playing a role in streptomycete cell wall metabolism [85]. Deletion of *tunRS* in *S. venezuelae* increases tunicamycin sensitivity relative to the wild-type but this may be due to polar effects on the downstream *tmrB* gene (our unpublished data).

#### CssRS: vnz_19335/vnz_19330

*Bacillus subtilis* copes with misfolded protein stress by activating the expression of HtrA-like proteases via the TCS CssRS [86]. This TCS was identified by searching the genome sequence of *S. coelicolor* [3] for the *B. subtilis* CssRS homologue. Homology was experimentally confirmed via deletion mutants. Removal of CssRS in *S. lividans* leads to the accumulation of misfolded proteins outside the cell and it was shown that expression of the HtrA-like proteases is downregulated in the *cssR* and *cssS* mutants [61]. The conservation of function in the very distant related bacteria *B. subtilis* and *S. lividans* indicates an overall conservation of this TSC.

#### PhoPR: vnz_19585/19585

Perhaps the best studied *Streptomyces* TCS, and the only one with a well-defined activating signal is PhoPR. It is not possible to cover the entirety of PhoPR research in a few short paragraphs, and thus what follows is a brief summary.

Inorganic phosphate is a vitally important macronutrient for biological life, necessary for genetic material (e.g. DNA,

RNA), energy transfer (e.g. ATP, GTP) and regulatory signalling, to name just a few of its roles. PhoP is a master regulator which governs both primary and secondary metabolism in streptomycetes [87]. It forms an operon with genes encoding a sensor kinase, PhoR, and another regulatory protein PhoU [25, 88]. PhoP binds to defined promoter sequences called PHO boxes which help define the PhoP regulon. These are 11 nucleotide direct repeat units (DRUs) with a consensus sequence of GTTCACC each recognized by a single PhoP monomer with a minimum of two DRUs required for PhoP-DNA binding. In *Escherichia coli* the DNA-binding domain (DBD) is permanently exposed, allowing efficient interaction with PHO boxes. This is in contrast to PhoP of *S. coelicolor,* which requires phosphorylation by PhoR to expose the DBD, suggesting a more tightly controlled response to phosphate [87, 88]. Under phosphate-limiting conditions the PhoP regulon works to scavenge phosphate from both the extracellular and the intracellular environments whilst also delaying or completely repressing morphological differentiation [87]. In *S. coelicolor* this includes expression of the alkaline phosphatase PhoA and a phospholipase PhoD to scavenge inorganic phosphate from phosphorylated proteins alongside a phytase (*vnz_00450*) to hydrolase phytate. Transport systems such as the high-affinity phosphate-specific transporter *pstSCAB* are also highly upregulated by PhoP when phosphate levels are low [89–91]. Glycerophosphodiesters from membrane phophlipids can be hydrolysed by glycerophosphodiester phosphodiesterases (GCPDs) such as *glpQ1* and *glpQ2* to form an alcohol and sn-glycerol-3-phosphate, a cellular phosphate source. PhoP directly activates both *glpQ1* and *2* [92, 93]. It also becomes imperative to store cellular phosphate and this can be done via a reversible polymerization catalysed by the polyphosphate kinase *ppK*, also activated by PhoP [94]. Finally, there are morphological differentiation genes controlled by PhoP, including five *bld* genes (*bldA/C/D/K/M*) and two ECF sigma-factors *sigU* and *chpC*, all of which are repressed to delay development whilst overcoming the phosphate limitation [95]. Little is known about the regulator PhoU except that expression of *phoU* is dependent on PhoP activation and it acts to negatively modulate *pho* regulon expression, forming a self-regulation feedback mechanism [96].

In general, when phosphate levels are high secondary metabolism is repressed. Deletion of *phoP* in *S. coelicolor* leads to varied metabolite biosynthesis dependant on the media with hastened ACT production in R5 but repressed ACT and undecylprodigiosin in defined starch media. PhoP acts at a higher level, repressing genes such as *afsS*: a positive regulator of *actII-ORF4* and *redZ* [97, 98]. PhoP also represses the expression of *scbR*, in turn repressing gamma-butyrolactones leading to downstream repression of coelimycin P1 biosynthesis as well as *cdaR*, and thus repressing CDA biosynthesis [87]. It has also been shown that *phoP* disruption increases production of the macrolide antifungal pimaricin in *Streptomyces natalensis*, a species that is extremely sensitive to media phosphate concentration, although the *pim* biosynthetic genes contain no PHO boxes [99].

**AbrC1/2/3: vnz_21260/vnz_21255/vnz_21250**

The branched TCS encoded by the *abrC123* operon encodes two histidine kinases (AbrC1 and AbrC2) and one RR (AbrC3) in *S. coelicolor*. Deletion of the *abrC123* genes, or overexpression on a high-copy-number plasmid, resulted in an *S. coelicolor* strain with reduced ability to produce the antibiotics ACT, RED and CDA. These strains also displayed decreased differentiation rates [51]. The aberration of antibiotic production has been linked to *abrC1* via a single deletion mutant. The SK genes are separated by a 114 bp region and DNA regions of this size can contain promoter regions, although quantitative reverse transcriptase (qRT)-PCR experiments did not detect any differential expression and *abrC12* was consistently expressed as a single transcript [62]. Differential RNA-seq of *S. coelicolor* A3(2) confirmed a single transcriptional start site preceeding *abrC3* (*SCO4596*) and no others within the operon [100]. By contrast, the RR *abrC3* was found to be expressed independently and regulates its own promoter but not that of abrC2 [62]. The true number of promoters present remains undefined.

DNA microarray analysis and qRT-PCR revealed that 16 of the 32 significantly downregulated genes in an Δ*abrC3* mutant corresponded to the entire *act* BGC. Chromatin immunoprecipitation followed by microarray (ChIP-chip) analysis of AbrC3 showed binding of the regulator to its own promoter, a mechanism which is not uncommon in RRs, and that of the *act* cluster-situated regulator *actII-ORF4* [101].

**EsrSR: vnz_22090/vnz_22095**

This is homologous to a TCS characterized in *Corynebacterium glutamicum* and named EsrSR (envelope stress response) when it was found to be homologous to the cell envelope stress-sensing three-component system LiaFSR from *B. subtilis*. One major system induced by cytoplasmic membrane disruption is the phage shock protein (Psp) system, found widely distributed through bacteria as a stress response mechanism, and LiaFSR is a three-component system which regulates this Psp response [102, 103]. The *esrSR* operon is divergently transcribed with the third component, the integral membrane protein EsrI. Both EsrS and EsrI contain PspC domains, important for the Psp response via the PspC protein, and the same holds true for the *S. venezuelae* homologues (EsrS: vnz_22090 and EsrI: vnz_20095) [103]. Interestingly, streptomycetes do not seem to encode any part of the Psp system other than PspA, which is an essential protein for growth and survival under membrane attack stress conditions [104]. Transcription of *esrISR* in *C. glutamicum* can be induced with cell-wall active antibiotics such as bacitracin and vancomycin as was demonstrated using *β*-galactosidase reporter assays with the *esrI* and *esrSR* promoter regions. EsrR binding domains were identified and subsequently confirmed using EMSA experiments. No *β*-galactosidase activity was observed when the same experiment was performed using an *esrSR* deletion mutant, and thus transcription is reliant on EsrSR. However, in the *esrI* deletion mutant there was a two-fold increase in the transcription of *esrSR*, suggesting EsrI may act as a repressor of *esrSR* transcription. Whilst

EsrSR showed no role in vancomycin resistance, deletion of *esrSR* resulted in a strain that was much more susceptible to bacitracin. This could be complemented with a plasmid carrying the *esrSR* genes [103]. Two EsrR target genes in *C. glutamicum* are ABC transport systems, and these systems have been previously been shown to play an important role in bacitracin resistance in *S. coelicolor* [105]. Thus, it appears that the EsrISR system senses and responds to cell envelope stress, orchestrating a broad response including induction of ABC transporter systems [103].

**AfsQ1/2: vnz_22605/vnz_22600**

AfsQ1/2 TCS is co-encoded with a partner lipoprotein AfsQ3, just like the MtrAB, EsrSR and CseBC TCSs. The operon encoding AfsQ1/2/3 is divergently transcribed from a gene encoding the ECF sigma factor SigQ and this is reminiscent of CseABC, which are co-encoded in an operon with the ECF sigma factor SigE [106, 107]. AfsQ1 was first described after heterologous expression in *S. lividans* from *S. coelicolor* and designated *afs* (A-factor synthesis) as it regulated A-factor production. Mapping of the sequence subsequently revealed the SK gene *afsQ2*. Phenotypically it was shown that expression of *afsQ1* activated ACT and RED production in *S. lividans* but disruption in *S. coelicolor* seemed to have no relatable effect [108]. It was later shown that on minimal media supplemented with glutamate an Δ*afsQ1/2* mutation in *S. coelicolor* repressed ACT, RED and CDA alongside a morphological change: increased aerial hyphae growth. However, disruption of *sigQ* proved to have the opposite effect, suggesting an antagonistic activity. RT-PCR data showed that expression of *sigQ* was significantly reduced in the *afsQ1/2* mutant but transcription of *afsQ1/2* was unchanged in the *sigQ* mutant. Consistent with the phenotypic appearance, expression of *actII-ORF4*, *redD* and *cdaR* were all reduced in the *afsQ1/2* mutant and increased in the *sigQ* mutant [109]. Interestingly it was shown that AfsQ2 does not seem to detect glutamine or glutamate even though the TCS seems to respond to glutamate supplementation. In the presence of excess nitrogen AfsQ1 will modulate nitrogen assimilation by competing with the orphan RR GlnR for the promoter regions of *glnA* (glutamine synthase) and *nirB* (nitrogen reductase). This highlights possible cross-talk between AfsQ1/2 and GlnR. Elucidation of the AfsQ1 binding sequence (GTnAC-$n_6$-GTnAC) allowed the mapping of the regulon to the genome and revealed a global range. Putative binding sites include the developmental genes *bldM* and *whiD* [106]. Heterologous constitutive expression of a gain-of-function phosphomimetic mutant of *afsQ1* in a range of wild streptomycete isolates led to lethality in 8/11 strains whilst the wild-type gene was not lethal in any of the 11 strains. An alternative thiostrepton-inducible promoter was used and, through titration, non-lethal levels of induction could be achieved. This led to the isolation of the novel class-I lasso peptide siamycin-I from WAC00263, which showed promising activity against both methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). When aligned to *S. nodosus* the probable siamycin-I cluster contains a TCS (SNOD34175-SK, SNOD34170-RR [110]).

