

## Importance of two component systems regulation in *Streptomyces* strains

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### Abstract

*Streptomyces* strains experience a number of changes within the complex hyperosmotic ecological niche they inhabit. A two-component regulatory system allows them to control the cascade of signals required to acclimate to these changes. These kinds of systems consist of a sensor domain (histidine kinase) and an effector response-regulating domain. The regulation process uses phosphotransfer, dephosphorylation and self-phosphorylation. Coupled with the system are proteins that control the activity of the target protein, and transcription factors that allow the expression of genes in response to stress. This brief review provides an updated and summarized vision of some two-component systems known in *Streptomyces*, and the advantages of understanding these systems for the biosynthesis of secondary metabolites. Comprehending these adaptation mechanisms expands the potential uses of *Streptomyces*, as these systems are frequently linked to the production of new substances of interest in biotechnology, considering that these mechanisms regulate two major processes -- cellular differentiation and secondary metabolite production -- to respond to their complex environment.-

**Keywords:** secondary metabolites, histidine kinase, response regulator.

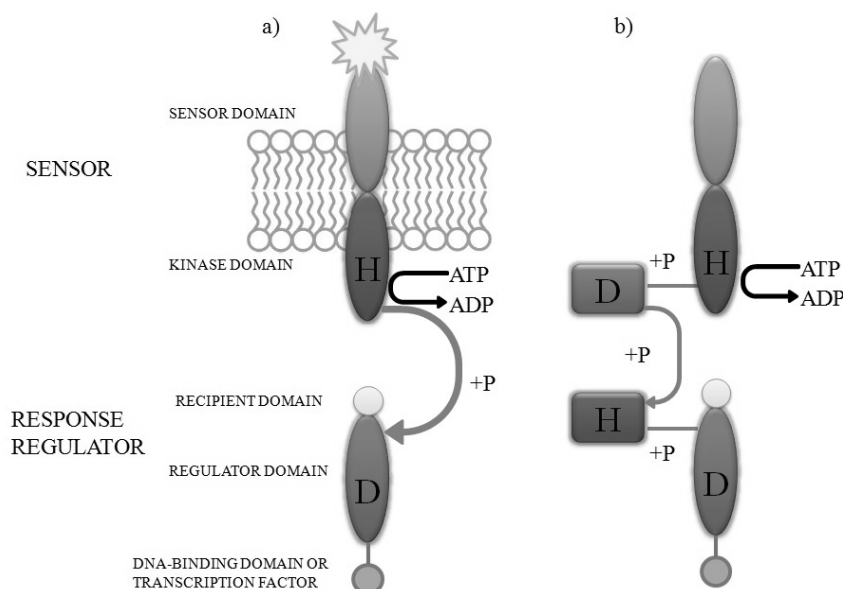
### 1. Introduction

Actinomycetes are saprophytic Gram-positive bacteria that live in terrestrial and marine environments [1]. They grow as vegetative multi-gene hyphae that subsequently produce spores [2]. Their morphological differentiation leads to the physiological one, which in turn results in the synthesis of a number of secondary metabolites of importance in industry and medicine as antifungal, antiviral, antiparasitic and antitumor agents, as well as herbicides and immunosuppressants, among others [3,4,5]. Both processes occur in response to environmental stress [6]. To communicate these changes, these processes use a variety of systems, including secondary metabolites, ions (Fe<sup>2+</sup>) and proteins (catabolite regulation protein, CRP). However, two-component systems (TCSs) are the most commonly used of all for signal transduction [7,8]. Although TCSs have not been classified to date, these can be grouped into three classes: 1) those involved in the biosynthesis of secondary metabolites; 2) those regulating pleiotropic mechanisms; and 3) both regulating mechanisms: pleiotropic and in the biosynthesis of secondary metabolites. The interaction and cascade of events between two-component systems and transcription factors is complex, and in some cases the signals that indicate the onset of regulation are unknown [9]. This paper briefly reviews general and recent aspects of the two-component system in actinomycetes, emphasizing their involvement in the production of secondary metabolites of industrial interest.