**OsaABC: vnz_26710/vnz_26715/vnz_26705**

The function of this TCS was discovered by transposon mutagenesis in *S. coelicolor* and *S. lividans* [28]. Deletion of the RR *osaB* resulted in strains unable to form aerial hyphae under osmotic stress. Additionally, the *osaB* mutant produces three to five times more actinorhodin and undecylprodigiosin in *S. coelicolor*. Surprisingly, the hybrid SK OsaA does not seem to be essential for osmoadaptation, as reflected in a much less severe phenotype of delayed osmoadaptation and development compared to the *osaA* mutant. The increased production of secondary metabolites in the deletion mutants is most likely caused by the disruption of the complex osmotic stress response rather than direct regulation of antibiotic synthesis. The authors speculate that the phosphorylation of OsaA could be controlled by a second hybrid orphan SK SCO7327 (vnz_01755) [28]. Also, the downstream RR and SK are not transcriptionally coupled, underlining the independence of the RR which interacts with other proteins rather than DNA due to its atypical coiled-coil output domain. The hybrid SK OsaC is divergently transcribed from *osaA*. This regulatory protein consists of SK kinase components (HATPase_c_2, PAS, 2 GAF domains) whereas the HATPase domain shares homology with RsbW-like anti-sigma factors. OsaC is required to return *osaB* and *sigB* expression back to constitutive levels after osmotic stress [111]. Disruption of *osaB* or *osaC* in the industrial strain *S. avermitilis* led to increased production of oligomycin and avermectin and lack of aerial hyphae formation under osmotic stress [112]. This indicates that the involvement of OsaABC in the regulation of antibiotic production in response to osmotic stress is conserved throughout *Streptomyces*.

**GluRK: vnz_26890/vnz_26895**

The GluRK TCS can be found located divergently from the glutamate uptake-encoding operon *gluABCD*. This genomic organization is highly conserved and widely distributed throughout *Actinobacteria*. Interestingly there is no GluRK homologue in *C. glutamicum*, the industrial producer of glutamate, even though it encodes the *gluABCD* uptake system. The operon encodes ATP and glutamate binding proteins (GluA and GluB respectively) and two glutamate permeases (GluC and GluD). RT-PCR and EMSA of *S. coelicolor* Δ*gluRK* and purified $His_6$-GluR protein confirmed direct activation of the transcription of the *gluABCD* operon. GluK was subsequently shown to be a glutamate sensor with biolayer interferometry (BLI). The extracellular domain of the sensor kinase GluK was his-tagged, immobilized on an Ni-NTA sensor and analysed for glutamate dissociation where it showed a significant shift (0.44 nm) whilst no such shift was observed for the glutamine control. When grown on complex media such as MS and R2YE there was no distinct phenotype of *S. coelicolor* Δ*gluRK*. However, on MM supplemented with low (10 mM) glutamate, the mutant strain had reduced RED and increased ACT production compared to the M145 wild-type strain. Under high (75 mM) glutamate supplementation the mutant had reduced yCPK and increased ACT alongside significant growth impairment. This regulation is likely to be indirect as EMSAs revealed $His_6$-GluR could not bind to

*actII-ORF4, redZ/red* and *kasO*, all of which are key regulators of ACT, RED and yCPK biosynthesis, respectively [65].

#### CutRS: vnz_27390/vnz_27395

Discovered in *Streptomyces lividans* in 1991, CutRS was the first TCS to be described in the genus *Streptomyces*. Originally, and erroneously as admitted by the authors, it was thought to be involved in the regulation of copper transport/metabolism and supressed melanin production *melC1* mutations [113]. Deletion of *cutRS* from *S. lividans* TK64 caused accelerated and increased production of ACT. The mutants also displayed a premature onset of ACT from 7 days to 3–4 days on solid media equivalent to a 15 h acceleration in liquid media. These could be complemented by the introduction of *cutR*. Temporal transcriptional analysis of *S. lividans* TK64 in liquid cultures detected *cutRS* transcripts during transition (19 h) and onset (40 h) phases but was unable to detect transcripts during the exponential phase. Consequently, actinorhodin biosynthesis (*act*) repression by *cutRS* mRNA lasted approximately 21 h after transcription [66].

### Other TCSs of interest

#### CseBC: vnz_15850/vnz_15855

The ECF sigma factor, $\sigma^E$, is encoded by the gene *sigE* and plays a role in stabilizing the cell envelope in *S. coelicolor*, with null mutants becoming more sensitive to cell wall lytic enzymes [55, 107]. The CseBC TCS is named for its control of *sigE* (control of *sigE*) with null mutants displaying the same phenotype as the *sigE* null mutant. It is encoded downstream of *sigE* along with *cseA*, a gene encoding a lipoprotein. Determining the function of TCS-associated lipoproteins has historically proven to be challenging, although it is clear that loss of CseA from the cytoplasmic cell membrane induces upregulation of the *sigE* promoter [114]. S1 nuclease mapping of the *sigE* promoter showed transcription was reliant on the presence of the response regulator CseB [115]. Further studies, using a kanamycin-resistance reporter gene fused to the promoter of the *sigE* operon, were used to investigate the role of CseBC in responding to various antibiotics. This was in response to work done in *B. subtilis* where the $\sigma^W$ regulon was induced in response to cell-wall-specific antibiotics [116, 117]. In *S. coelicolor* the *sigE* promoter was transiently induced by the addition of the glycopeptide antibiotic vancomycin, increasing six-fold 30 min after exposure to vancomycin at 10 μg $ml^{-1}$ but subsequently reducing to uninduced levels by 90 min. It seems likely that CseBC is detecting and responding to cell wall disruption, specifically to an intermediate in peptidoglycan degradation/biosynthesis or a physical characteristic of cell wall disruption [117].

#### EcrE1/2: vnz_16945/vnz_16940

Named EcrE1/2 (vnz_16945/16940) due to having expression coordinated with RED from DNA microarrays in *Streptomyces coelicolor* there were in fact two TCSs found to be expressed, EcrA1/2 being the other but is not present in *S. venezuelae* or many other streptomycetes [118]. The SKs EcrE1 and EcrA2 share 33.33 % amino acid identity whilst the RRs EcrE2 and EcrA1 share 56.46 %. Disruption of *ecrE1/2* in *S. coelicolor* revealed no morphological or ACT production variation from the wild-type although RED production was shown to be ~40 % reduced. Northern blotting confirmed that transcription of the transcriptional regulator genes *redD* and *redZ* were markedly reduced in the *ecrE1/2* disruption mutant compared to the wild-type control, suggesting that EcrE1/2 plays a role in the transcriptional activation of RED biosynthesis [57]. EcrA1/2 were previously shown to be playing a similar role in *S. coelicolor*, reducing RED production by 60 % and displaying no difference in morphology or ACT production [118]. No work has yet been done to investigate the interplay between two analogous TCSs. It is possible that their activities are interchangeable, detecting different signals but resulting in the same response: positively regulating RED biosynthesis. However, the presence of only EcrE1/2 in a more diverse range of streptomycetes suggests it may play a more important role.

#### CagRS: vnz_18555/vnz_18560

Clavulanic acid (CA) is a potent *β*-lactamase inhibitor used in the clinical treatment of *β*-lactam-resistant microbial infections. *Streptomyces clavuligerus* is an industrialy important producer of CA [119]. Originally designated *orf22* (SK) and *orf23* (RR), this TCS was found adjacent to the CA BGC [58]. These were later renamed *cagS* and *cagR* respectively [59]. Disruption of *cagS* slowed growth and sporulation on/in solid and liquid media and decreased CA production whilst the opposite proved true during overexpression of *cagS* [58]. Later investigation revealed *cagRS* as a global regulator. ChIP-seq revealed CagR primarily regulates genes involved in CA biosynthesis, fatty acid degradation, arginine metabolism and glycerol-3-phosphate production. The latter two are important CA biosynthesis precursors whilst it was shown that CagR could bind the promoter region of the γ-butyrolactone biosynthesis protein *BB341_RS25520 (avaA2)* through which CA production can also be regulated, highlighting a complex and multiplex control of CA production at various metabolic levels [59].

#### RagKR: vnz_18850/vnz_18845

This TCS was identified using DNA microarrays as being part of the operon *ragABKR* regulated by the orphan RR RamR, thus named RagK and RagR (*ramR*-activated genes). Unlike the *ramR* mutant, the *S. coelicolor ΔragABKR* mutant did not display a *bld* phenotype, although there was an obvious and significant delay in sporulation on R2YE solid media. It also presented with long, straight, undifferentiated aerial hyphae lacking commitment to sporulation, similar to the *whiG* developmental mutant. Overexpression of *ragK* and *ragR* in developmental mutants showed that both were essential for recovery of *ramR*, *ramS* or *bldK* mutant phenotypes. In addition, complementation was possible with RagR(D53E), a constitutively active version of RagR. The *ramR* mutant is also unable to synthesize SapB, a small secreted surfactant which can act at the air–water interface to release surface tension [60, 120]. SapB synthesis could be restored by RagR(D53E), suggesting that *ragAB* are not required for SapB production

and it is in fact *ragR* regulon elements which are key. Interestingly, it has long been thought that SapB is the ultimate inducer of aerial hyphae formation following an extracellular signalling cascade as there is impaired SapB production in all the *bld* mutants. Whilst SapB synthesis could not be restored via the phosphorylation-negative RagR(D53N) construct, it was still able to rescue the *ram/bld* developmental mutants, suggesting a SapB-independent induction of morphogenesis. This diffusible extracellular signalling factor could be similar to the γ-butyrolactone A-factor of *S. grisues* or paramycin in *S. alboniger* [60].

#### SenSR: vnz_19885/vnz_19880

SenSR was first described in 2005 in *Streptomyces reticuli,* a cellulose-degrading species of *Streptomcyes* which exhibits catalase and manganese-peroxidase activity through the mycelia-associated haem-containing enzyme CpeB (vnz_36165) [121]. This peroxidase activity is controlled by the FurS protein, which binds to its cognate operator upstream of the *furS* gene (*vnz_36170*) when in its thiol state [122]. Using a *senSR* disruption mutant in *S. reticuli* and *S. lividans* transformants it was shown that SenSR reduces *cpeB, furS* transcripts and that of a downstream gene, *hbpS* (*vnz_29470*). The disruption mutant exhibited increased sensitivity to haemin, an iron-containing porphyrin with antibacterial activity, and plumbagin, another redox-cycling compound known to generate reactive oxygen species (ROS). It is thought that SenSR could be involved in the sensing of redox-triggered event signals. Sensitivity to $H_2O_2$ and diamide was increased in the *senSR* mutant but relatively less than the sensitivity to haemin and porphyrin (26 and 12 % compared to 66 and 41 % respectively). Further protein–protein interaction studies revealed that SenS interacted with an extracellular protein revealed to be HbpS [123]. This haem-binding protein was proposed to be of importance in the SenS signal-transduction cascade leading to increased CpeB synthesis as *S. reticuli* mutants lacking active HpbS had reduced CpeB levels [124]. Further studies propose the HpbS–SenS complex acts by HbpS binding and degrading haem in an $H_2O_2$-dependent manner consequently displaying iron on its surface, disrupting the HpbS–SenS extracellular interaction and triggering the signalling cascade [125]. In addition, the ability of HbpS to oxidize ferrous iron ($Fe^{2+}$) to ferric iron ($Fe^{3+}$) prevents the formation of the highly reactive products of the Fenton reaction, protecting *S. reticuli* from hazardous haem concentrations via iron sequestration [126].

#### ChiRS: vnz_24860/vnz_24865

The polysaccharide of *N*-acetylglucosamine, chitin, is the most abundant natural biopolymer derived from the exoskeletons of crustaceans, fungi and insects, and is highly insoluble [127]. Streptomycetes rely significantly on polymers such as chitin, cellulose and xylan as food sources in their ecological niches, be that soil or sea, due to their worldwide abundance [128]. Chitinases hydrolyse the $\beta$1,4 linkage to produce chitooligosaccharides which are then converted to GlcNAc by *N*-acetylglucosaminidase. These can be used as a carbon and nitrogen source by the cell. ChiRS was first discovered in *Streptomyces thermoviolaceus* OPC-520 in 1999 as a TCS found upstream of the chitinase locus (*chi40*). Introduction of this *chiRS* operon into *S. lividans* 66 resulted in vastly increased production of Chi40 when induced with chitobiose and chitin whilst GlcNAc had no effect [63]. Follow-up experiments including EMSAs could not confirm the specificity of ChiR for the chitinase *chiC* promoter in *S. coelicolor,* but S1-nuclease mapping showed a significant decrease in promoter RNA-protected fragment between M145 and a *chiR::tsr* disruption mutant, suggesting that *chiR,* at least indirectly, influenced the regulation of *chiC* [129]. It is suggested, in corroboration with evidence from the chitinolytic system in *Vibrio* species, that in *S. coelicolor*, DasA, a chitobioase-binding protein, binds to the extracellular domain of ChiS, inactivating it and repressing the chitinolytic genes. The presence of extracellular chitin and thus $(GlcNAc)_2$ creates competition for DasA, and ChiS cannot bind DasA-$(GlcNAc)_2$, resulting in the chitinolytic phenotype via ChiR. Deletion of DasA results in a vastly increased chitinolytic activity which may confirm the ability of DasA to interfere with the start of the ChiS signalling cascade [128].

#### AbsA1/2: vnz_25235/vnz_25240

Identified during a screen for global antibiotic regulators in *S. coelicolor* (controlling the four antibiotics ACT, RED, CDA and MMY), four mutants were found to block antibiotic production without adverse morphological development beyond the lack of pigmentation you would expect from disrupted antibiotic production. These mutations were located in the *absA* locus, which contains the SK *absA1* and its cognate RR *absA2* [64]. Further investigation revealed that all the mutations were located in the histidine kinase sensor-transmitter domain. These strains could spontaneously revert to a pigmented, antibiotic phenotype via *sab* (suppressor of *abs*) mutations. A second phenotype (type-II) was described, also pigmented but with 1 day earlier expression of ACT and RED, these were known as Pha (precocious hyperproduction of antibiotics [130]). In *S. coelicolor* the *absA1/2* operon is located within the CDA biosynthetic gene cluster. In *S. venezuelae* only a homologe of the RR *absA2* is present, located within a γ-butyrolactone (GBL) BCG. These are used as auto-inducers, intraspecies quorum-sensing signalling molecules. Deletion of GBL genes in *Streptomyces chattanoogensis* led to abolishment of morphological differentiation and antibiotic production on solid media. Control of GBL biosynthesis is also critical for the correct timing of metabolic switch events [131].

#### KdpDE: vnz_27435/vnz_27440

KdpDE is a homeostasis regulating TCS most extensively studied in *E. coli* with homologous systems found almost ubiquitously among bacteria. Potassium is the major intracellular cation and, whilst essential for life, it is also key in regulating cellular electrolyte and fluid balance [132]. In *E. coli kdpDE* are part of the *kdpFABCDE* operon where *kdpFABC* encode a P-type ATPase: a high-affinity $K^+$ transporter. KdpD is an internally orientated SK with four transmembrane domains forming two very short extracellular loops. It senses changes

in cytoplasmic $K^+$ and ATP concentration as well as osmolarity with a C-terminal $K^+$ sensor [133, 134]. KdpA is a unique subunit of the ATPase in *E. coli* with no other similar units present in any other P-type ATPase. It does, however, share structural and functional homology to KcsA, an MPM-type potassium channel found in a few streptomycetes including *S. lividans* [135]. Our analysis found three streptomycetes that do not contain a KdpDE homologue, raising the question of how they sense and regulate $K^+$ concentration. It is conceivable that systems such as KcsA make *kdpFABCDE* redundant and are regulated by an alternative TCS. This TCS is also a key adaptive regulation system for bacteria virulence with a KdpDE deletion in *M. tuberculosis* displaying hypervirulence in mice. Similar effects have been observed in *E. coli* and *Staphylococcus aureus* [136]. In *Mycobacterium* KdpE binds to an A-rich 22 bp promoter sequence with much greater affinity when phosphorylated. In *M. tuberculosis* KdpD interacts with two lipoproteins, LprJ and LprF, which probably act as ligand-binding proteins or are involved in the phosphor-relay system [137, 138].

#### HrrAS: vnz_30150/vnz_30155

Little is known about *vnz_30150/55,* although it shares the highest homology with *DIP2267/8* from the strict human pathogen *Corynebacterium diphtheriae* which encodes a TCS named HrrAS [67]. In this organism the haem oxygenase (HmuO) is key for iron uptake and utilization in the infectious environment, where iron is scarce. This is activated by two TCSs: ChrAS (no clear homologue in *S. venezuelae*) and HrrAS with crosstalk also able to counteract deletions in single KO strains [67]. Comparative transcriptome analysis alongside DNA-protein interaction assays revealed that haem-containing subunits of the respiratory chain such as *ctaD* and the *ctaE-qcrCAB* operon are transcriptionally activated by HrrA. Conversely, HrrA represses most of the genes involved in haem biosynthesis, including glutamyl-tRNA reductase (*hemA*), uroporphyrinogen decarboxylase (*hemE*) and ferrochetolase (*hemH*) [139]. Homologues of these in *S. venezuelae* are *vnz_15635*, *vnz_28830* and *vnz_27380,* respectively. The *hrrA* deletion mutant displayed a depleted growth rate on solid and in liquid cultures containing haem because of the reduction in activation of *hmuO* [139]. HrrSA and ChrAS have both been shown to play crucial roles in the detection and regulation of haem utilization but they appear to interfere in each other's activities with both HrrA and ChrA directly repressing the transcription of the *chrSA* operon and *hrrA* [140].

#### VanRS: vnz_33685/vnz_33690

Vancomycin is a glycopeptide-class antibiotic whose resistance can be found throughout a wide diversity of microorganisms including human pathogens such as *Staphylococcus aureus* and actinomycetes including *S. coelicolor*. Like many other glycopeptide antibiotics, vancomycin inhibits cell wall biosynthesis by binding to extracytoplasmic lipid-attached peptidoglycan precursors via their D-Ala–D-Ala terminus. Resistance arises through two main mechanisms: either the alteration of D-Ala–D-Ala to D-Ala–D-Lac or to D-Ala–D-Aer. Unlike other glycopeptide-resistant actinomycetes such as *Streptomyces toyocaensis* and *Actinoplanes teichomyceticus, S. coelicolor* does not produce a glycopeptide itself [141]. In *S. coelicolor* resistance arises through seven genes in four transcriptional operons: the TCS *vanRS*, *vanJ*, *vanK* and *vanHAX*. Interestingly the *van* cluster confers resistance to another glycopeptide, teicoplanin, but only when first induced by vancomycin. In isolation *S. coelicolor* is not resistant to teicoplanin. Transcriptional analysis revealed that the *van* operon is completely dependent on *vanR* and conversely proves its entire regulon, and that the *vanRS* transcript is undetectable without induction via vancomycin. Remarkably there was also a startling increase in the presence of *sigE* (the ECF sigma factor mentioned in regard to CseBC) in the *vanR* mutant. It is proposed that without the cell wall remodelling that occurs in response to vancomycin-induced *vanR* expression, the SigE-activating cell wall intermediates build up and induce a response [141, 142]. In the absence of vancomycin, dephosphorylated VanR can be phosphorylated by the small molecule phosphodonor acetyl phosphate at which point VanS acts as balance, using its kinase activity to remove the phosphate group from VanR. *S. coelicolor* and *S. toyocaensis* have different VanRS induction spectra and simply by swapping the VanRS systems over their respective spectra can be altered, suggesting different substrate specificities [142]. In *S. coelicolor* it is proposed that VanS is activated by a complex formed of vancomycin-bound vancosamine sugars present in peptidoglycan precursors, initiating the signalling cascade and inducing glycopeptide resistance [143].