## 2. Two-Component Systems. Overview

Two-component systems (TCSs) are widely distributed among the three domains of life, i.e. Eukaria, Bacteria and Archea [10]. In bacteria, TCSs are regulation systems that frequently control the adaptive response to different stressors. These usually consist of two core elements: a sensor protein -- histidine kinase (HK) -- and a response regulator (RR) akin to it [7]. Generally, the sensor protein uses adenosine triphosphate (ATP) for self-phosphorylating a conserved histidine residue in response to a specific signal. The high-energy phosphoryl group is transferred to a conserved aspartate residue located in the response regulator (RR), causing the binding to specific genes within DNA [7]. Although many TCSs use the Histidine-Aspartate phosphotransfer event between HK and RR, there is another group that may display two additional phosphotransfers Histidine-Aspartate-Histidine-Aspartate [7, 8].

In actinomycetes, the complete sequencing of the model organism *Streptomyces (S.) coelicolor* has provided most of the information on regulatory mechanisms and TCSs [11]. A total of 965 proteins (12.5%) have been identified as having a regulation role, including 85 histidine kinases and 79 response regulators (with 53 HK-RR pairs) potentially involved in physiological and environmental fluctuations [11]. Particularly, in *S. coelicolor* it has been shown that the HK-RR pair called *osaAB* is involved in morphological differentiation and synthesis of actinorhodin and undecylprodigiosin when the organism grows under hyperosmotic conditions; this feature has been defined as an osmoadaptive response [12, 13]. On the other hand, in *Streptomyces avermitilis*, whose importance in the pharmaceutical industry has fostered the complete sequencing of its genome [3], 91 HK and 72 RR have been reported [9]. For its part, *Streptomyces bingchenggensis*, which produces milbemycin, contains 125 HK and 117 RR in its genome [9]. These figures contrast with those reported for other bacteria, like *E. coli* (32 HK and 23 RR) and *Bacillus (B.) subtilis* (36 HK and 34 RR). The difference in the composition and number of TCSs in species of the genus *Streptomyces* relative to other bacteria seems to reflect a certain ability to respond to a wide variety of environmental stimuli and changes in their natural habitats, and also gives them the ability to produce secondary metabolites that allow them to compete and survive in complex environments (Figure 1).



**Figure 1.** The two-component system consists of a sensor protein and a response regulator. a) The sensor protein is self-phosphorylated in a conserved histidine (H) residue in the kinase domain in response to a signal from the sensor domain (marked with the “explosion” symbol). The high-energy phosphoryl group (+P) is transferred to a conserved aspartate residue (D) in the receptor domain of the response regulator, initiating the cascade of signals that activate a transcription factor or a DNA binding domain. A second pathway b) involves the transfer of +P to auxiliary aspartate (D) and histidine (H) domains. There are various arrangements in the transfer of +P from the sensor to the response regulator as regards the pathway being regulated.

### 3. Histidine kinases. Structure and function

Histidine kinases (HKs) possess a modular structure with various input domains bounded to a conserved catalytic core. This design allows the coupling of a wide variety of input signals leading to appropriate responses through self-phosphorylation and phosphotransfer [14]. The typical HK structure consists of a homodimeric membrane that contains a sensor domain formed by an extracellular loop with at least two segments covering the membrane, and a transmitting domain located in the cytoplasm [15]. In a typical HK, the cytoplasmic core consists of two different structures: a) the ATP-binding domain (DATP), also known as HATPase, and b) the histidinephosphotransferase domain (DHFT) or Histidine kinase A [16]. DHFT contains the conserved histidine residue for phosphorylation while DATP acts as a catalyzer by transferring the phosphoryl group from ATP to the histidine residue. HKs have multiple enzymatic activities, including autokinase, phosphotransferase and phosphatase [17]. In the actinomycetes *S. thermoviolaceus* and *S. coelicolor* the *chiRSK* operon has been identified, which encodes a chitinase – the enzyme responsible for hydrolyzing chitin toward chito-oligosaccharides that, through the action of other enzymes, lead the biosynthesis of N-acetylglucosamine, a major source of carbon and nitrogen [18]. Interestingly, the *in-silico* analysis of *chiS* in *S. peucetius* revealed the similarity with HK sequences of other actinomycetes such as *S. avermitilis*; it was subsequently confirmed that it was a HK [19]. On the other hand, it was recently reported that two HKs from *S. coelicolor*, AbrC1 and AbrC2, are necessary to regulate the production of antibiotics. The *abrC1* mutant, obtained in the referred investigation, overproduces the three antibiotics identified in *S. coelicolor*, making it an interesting strain for the heterologous biosynthesis of secondary metabolites [20]. Likewise, in *Streptomyces acidiscabies* -- a producer of thaxtomin A and the secondary metabolite aromatic angucyclinonepolyketide WS5995B -- an HK (*tcsK*) was identified that, when over-expressed, increases 7.1 times the synthesis of WS5995B without affecting the production of thaxtomin A. Besides, it was shown that *tcsK* produces a 120-fold decrease in the production of spores in this species [21].