## UNPAIRED SKS

P2RP analysis predicted 27 unpaired SKs in *S. venzuelae,* all of which are classic SKs except the hybrid vnz_01755. Fourteen of the unpaired SKs are probably incomplete, meaning that they lack an obvious phosphorylatable His residue. The hybrid SK vnz_01755 was suggested for crosstalk with the OsaABC system because deletion of the hybrid SK *osaA* only displays a mild phenotype [28].

The only unpaired SK described in detail is OhkA, which is a classic SK containing one PAS domain, the SK typical dimerization domain HisKA and the ATPase domain (Table 3). This structure is similar to the *E. coli* SK NtrB [144]. Neither of these SKs contain any transmembrane domains, and thus they are probably cytoplasmic-sensing SKs. Not only is the structure of OhKA highly conserved throughout *Streptomyces* (see Fig. 2, *vnz_05825*) but so too is the genomic context as well [145]. The SK OhKA forms an operon with the upstream putative rRNA methylase [145]. Downstream are *pheS* (*SCO1595*) and *pheT* (*SCO1594*), which encode alpha and beta chains of a putative phenylalanyl-tRNA synthetase respectively. To investigate the role of OhkA in *Streptomyces* the genes were deleted in *S. coelicolor* and *S. avermitilis*. Both strains showed a similar phenotype of compromised aerial hyphae and spore formation and increased production of ACT and CDA in *S. coelicolor* and oligomycin A in *S. avermitilis* [145]. Additionally, the deletion of *ohkA* in *Streptomyces peucetius*

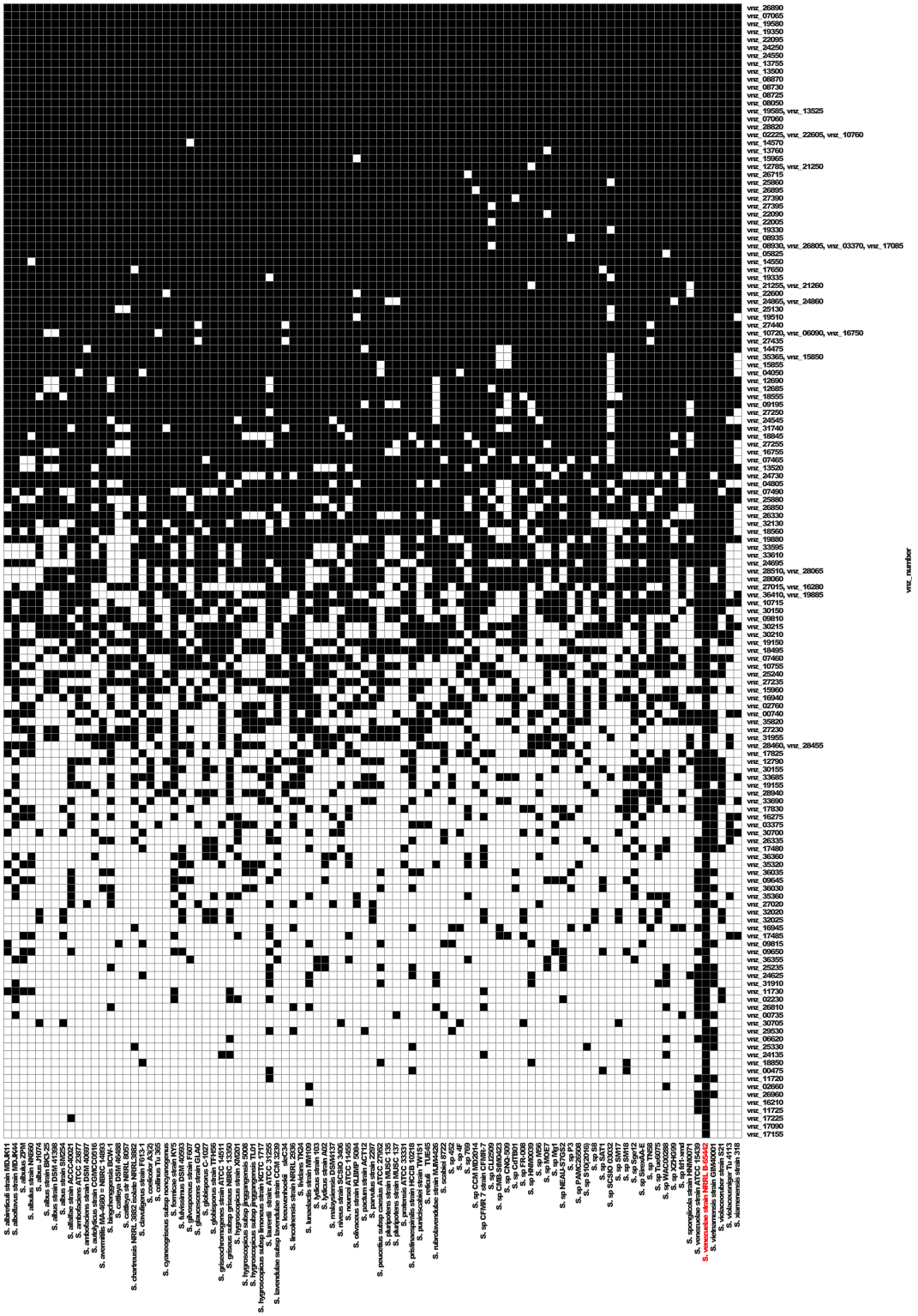


**Fig. 2.** Conservation of *S. venezuelae* TCS genes in other streptomycetes. The TCS genes are sorted according to their conservation and the species are sorted in alphabetical order. *S. venezuelae* genes represent the reference for each orthologue cluster and those separated by commas share high identity and could be paralogues

led to increased production of doxorubicin and daunorubicin. However, OhkA is not paired with an RR in the DOX or DNR clusters [30].

Unpaired SKs can be part of conservons (conserved operons). The *S. coelicolor* genome contains 13 conservons (*cvn1-13*) each containing four genes *cvnA-D*: the first gene encodes a membrane sensor kinase, the second and third genes are of unknown function and the fourth encodes a possible ATP/GTP-binding protein. In four of the conservons in *S. coelicolor* a predicted cytochrome P-450 lies downstream of the operon. *S. venezuelae* contains eight conservons (*cvn1-7* and *cvn9*). In *S. coelicolor cvn1* and *9* display altered formation of aerial hyphae and antibiotic production upon deletion [29, 31] The proteins of *cvn9* interact with each other and form a membrane-bound heterocomplex in an adenosine phosphate-dependent manner. The authors suggest that the Cnv9 complex may act as a signal transducer which transfers an environmental signal to an intracellular response; in the case of *cvnA,* this is the different expression of the *bld* cascade [29]. In a later study all 13 conservons in *S. coelicolor* were deleted but only *cvn1* displayed a phenotype under the tested conditions. Aerial hyphae formation and antibiotic production were disrupted not only in the *cvn1* mutant but also in the *cvnA1* mutant. Transcriptional analysis showed that *cvn1* represses the vegetative sigma factor $\sigma^{SigU}$ [31].

Unpaired SKs probably originate from duplication events [45] and add additional complexity to the gene regulation by TCSs, allowing bacteria to adapt to complex environmental stimuli. Identification of RR partners of unpaired SK is not trivial. Several bioinformatics approaches of identifying interaction partners of unpaired SK have been published [146–148] which are based on chromosomally coupled RRs and SKs. Therefore, the identification of interactions of distinct unpaired SKs is limited [149].

## ORPHAN RRS

In addition to the 27 unpaired SKs are the 18 orphan RRs of *S. venezuelae* that have been identified by P2RP (Table 4). Orphan RRs are capable of effecting transcriptional regulation independent of a cognate sensor kinase although this does not necessarily imply disregarding phosphorylation state. These fall into two broad categories, typical and atypical, with six of the 18 falling into the typical category and 12 proven or putative atypical orphan RRs.

Atypical response regulators are so categorized due to a lack of essential phosphorylation residues within the conserved phosphorylation pocket. These RRs include important developmental regulators such as the NarL/FixJ family BldM and WhiI, which are required for the formation of aerial hyphae and spore maturation respectively. Whilst BldM contains the conserved pocket, a D54A mutation at the conserved phosphorylation site is fully functional, hence being categorized as atypical. WhiI lacks a pair of adjacent aspartates and one highly conserved lysine forming a degenerate phosphorylation pocket incapable of binding $Mg^{2+}$ [38, 150]. The BldM regulon included two distinct groups of genes based on *whi* gene dependency. Group I genes are regulated by a BldM homodimer, independently of WhiI, and are important for early-stage development, including *whiB* which is vital for normal aerial tip growth. The BldM–WhiI heterodimer controls Group II genes, which include late-stage developmental genes such as the DNA translocase *smeA-sffA*. These target chromosomes into spores during septa formation and the *whiE* operon, which encodes the spore pigment [150]. BldM and WhiI are both highly conserved in *Streptomyces*. This stringent conservation could suggest interaction with as yet undiscovered partners. Another atypical RR is JadR, the OmpR-family transcriptional activator of jadomycin B biosynthesis in *S. venezuelae*. The dual aspartates of JadR at residues 49 and 50 have been replaced with a glutamate and serine. Instead of being activated by phosphorylation, JadR actually binds the small molecule jadomycin B, leading to the conformational change required for DNA binding. This is similar to the mechanism shown by the NarL-family atypical orphan RR RedZ of *S. coelicolor*, which regulates production of the RED antibiotic but is unable to bind the *redD* promoter in the presence of RED [36]. It remains unanswered whether BldM and/or WhiI sense such effector molecules, or merely exert gene regulation based on their concentration inside the cell.

Typical orphan RRs are simply RRs without a partner SK encoded adjacent on the genome. There has been little work done on typical orphan RRs in *S. venezuelae* but the OmpR-like orphan response regulator GlnR of *S. coelicolor* has cross-talk with AfsQ1/2 as previously mentioned. It regulates expression of the glutamine synthetase (GS) I-b-subtype enzyme, *glnA,* and a putative ammonium transporter *amtB* in *S. coelicolor*. Disruption of *glnR* results in a glutamine auxotrophic phenotype. The transcriptional regulator, a close GlnRI homologue (31 % homology), GlnRII recognizes the same promoters as GlnR, although the *glnRII* disruption mutant proved to be prototrophic to glutamine. GlnRII lacks many of the conserved residues in the phosphorylation pocket, including serine/threonine, tyrosine and the phosphorylatable aspartate. Due to the observable phenotype differences and lack of important residues, it is likely that GlnRII is not a true functional RR [151, 152]. In addition to its role in nitrogen assimilation, GlnR has been implicated in the regulation of cellobiose degradation, upregulating *β*-glucosidase genes under nitrogen-limited conditions in *Saccharopolyspora erythraea*. The cellobiose cluster in *S. coelicolor* A3(2) (*SCO2795-2798*) was also found to contain putative GlnR-binding boxes in the promoter regions. Similar results were found in other streptomycetes, *Mycobacterium smegmatis* and *Amycolatopsis mediterranei*, suggesting a level of conservation within *Actinobacteria* [153].

## FUTURE PROSPECTS

In our previous paper [25] there was sufficient knowledge to review eight specific TCSs. Just 15 years later that number has risen to 22. With a suite of knockout and mutagenesis tools

available to use within streptomycetes including the CRISPR-Cas9 system, it is possible to rapidly and flawlessly not only make knockouts but *cis* single nucleotide changes which are vital tools for the study of TCSs [154]. Combined with the plethora of next-generation whole genome sequences and the modern suites of powerful *in silico* tools that can expedite the analysis of such data, this has made the identification of TCSs of interest almost trivial. The real challenge lies in two areas: identifying the activating signal(s) of the SK and translating the basic research into applicable tools and techniques. The industrial and clinical importance of *Actinobacteria* cannot be understated and the work discussed in this review provides a clear and valuable foundation of TCS knowledge which can be used to uncover and increase antibiotic production in these 'talented' bacteria.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Koshland DE. The seven pillars of life. *Science* 2002;295:2215 LP–2216.
2. Galperin MY. Structural classification of bacterial response regulators: diversity of output domains and domain combinations. *J Bacteriol* 2006;188:4169–4182.
3. Bentley SD, Chater KF, Cerdeño-Tárraga AM, Challis GL, Thomson NR *et al*. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* 2002;417:141–147.
4. Nixon BT, Ronson CW, Ausubel FM. Two-component regulatory systems responsive to environmental stimuli share strongly conserved domains with the nitrogen assimilation regulatory genes *ntrB* and *ntrC*. *Proc Natl Acad Sci USA* 1986;83:7850–7854.
5. Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. *Annu Rev Biochem* 2000;69:183–215.
6. Quon KC, Marczynski GT, Shapiro L. Cell cycle control by an essential bacterial two-component signal transduction protein. *Cell* 1996;84:83–93.
7. Fol M, Chauhan A, Nair NK, Maloney E, Moomey M *et al*. Modulation of *Mycobacterium tuberculosis* proliferation by MtrA, an essential two-component response regulator. *Mol Microbiol* 2006;60:643–657.
8. Stephenson K, Hoch JA. Virulence- and antibiotic resistance-associated two-component signal transduction systems of gram-positive pathogenic bacteria as targets for antimicrobial therapy. *Pharmacol Ther* 2002;93:293–305.
9. Groisman EA. Feedback control of two-component regulatory systems. *Annu Rev Microbiol* 2016;70:103–124.
10. Park H, Saha SK, Inouye M. Two-domain reconstitution of a functional protein histidine kinase. *Proc Natl Acad Sci U S A* 1998;95:6728–6732.
11. Tanaka T, Saha SK, Tomomori C, Ishima R, Liu D *et al*. NMR structure of the histidine kinase domain of the *E. coli* osmosensor EnvZ. *Nature* 1998;396:88–92.
12. Hopwood DA. Forty years of genetics with *Streptomyces*: from *in vivo* through *in vitro* to *in silico*. *Microbiology* 1999;145:2183–2202.
13. Bystrykh LV, Fernández-Moreno MA, Herrema JK, Malpartida F, Hopwood DA *et al*. Production of actinorhodin-related "blue pigments" by *Streptomyces coelicolor* A3(2). *J Bacteriol* 1996;178:2238–2244.
14. Feitelson JS, Malpartida F, Hopwood DA. Genetic and biochemical characterization of the red gene cluster of *Streptomyces coelicolor* A3(2). *Microbiology* 1985;131:2431–2441.
15. Hojati Z, Milne C, Harvey B, Gordon L, Borg M *et al*. Structure, biosynthetic origin, and engineered biosynthesis of calcium-dependent antibiotics from *Streptomyces coelicolor*. *Chem Biol* 2002;9:1175–1187.
16. Gomez-Escribano JP, Song L, Fox DJ, Yeo V, Bibb MJ *et al*. Structure and biosynthesis of the unusual polyketide alkaloid coelimycin P1, a metabolic product of the *cpk* gene cluster of *Streptomyces coelicolor* M145. *Chem. Sci.* 2012;3:2716.
17. Gottelt M, Kol S, Gomez-Escribano JP, Bibb M, Takano E. Deletion of a regulatory gene within the cpk gene cluster reveals novel antibacterial activity in *Streptomyces coelicolor* A3(2). *Microbiology* 2010;156:2343–2353.
18. O'Rourke S, Wietzorrek A, Fowler K, Corre C, Challis GL *et al*. Extracellular signalling, translational control, two repressors and an activator all contribute to the regulation of methylenomycin production in *Streptomyces coelicolor*. *Mol Microbiol* 2009;71:763–778.
19. Glazebrook MA, Doull JL, Stuttard C, Vining LC. Sporulation of *Streptomyces venezuelae* in submerged cultures. *J Gen Microbiol* 1990;136:581–588.
20. Schlimpert S, Flärdh K, Buttner MJ. Fluorescence time-lapse imaging of the complete *S. venezuelae* life cycle using a microfluidic device. *JoVE* 2016;108:e53863.
21. Inahashi Y, Zhou S, Bibb MJ, Song L, Al-Bassam MM *et al*. Watasemycin biosynthesis in *Streptomyces venezuelae*: thiazoline C-methylation by a type B radical-SAM methylase homologue. *Chem Sci* 2017;8:2823–2831.
22. Bush MJ, Chandra G, Al-Bassam M, Findlay K, Buttner M. BldC delays entry into development to produce a sustained period of vegetative growth in *Streptomyces venezuelae*. *bioRxiv* 2018;194126.
23. Barakat M, Ortet P, Whitworth DE. P2RP: a web-based framework for the identification and analysis of regulatory proteins in prokaryotic genomes. *BMC Genomics* 2013;14:269.
24. Ortet P, De Luca G, Whitworth DE, Barakat M. P2TF: a comprehensive resource for analysis of prokaryotic transcription factors. *BMC Genomics* 2012;13:628.
25. Hutchings MI, Hoskisson PA, Chandra G, Buttner MJ. Sensing and responding to diverse extracellular signals? Analysis of the sensor kinases and response regulators of *Streptomyces coelicolor* A3(2). *Microbiology* 2004;150:2795–2806.
26. Ulrich LE, Koonin E V, Zhulin IB. One-component systems dominate signal transduction in prokaryotes. *Trends Microbiol* 2005:13.
27. Jiang M, Shao W, Perego M, Hoch JA. Multiple histidine kinases regulate entry into stationary phase and sporulation in *Bacillus subtilis*. *Mol Microbiol* 2000;38:535–542.
28. Bishop A, Fielding S, Dyson P, Herron P. Systematic insertional mutagenesis of a streptomycete genome: a link between osmoadaptation and antibiotic production. *Genome Res* 2004;14:893–900.
29. Komatsu M, Takano H, Hiratsuka T, Ishigaki Y, Shimada K *et al*. Proteins encoded by the conservon of *Streptomyces coelicolor* A3(2) comprise a membrane-associated heterocomplex that resembles eukaryotic G protein-coupled regulatory system. *Mol Microbiol* 2006;62:1534–1546.
30. Pokhrel AR, Nguyen HT, Dhakal D, Chaudhary AK, Sohng JK. Implication of orphan histidine kinase (OhkAsp) in biosynthesis of doxorubicin and daunorubicin in *Streptomyces peucetius* ATCC 27952. *Microbiol Res* 2018;214:37–46.