### 4. Response Regulators. Structure and function

Response Regulators (RR) are the end components in a signaling pathway; they operate as "switches" activated by phosphorylation to produce an adaptive response [7]. RRs catalyze the transfer of phosphate groups (phosphoryl) from the HK phospho-histidine complex to a conserved aspartate residue within its own regulation domain (RD); molecules such as acetyl phosphate, carbamoyl phosphate and phosphate imidazole can serve as phosphate donors to RRs [7]. The RR's basic structure contains: a) an N-terminal RD and b) an effector domain (ED) [22]. The RD is involved in the catalysis of the transfer of phosphoryl groups from HK toward itself and regulates the phosphorylation-dependent activity of the effector domain; in this way, it allows the output response with various effector domains, thus allowing a huge arrangement of responses that are regulated through the TCS [22]. An example of the role of RR in actinomycetes is the one reported in the cluster *ram* (rapid aerial mycelium), which plays a key role in the development of the vegetative to aerial hyphae in *S. coelicolor*, since these encode a number of proteins including RamR and RamC. The respective *ramR* and *ramC* genes are required for the formation of aerial hyphae, but are not essential for vegetative growth in other actinomycete species [23]. Other studies have demonstrated the role of BldM and WhiI (hyphae formation and spore production) in the differentiation of several actinomycete species, and the importance of response regulators in signal transduction [24]. As regards secondary metabolism in actinomycetes, response regulators may affect biosynthesis either positively or negatively. In the case of *Streptomyces botropencis*, it was demonstrated that the response regulator Tnx11 activates the biosynthesis of trioxacarcin A, a chemical with antibacterial, antimalarial and antitumor activity [25]. On the other hand, rapamycin biosynthesis by *Streptomyces rapamycinicus* drops when the response regulator RapR is overexpressed [26].

## 5. GAF and PAS Domains. Relationship with the Two-Component System

The GAF domain has been identified in many bacterial proteins (adenylatecyclase, phosphotransferases and HKs), and seem to function as binding sites for small ligands, thereby regulating the catalytic activity of the target protein [27]; the presence of GAF domains in certain genes induces the self-phosphorylation of HK and, subsequently, the transduction of signals to activate or repress the expression of the respective genes [18]. For its part, the PAS domain is typically found in proteins involved in mechanisms of adaptation or detection of environmental changes. In bacteria, these are normally found in proteins involved in the regulation of oxygen (fixI), sporulation (Kina), nitrogen fixation (NTRB) and negative phototropism (PYP) [28]. Both domains -- GAF and PAS -- are involved in regulation and signaling pathways; however, the GAF domain allows the binding of molecules such as nucleotides, while the PAS domain allows the binding of larger molecules such as flavins, heme groups and chromophores. In actinomycetes, these domains are involved in various functions, operating mainly as regulatory proteins in species such as *S. coelicolor* (A3), *S. avermitilis*, *S. griseus* and *S. bingchengensis*, with PAS and GAF sensor domains, as well as phosphatase-type domains [29]. In *S. coelicolor*, the *osaC* gene possesses one PAS and two GAF domains, being essential in the response to osmoadaptation. It has been experimentally shown that null mutants in both domains are unable to differentiate in the presence of an osmolyte [30]. On the other hand, as to the biosynthesis of secondary metabolites, it was demonstrated that protein PimM of *Streptomyces natalensis* that combines a PAS domain in the N-terminal end and an HTH (*helix-turn-helix*) motif in the C-terminal end is a positive regulator for the biosynthesis of pimaricin [31].