31. Takano H, Hashimoto K, Yamamoto Y, Beppu T, Ueda K. Pleiotropic effect of a null mutation in the *cvn1* conservon of *Streptomyces coelicolor* A3(2). *Gene* 2011;477:12–18.
32. Yagüe P, Rodríguez-García A, López-García MT, Rioseras B, Martín JF *et al*. Transcriptomic analysis of liquid non-sporulating *Streptomyces coelicolor* cultures demonstrates the existence of a complex differentiation comparable to that occurring in solid sporulating cultures. *PLoS One* 2014;9:e86296.
33. Zhao Y, Li L, Zheng G, Jiang W, Deng Z *et al*. CRISPR/dCas9-mediated multiplex gene repression in *Streptomyces*. *Biotechnol J* 2018;13:1800121.
34. Xu Z, Wang Y, Chater KF, Ou HY, Xu HH *et al*. Large-scale transposition mutagenesis of *Streptomyces coelicolor* identifies hundreds of genes influencing antibiotic biosynthesis. *Appl Environ Microbiol* 2017;83:1–16.
35. Romero-Rodríguez A, Rocha D, Ruiz-Villafan B, Tierrafría V, Rodríguez-Sanoja R *et al*. Transcriptomic analysis of a classical model of carbon catabolite regulation in Streptomyces coelicolor. *BMC Microbiol* 2016;16:77.
36. Wang L, Tian X, Wang J, Yang H, Fan K *et al*. Autoregulation of antibiotic biosynthesis by binding of the end product to an atypical response regulator. *Proc Natl Acad Sci U S A* 2009;106:8617–8622.
37. Patkari M, Mehra S. Transcriptomic study of ciprofloxacin resistance in Streptomyces coelicolor A3(2). *Mol Biosyst* 2013;9:3101.
38. Molle V, Buttner MJ. Different alleles of the response regulator gene bldM arrest *Streptomyces coelicolor* development at distinct stages. *Mol Microbiol* 2000;36:1265–1278.
39. Nguyen THK, Kumagai T, Matoba Y, Suzaki T, Sugiyama M. Molecular cloning and functional analysis of minD gene from Streptomyces lavendulae ATCC25233. *J Biosci Bioeng* 2008;106:303–305.
40. Lu T, Zhu Y, Zhang P, Sheng D, Cao G *et al*. SCO5351 is a pleiotropic factor that impacts secondary metabolism and morphological development in Streptomyces coelicolor. *FEMS Microbiol Lett* 2018;365.
41. Aínsa JA, Parry HD, Chater KF. A response regulator-like protein that functions at an intermediate stage of sporulation in Streptomyces coelicolor A3(2). *Mol Microbiol* 1999;34:607–619.
42. Al-Bassam MM, Haist J, Neumann SA, Lindenberg S, Tschowri N. Expression patterns, genomic conservation and input into developmental regulation of the GGDEF/EAL/HD-GYP domain proteins in *Streptomyces*. *Front Microbiol* 2018;9:2524.
43. Chandra G, Chater KF. Developmental biology of *Streptomyces* from the perspective of 100 actinobacterial genome sequences. *FEMS Microbiol Rev* 2014;38:345–379.
44. Galperin MY. A census of membrane-bound and intracellular signal transduction proteins in bacteria: bacterial IQ, extroverts and introverts. *BMC Microbiol* 2005;5:35.
45. Capra EJ, Laub MT. Evolution of two-component signal transduction systems. *Annu Rev Microbiol* 2012;66:325–347.
46. Alm E, Huang K, Arkin A. The evolution of two-component systems in bacteria reveals different strategies for niche adaptation. *PLoS Comput Biol* 2006;2:e143–42.
47. Alvarez-Álvarez R, Rodríguez-García A, Santamarta I, Pérez-Redondo R, Prieto-Domínguez A *et al*. Transcriptomic analysis of Streptomyces clavuligerus ΔccaR::tsr: effects of the cephamycin C-clavulanic acid cluster regulator CcaR on global regulation. *Microb Biotechnol* 2014;7:221–231.
48. Kim YJ, Moon MH, Song JY, Smith CP, Hong S-K *et al*. Acidic pH shock induces the expressions of a wide range of stress-response genes. *BMC Genomics* 2008;9:604.
49. Yagüe P, Rodríguez-García A, López-García MT, Rioseras B, Martín JF *et al*. Transcriptomic analysis of liquid non-sporulating Streptomyces coelicolor cultures demonstrates the existence of a complex differentiation comparable to that occurring in solid sporulating cultures. *PLoS One* 2014;9:e86296.
50. Liu M, Zhang P, Zhu Y, Lu T, Wang Y *et al*. Novel two-component system macrs is a pleiotropic regulator that controls multiple morphogenic membrane protein genes in *Streptomyces coelicolor*. *Appl Environ Microbiol* 2019;85:e02178–18.
51. Yepes A, Rico S, Rodríguez-García A, Santamaría RI, Díaz M. Novel two-component systems implied in antibiotic production in *Streptomyces* coelicolor. *PLoS One* 2011;6:e19980.
52. Som NF, Heine D, Holmes NA, Munnoch JT, Chandra G *et al*. The conserved actinobacterial two-component system MtrAB coordinates chloramphenicol production with sporulation in *Streptomyces venezuelae* NRRL B-65442. *Front Microbiol* 2017;8:1–11.
53. Yu Z, Zhu H, Dang F, Zhang W, Qin Z *et al*. Differential regulation of antibiotic biosynthesis by DraR-K, a novel two-component system in *Streptomyces coelicolor*. *Mol Microbiol* 2012;85:535–556.
54. Yeo KJ, Han YH, Eo Y, Cheong HK. Expression, purification, crystallization and preliminary X-ray analysis of the extracellular sensory domain of DraK histidine kinase from *Streptomyces coelicolor*. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2013;69:909–911.
55. Paget MS, Chamberlin L, Atrih A, Foster SJ, Buttner MJ. Evidence that the extracytoplasmic function sigma factor sigmaE is required for normal cell wall structure in *Streptomyces coelicolor* A3(2). *J Bacteriol* 1999;181:204–211.
56. Wyszynski FJ, Hesketh AR, Bibb MJ, Davis BG. Dissecting tunicamycin biosynthesis by genome mining: cloning and heterologous expression of a minimal gene cluster. *Chem Sci* 2010;1:581–589.
57. Wang C, Ge H, Dong H, Zhu C, Li Y *et al*. A novel pair of two-component signal transduction system *ecrE1/ecrE2* regulating antibiotic biosynthesis in *Streptomyces coelicolor*. *Biologia* 2007;62:511–516.
58. Song JY, Kim ES, Kim DW, Jensen SE, Lee KJ. A gene located downstream of the clavulanic acid gene cluster in *Streptomyces clavuligerus* ATCC 27064 encodes a putative response regulator that affects clavulanic acid production. *J Ind Microbiol Biotechnol* 2009;36:301–311.
59. Fu J, Qin R, Zong G, Liu C, Kang N *et al*. The CagRS two-component system regulates clavulanic acid metabolism via multiple pathways in *Streptomyces clavuligerus* F613-1. *Front Microbiol* 2019;10:244.
60. San Paolo S, Huang J, Cohen SN, Thompson CJ. *rag* genes: novel components of the RamR regulon that trigger morphological differentiation in *Streptomyces coelicolor*. *Mol Microbiol* 2006;61:1167–1186.
61. Rozas D, Gullón S, Mellado RP. A novel two-component system involved in the transition to secondary metabolism in *Streptomyces coelicolor*. *PLoS One* 2012;7:e31760.
62. Rodriguez H, Rico S, Yepes A, Franco-EchevarrÃa E, Antoraz S *et al*. The two kinases, AbrC1 and AbrC2, of the atypical two-component system AbrC are needed to regulate antibiotic production and differentiation in *Streptomyces coelicolor*. *Front Microbiol* 2015;6:1–9.
63. Tsujibo H, Hatano N, Okamoto T, Endo H, Miyamoto K *et al*. Synthesis of chitinase in *Streptomyces thermoviolaceus* is regulated by a two-component sensor-regulator system. *FEMS Microbiol Lett* 1999;181:83–90.
64. Brian P, Riggle PJ, Santos RA, Champness WC. Global negative regulation of *Streptomyces coelicolor* antibiotic synthesis mediated by an *absA*-encoded putative signal transduction system. *J Bacteriol* 1996;178:3221–3231.
65. Li L, Jiang W, Lu Y. A novel two-component system, GluR-GluK, involved in glutamate sensing and uptake in *Streptomyces coelicolor*. *J Bacteriol* 2017;199:e00097–17.
66. Chang H-M, Chen M-Y, Shieh Y-T, Bibb MJ, Chen CW. The *cutRS* signal transduction system of *Streptomyces lividans* represses the biosynthesis of the polyketide antibiotic actinorhodin. *Mol Microbiol* 1996;21:107–108.
67. Bibb LA, Kunkle CA, Schmitt MP. The ChrA-ChrS and HrrA-HrrS signal transduction systems are required for activation of the *hmuO* promoter and repression of the *hemA* promoter in *Corynebacterium diphtheriae*. *Infect Immun* 2007;75:2421–2431.