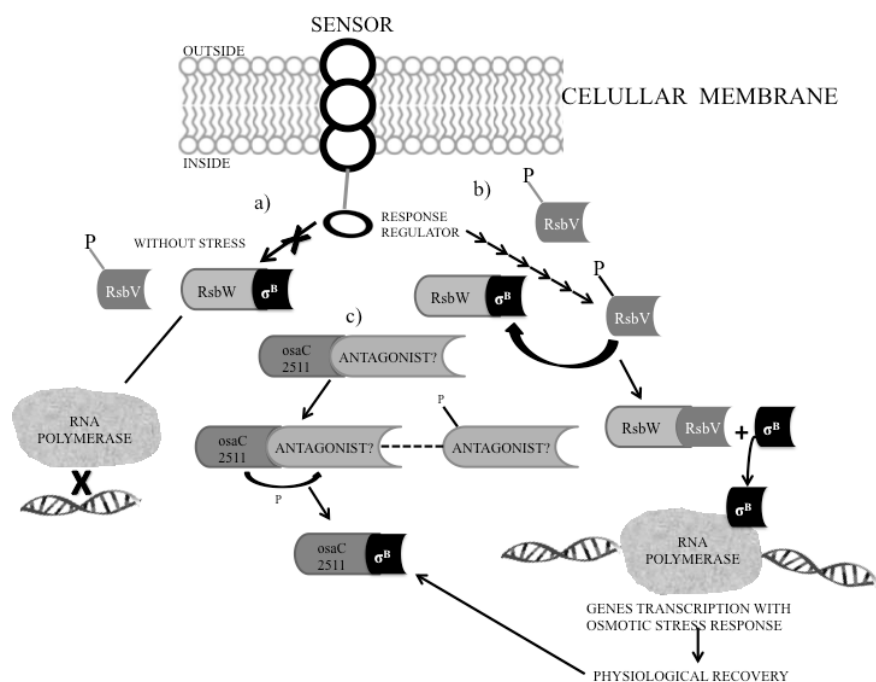
## 6. Sigma ( $\sigma$ ) Regulatory Factors

Another strategy of bacteria for controlling gene expression is the alternative use of transcription factors, called sigma factors [32, 33, 34]. During exponential growth, transcription is mediated by sigma factors; however, under specific conditions (environmental stress), RNA polymerase is replaced by factors that recognize specific promoters and control specialized regulons such as the two-component systems [35]. An example of this particular regulation has been reported for *S. griseus*, whose sigma factor null mutants ( $\sigma^{\text{ShbA}}$ ) showed severe growth defects (undifferentiated hyphae); proteomics analyses showed that these defects were due to a low transcription rate of constitutive genes required for differentiation [36]. One of the best-studied sigma factors is  $\sigma^{\text{B}}$ , present under diverse stressing situations in gram-positive bacteria of the genera *Bacillus*, *Listeria* and *Staphylococcus* [37, 38]. Other sigma factors equivalent to  $\sigma^{\text{B}}$  have been found in other gram-positive bacteria, including the genera *Mycobacterium* and *Streptomyces* [39]. In *S. coelicolor*,  $\sigma^{\text{B}}$  acts within a complex network of several sigma factors, playing an important role in the response to osmotic and oxidative stress, cell differentiation and antibiotics production [40, 41, 42]. Another example is reported in *Streptomyces coelicolor*, where the induction of saline stress affects the biosynthesis of actinorhodin and undecylprodigiosin, as well as the morphological differentiation mediated by  $\sigma^{\text{H}}$  [43, 44, 6]. In actinomycetes of industrial importance such as *S. avermitilis* and *S. clavuligerus*,  $\sigma^{\text{B}}$  is involved in the synthesis of avermectin, oligomycin and clavulanic acid (unpublished data). In *S. hygroscopicus* (Validamycin A producer), the decrease in reactive oxygen species has been reported to increase the transcription levels of  $\sigma^{\text{H}}$  and  $\sigma^{\text{B}}$  factors (response factors to oxidative stress), as well as the global regulator PhoRP; these results suggest that, as a whole, these factors could act as a global regulator in response to oxidative stress [45]. In the case of *S. avermitilis*, the role of  $\sigma$  (25) in controlling the synthesis of avermectin and oligomycin has been reported [46]. On the other hand, it was recently reported that the set of three sigma factors  $\text{WhiG}_{\text{ch}}$ , generally regarded as a morphological regulation factor, positively regulates the synthesis of natamycin in *Streptomyces chattanoogensis* L10, while inhibiting the biosynthesis of migrastatin and jadomycin [47].