68. Bihlmaier C, Welle E, Hofmann C, Welzel K, Vente A *et al*. Biosynthetic gene cluster for the polyenoyltetramic acid alpha-lipomycin. *Antimicrob Agents Chemother* 2006;50:2113–2121.
69. van Keulen G, Alderson J, White J, Sawers RG. The obligate aerobic actinomycete *Streptomyces coelicolor* A3(2) survives extended periods of anaerobic stress. *Environ Microbiol* 2007;9:3143–3149.
70. Hong H-J, Hutchings MI, Neu JM, Wright GD, Paget MSB *et al*. Characterization of an inducible vancomycin resistance system in *Streptomyces coelicolor* reveals a novel gene (*vanK*) required for drug resistance. *Mol Microbiol* 2004;52:1107–1121.
71. Perez JC, Groisman EA. Evolution of transcriptional regulatory circuits in bacteria. *Cell* 2009;138:233–244.
72. Zahrt TC, Deretic V. An essential two-component signal transduction system in *Mycobacterium tuberculosis*. *J Bacteriol* 2000;182:3832–3838.
73. Möker N, Brocker M, Schaffer S, Krämer R, Morbach S *et al*. Deletion of the genes encoding the MtrA-MtrB two-component system of *Corynebacterium glutamicum* has a strong influence on cell morphology, antibiotics susceptibility and expression of genes involved in osmoprotection. *Mol Microbiol* 2004;54:420–438.
74. Brocker M, Bott M. Evidence for activator and repressor functions of the response regulator MtrA from *Corynebacterium glutamicum*. *FEMS Microbiol Lett* 2006;264:205–212.
75. Purushotham G, Sarva KB, Blaszczyk E, Rajagopalan M, Madiraju MV. *Mycobacterium tuberculosis* oriC sequestration by MtrA response regulator. *Mol Microbiol* 2015;98:586–604.
76. Kang C-M, Abbott DW, Park ST, Dascher CC, Cantley LC *et al*. The *Mycobacterium tuberculosis* serine/threonine kinases PknA and PknB: substrate identification and regulation of cell shape. *Genes Dev* 2005;19:1692–1704.
77. Plocinska R, Purushotham G, Sarva K, Vadrevu IS, Pandeeti EVP *et al*. Septal localization of *the Mycobacterium tuberculosis* MtrB sensor kinase promotes MtrA regulon expression. *J Biol Chem* 2012;287:23887–23899.
78. Plocinska R, Martinez L, Gorla P, Pandeeti E, Sarva K *et al*. *Mycobacterium tuberculosis* MtrB sensor kinase interactions with FtsI and Wag31 proteins reveal a role for MtrB distinct from that regulating MtrA activities. *J Bacteriol* 2014;196:4120–4129.
79. Nguyen KT, Piastro K, Gray TA, Derbyshire KM. Mycobacterial biofilms facilitate horizontal DNA transfer between strains of *Mycobacterium smegmatis*. *J Bacteriol* 2010;192:5134–5142.
80. Gao B, Paramanathan R, Gupta RS. Signature proteins that are distinctive characteristics of actinobacteria and their subgroups. *Antonie Van Leeuwenhoek* 2006;90:69–91.
81. Zhang P, Wu L, Zhu Y, Liu M, Wang Y *et al*. Deletion of MtrA inhibits cellular development of *Streptomyces coelicolor* and alters expression of developmental regulatory genes. *Front Microbiol* 2013;2017:8.
82. Clark LC, Seipke RF, Prieto P, Willemse J, van Wezel GP *et al*. Mammalian cell entry genes in *Streptomyces* may provide clues to the evolution of bacterial virulence. *Sci Rep* 2013;3:1109.
83. Noda Y, Yoda K, Takatsuki A, Yamasaki M. TmrB protein, responsible for tunicamycin resistance of *Bacillus subtilis*, is a novel ATP-binding membrane protein. *J Bacteriol* 1992;174:4302–4307.
84. Munnoch JT, Martinez MTP, Svistunenko DA, Crack JC, Le Brun NE *et al*. Characterization of a putative NsrR homologue in *Streptomyces venezuelae* reveals a new member of the Rrf2 superfamily. *Sci Rep* 2016;6:31597.
85. Kwon S-Y, Kwon H-J. The possible role of SCO3388, a *tmrB*-like gene of *Streptomyces coelicolor*, in germination and stress survival of spores. *J Appl Biol Chem* 2013;56:165–170.
86. Darmon E, Noone D, Masson A, Bron S, Kuipers OP *et al*. A novel class of heat and secretion stress-responsive genes is controlled by the autoregulated CssRS two-component system of *Bacillus subtilis*. *J Bacteriol* 2002;184:5661–5671.
87. Allenby NEE, Laing E, Bucca G, Kierzek AM, Smith CP. Diverse control of metabolism and other cellular processes in *Streptomyces coelicolor* by the PhoP transcription factor: genome-wide identification of in vivo targets. *Nucleic Acids Res* 2012;40:9543–9556.
88. Sola-Landa A, Rodríguez-García A, Franco-Domínguez E, Martín JF. Binding of PhoP to promoters of phosphate-regulated genes in *Streptomyces coelicolor:* identification of PHO boxes. *Mol Microbiol* 2005;56:1373–1385.
89. Apel AK, Sola-Landa A, Rodríguez-García A, Martín JF. Phosphate control of *phoA*, *phoC* and *phoD* gene expression in *Streptomyces coelicolor* reveals significant differences in binding of PhoP to their promoter regions. *Microbiology* 2007;153:3527–3537.
90. Sola-Landa A, Rodríguez-García A, Apel AK, Martín JF. Target genes and structure of the direct repeats in the DNA-binding sequences of the response regulator PhoP in *Streptomyces coelicolor*. *Nucleic Acids Res* 2008;36:1358–1368.
91. Boukhris I, Dulermo T, Chouayekh H, Virolle M-J. Evidence for the negative regulation of phytase gene expression in *Streptomyces lividans* and *Streptomyces coelicolor*. *J Basic Microbiol* 2016;56:59–66.
92. Brzoska P, Boos W. The ugp-encoded glycerophosphoryldiester phosphodiesterase, a transport-related enzyme of *Escherichia coli*. *FEMS Microbiol Rev* 1989;63:115–124.
93. Santos-Beneit F, Rodríguez-García A, Sola-Landa A, Martín JF. Cross-talk between two global regulators in *Streptomyces*: PhoP and AfsR interact in the control of *afsS*, *pstS* and *phoRP* transcription. *Mol Microbiol* 2009;72:53–68.
94. Ghorbel S, Kormanec J, Artus A, Virolle MJ. Transcriptional studies and regulatory interactions between the *phoR-phoP* operon and the *phoU*, *mtpA*, and PPK genes of *Streptomyces lividans* TK24. *J Bacteriol* 2006;188:677–686.
95. Rodríguez-García A, Barreiro C, Santos-Beneit F, Sola-Landa A, Martín JF. Genome-wide transcriptomic and proteomic analysis of the primary response to phosphate limitation *in Streptomyces coelicolor* M145 and in a DeltaphoP mutant. *Proteomics* 2007;7:2410–2429.
96. Martín-Martín S, Rodríguez-García A, Santos-Beneit F, Franco-Domínguez E, Sola-Landa A *et al*. Self-control of the PHO regulon: the PhoP-dependent protein PhoU controls negatively expression of genes of PHO regulon in *Streptomyces coelicolor*. *J Antibiot* 2017;71:113–122.
97. Santos-Beneit F, Rodríguez-García A, Apel AK, Martín JF. Phosphate and carbon source regulation of two PhoP-dependent glycerophosphodiester phosphodiesterase genes of *Streptomyces coelicolor*. *Microbiology* 2009;155:1800–1811.
98. Fernández-Martínez LT, Santos-Beneit F, Martín JF. Is PhoR-PhoP partner fidelity strict? PhoR is required for the activation of the PHO regulon in *Streptomyces coelicolor*. *Mol Genet Genomics* 2012;287:565–573.
99. Mendes MV, Tunca S, Antón N, Recio E, Sola-Landa A *et al*. The two-component *phoR-phoP* system of *Streptomyces natalensis*: Inactivation or deletion of *phoP* reduces the negative phosphate regulation of pimaricin biosynthesis. *Metab Eng* 2007;9:217–227.
100. Jeong Y, Kim J-N, Kim MW, Bucca G, Cho S *et al*. The dynamic transcriptional and translational landscape of the model antibiotic producer *Streptomyces coelicolor* A3(2). *Nat Commun* 2016;7:11605.
101. Rico S, Santamaría RI, Yepes A, Rodríguez H, Laing E *et al*. Deciphering the regulon of *Streptomyces coelicolor* AbrC3, a positive response regulator of antibiotic production. *Appl. Environ. Microbiol.* 2014;80:2417–2428.
102. Joly N, Engl C, Jovanovic G, Huvet M, Toni T *et al*. Managing membrane stress: the phage shock protein (PSP) response, from molecular mechanisms to physiology. *FEMS Microbiol Rev* 2010;34:797–827.
103. Kleine B, Chattopadhyay A, Polen T, Pinto D, Mascher T *et al*. The three-component system EsrISR regulates a cell envelope

stress response in *Corynebacterium glutamicum*. *Mol Microbiol* 2017;106:719–741.

104. Vrancken K, Van Mellaert L, Anné J. Characterization of the *Streptomyces lividans* PspA response. *J Bacteriol* 2008;190:3475–3481.
105. Hesketh A, Hill C, Mokhtar J, Novotna G, Tran N *et al*. Genome-wide dynamics of a bacterial response to antibiotics that target the cell envelope. *BMC Genomics* 2011;12:226.
106. Wang R, Mast Y, Wang J, Zhang W, Zhao G *et al*. Identification of two-component system AfsQ1/Q2 regulon and its cross-regulation with GlnR in *Streptomyces coelicolor*. *Mol Microbiol* 2013;87:30–48.
107. Tran NT, Huang X, Hong Hee-Jeon, Bush MJ, Chandra G *et al*. Defining the regulon of genes controlled by σE, a key regulator of the cell envelope stress response in *Streptomyces coelicolor*. *Mol Microbiol* 2019;359.
108. Ishizuka H, Horinouchi S, Kieser HM, Hopwood DA, Beppu T. A putative two-component regulatory system involved in secondary metabolism in *Streptomyces* spp. *J Bacteriol* 1992;174:7585–7594.
109. Shu D, Chen L, Wang W, Yu Z, Ren C *et al*. *afsQ1-Q2-sigQ* is a pleiotropic but conditionally required signal transduction system for both secondary metabolism and morphological development in *Streptomyces coelicolor*. *Appl Microbiol Biotechnol* 2009;81:1149–1160.
110. Daniel-Ivad M, Hameed N, Tan S, Dhanjal R, Socko D *et al*. An engineered Allele of *afsQ1* facilitates the discovery and investigation of cryptic natural products. *ACS Chem Biol* 2017;12:628–634.
111. Fernández Martínez L, Bishop A, Parkes L, Del Sol R, Salerno P *et al*. Osmoregulation in *Streptomyces coelicolor*: modulation of SigB activity by OsaC. *Mol Microbiol* 2009;71:1250–1262.
112. Godinez O, Dyson P, del Sol R, Barrios-Gonzalez J, Millan-Pacheco C *et al*. Targeting the osmotic stress response for strain improvement of an industrial producer of secondary metabolites. *J Microbiol Biotechnol* 2015;25:1787–1795.
113. Tseng HC, Chen CW. A cloned *ompR*-like gene of *Streptomyces lividans* 66 suppresses defective MelC1, a putative copper-transfer gene. *Mol Microbiol* 1991;5:1187–1196.
114. Hutchings MI, Hong H-J, Leibovitz E, Sutcliffe IC, Buttner MJ. The sigma(E) cell envelope stress response of *Streptomyces coelicolor* is influenced by a novel lipoprotein, CseA. *J Bacteriol* 2006;188:7222–7229.
115. Paget MS, Leibovitz E, Buttner MJ. A putative two-component signal transduction system regulates sigmaE, a sigma factor required for normal cell wall integrity in *Streptomyces coelicolor* A3(2). *Mol Microbiol* 1999;33:97–107.
116. Cao M, Wang T, Ye R, Helmann JD. Antibiotics that inhibit cell wall biosynthesis induce expression of the *Bacillus* subtilis σW and σM regulons. *Mol Microbiol* 2002;45:1267–1276.
117. Hong H-J, Paget MSB, Buttner MJ. A signal transduction system in *Streptomyces coelicolor* that activates the expression of a putative cell wall glycan operon in response to vancomycin and other cell wall-specific antibiotics. *Mol Microbiol* 2002;44:1199–1211.
118. Yong-quan L, Pei-lin C, Shi-fei C, Dan W, Jing Z. A pair of two-component regulatory genes *ecrA1/A2* in *S. coelicolor*. *J Zhejiang Univ A*;5:173–179.
119. Brown AG, Butterworth D, Cole M, Hanscomb G, Hood JD *et al*. Naturally-occurring beta-lactamase inhibitors with antibacterial activity. *J Antibiot* 1976;29:668–669.
120. Willey J, Schwedock J, Losick R. Multiple extracellular signals govern the production of a morphogenetic protein involved in aerial mycelium formation by *Streptomyces coelicolor*. *Genes Dev* 1993;7:895–903.
121. Zou P, Schrempf H. The heme-independent manganese-peroxidase activity depends on the presence of the C-terminal domain within the *Streptomyces reticuli* catalase-peroxidase CpeB. *Eur J Biochem* 2000;267:2840–2849.
122. Ortiz de Orué Lucana D, Tröller M, Schrempf H. Amino acid residues involved in reversible thiol formation and zinc ion binding in the *Streptomyces reticuli* redox regulator FurS. *Mol Genet Genomics* 2003;268:618–627.
123. Ortiz de Orué Lucana D, Zou P, Nierhaus M, Schrempf H, Lucana DOdO. Identification of a novel two-component system SenS/SenR modulating the production of the catalase-peroxidase CPEB and the haem-binding protein HbpS in *Streptomyces reticuli*. *Microbiology* 2005;151:3603–3614.
124. Ortiz de Orué Lucana D, Schaa T, Schrempf H. The novel extracellular *Streptomyces reticuli* haem-binding protein HbpS influences the production of the catalase-peroxidase CpeB. *Microbiology* 2004;150:2575–2585.
125. Ortiz de Orué Lucana D, Groves MR. The three-component signalling system HbpS-SenS-SenR as an example of a redox sensing pathway in bacteria. *Amino Acids* 2009;37:479–486.
126. Wedderhoff I, Kursula I, Groves MR, Ortiz de Orué Lucana D. Iron binding at specific sites within the octameric HbpS protects streptomycetes from iron-mediated oxidative stress. *PLoS One* 2013;8:e7157927.
127. Taylor P, Tharanathan RN, Kittur FS. Critical reviews in food Science and nutrition chitin — the undisputed biomolecule of great potential. *Crit Rev Food Sci Nutr* 2003;43:61–87.
128. Colson S, van Wezel GP, Craig M, Noens EEE, Nothaft H *et al*. The chitobiose-binding protein, DasA, acts as a link between chitin utilization and morphogenesis in *Streptomyces coelicolor*. *Microbiology* 2008;154:373–382.
129. Homerová D, Knirschová R, Kormanec J. Response regulator ChiR regulates expression of chitinase gene, *chiC*, *in Streptomyces coelicolor*. *Folia Microbiol* 2002;47:499–505.
130. Anderson T, Brian P, Riggle P, Kong R, Champness W. Genetic suppression analysis of non-antibiotic-producing mutants of the *Streptomyces coelicolor absA* locus. *Microbiology* 1999;145:2343–2353.
131. Du YL, Shen XL, Yu P, Bai LQ, Li YQ *et al*. Gamma-butyrolactone regulatory system of *Streptomyces chattanoogensis* links nutrient utilization, metabolism, and development. *Appl Environ Microbiol* 2011;77:8415–8426.
132. Epstein WBT-P in NAR and MB. The roles and regulation of potassium in bacteria. *Prog Nucleic Acid Res Mol Bio* 2003;75:293–320.
133. Rothenbücher MC, Facey SJ, Kiefer D, Kossmann M, Kuhn A. The cytoplasmic C-terminal domain of the *Escherichia coli* KdpD protein functions as a K+ sensor. *J Bacteriol* 2006;188:1950–1958.
134. Heermann R, Jung K. The complexity of the 'simple' two-component system KdpD/KdpE in *Escherichia coli*. *FEMS Microbiol Lett* 2010;304:97–106.
135. Greie J-C, Altendorf K. The K+-translocating KdpFABC complex from *Escherichia coli:* a P-type ATPase with unique features. *J Bioenerg Biomembr* 2007;39:397–402.
136. Freeman ZN, Dorus S, Waterfield NR. The KdpD/KdpE two-component system: integrating $K^+$ homeostasis and virulence. *PLoS Pathog* 2013;9:e1003201.
137. Steyn AJC, Joseph J, Bloom BR. Interaction of the sensor module of *Mycobacterium tuberculosis* H37Rv KdpD with members of the Lpr family. *Mol Microbiol* 2003;47:1075–1089.
138. Ali MK, Li X, Tang Q, Liu X, Chen F *et al*. Regulation of Inducible Potassium Transporter KdpFABC by the KdpD/KdpE Two-Component System in *Mycobacterium smegmatis*. *Front Microbiol* 2017;8:570.
139. Frunzke J, Gätgens C, Brocker M, Bott M. Control of heme homeostasis in *Corynebacterium glutamicum* by the two-component system HrrSA. *J Bacteriol* 2011;193:1212–1221.
140. Heyer A, Gätgens C, Hentschel E, Kalinowski J, Bott M *et al*. The two-component system ChrSA is crucial for haem tolerance and interferes with HrrSA in haem-dependent gene regulation in *Corynebacterium glutamicum*. *Microbiology* 2012;158:3020–3031.
141. Hong H-J, Hutchings MI, Buttner MJ. Vancomycin resistance VanS/VanR two-component systems. *Adv Exp Med Biol* 2008;631:200–213.

142. Hutchings MI, Hong HJ, Buttner MJ. The vancomycin resistance VanRS two-component signal transduction system of *Streptomyces coelicolor*. *Mol Microbiol* 2006;59:923–935.
143. Kwun MJ, Novotna G, Hesketh AR, Hill L, Hong HJ. *In* vivo studies suggest that induction of VanS-dependent vancomyc*in* resistance requires binding of the drug to D-Ala-D-Ala termini *in* the peptidoglycan cell wall. *Antimicrob Agents Chemother* 2013;57:4470–4480.
144. Mascher T, Helmann JD, Unden G. Stimulus perception in bacterial signal-transducing histidine kinases. *Microbiol Mol Biol Rev* 2006;70:910–938.
145. Lu Y, He J, Zhu H, Yu Z, Wang R *et al*. An orphan histidine kinase, OhkA, regulates both secondary metabolism and morphological differentiation in *Streptomyces coelicolor*. *J Bacteriol* 2011;193:3020–3032.
146. White RA, Szurmant H, Hoch JA, Hwa T. Features of protein-protein interactions in two-component signaling deduced from genomic libraries. *Methods Enzymol* 2007;422:75–101.
147. Burger L, van Nimwegen E. Accurate prediction of protein-protein interactions from sequence alignments using a Bayesian method. *Mol Syst Biol* 2008;4:165.
148. Procaccini A, Lunt B, Szurmant H, Hwa T, Weigt M. Dissecting the specificity of protein-protein interaction in bacterial two-component signaling: orphans and crosstalks. *PLoS One* 2011;6:e19729.
149. Zschiedrich CP, Keidel V, Szurmant H. Molecular mechanisms of two-component signal transduction. *J Mol Biol* 2016;428:3752–3775.
150. Al-Bassam MM, Bibb MJ, Bush MJ, Chandra G, Buttner MJ. Response regulator heterodimer formation controls a key stage in *Streptomyces* development. *PLoS Genet* 2014;10:e1004554.
151. Fink D, Weissschuh N, Reuther J, Wohlleben W, Engels A. Two transcriptional regulators GlnR and GlnRII are involved in regulation of nitrogen metabolism in *Streptomyces coelicolor* A3(2). *Mol Microbiol* 2002;46:331–347.
152. Reuther J, Wohlleben W. Nitrogen metabolism in *Streptomyces coelicolor*: transcriptional and post-translational regulation. *J Mol Microbiol Biotechnol* 2007;12:139–146.
153. Xu Y, Ye BC. GlnR and PhoP regulate $\beta$-glucosidases involved in cellulose digestion in response to nitrogen and phosphate availability. *Microbiology* 2018;164:779–789.
154. Cobb RE, Wang Y, Zhao H. High-efficiency multiplex genome editing of *Streptomyces* species using an engineered CRISPR/Cas system. *ACS Synth Biol* 2015;4:723–728.