## 7. TCS - Sigma B Relationship

The majority of regulation domains (DR) display DNA-binding activity and function to activate and/or repress the transcription of specific genes. However, its binding to DNA is conditioned by three features: a) recognition of specific DNA sequences; (b) arrangements of binding sites; and c) specific transcriptional regulation mechanisms [7]. In some cases, the RR has an RD devoid of a DNA binding pattern; however, the presence of a supercoiled helix pattern suggests that the RD interacts with other proteins with a supercoiled helix, what might be critical for signal transduction [13].

On the other hand, one of the roles of sigma factors is the regulation of gene expression in response to physiological and environmental changes [11,41]. It has been proposed that  $\sigma^B$  is a master regulator present in both oxidative and osmotic stress, controlling the expression of other factors and proteins involved in the regulation cascade [41]. The  $\sigma^B$  factor has been widely studied in *B. subtilis*, whose activity is controlled by the module (regulon) RsbVW, a highly conserved mechanism in species that have  $\sigma^B$  [48]. Under unstressed conditions,  $\sigma^B$  remains inactive by the anti-sigma factor RsbW; the anti-anti-sigma factor RsbV remains phosphorylated and unable to capture RsbW, which acts as a RsbV kinase, thus providing a negative feedback in  $\sigma^B$  activation. Under stress conditions, RsbV is dephosphorylated by one or more phosphatases, resulting in the uptake of RsbW, allowing the release and activation of  $\sigma^B$  (**Figure2**) [37,38]. In *S. coelicolor*, the RsbK-type HK (called *osaA*) and its respective RR, *osaB*, have been involved in osmoadaptation, cell differentiation and production of antibiotics [12], the processes of which are controlled by a complex network of  $\sigma^B$  factors [40, 41, 42]. Experimental data support the functional binding between *osaAB* and the  $\sigma^B$  factor [13]. For its part, experimental results in *S. avermitilis* support the relationship between RR and  $\sigma^B$  (unpublished data). The proposed model TCS -  $\sigma^B$  is supported for other organisms, such as *S. griseus*, *Thermobifidafusca*, *Salinisporatropica*, *Salinisporaarenicola* and several *Frankia* species [48].



**Figure 2.** Model for the regulation of the activity of  $\sigma^B$  in *S. coelicolor* and *S. avermitilis*, under osmotic stress (a), the anti-sigma factor RsbW is bound to  $\sigma^B$ , and the anti-anti-sigma factor RsbV remains phosphorylated;  $\sigma^B$  does not bind to RNA polymerase and there is no gene transcription in response to osmotic stress. Under stress (b), RsbV is dephosphorylated and binds to RsbW, allowing  $\sigma^B$  allowing to bind to RNA polymerase and initiate the transcription. Solid arrows indicate the presence of a supercoiled helix domain or sigma factors.

Physiological recovery begins with the activation of kinase-type proteins (*osaB* and *sav2511*), which phosphorylate its potential antagonist, binding to  $\sigma^B$  and avoiding the continued expression of the regulon.

## 8. Conclusions

The two-component systems control a wide variety of cell processes in gram-positive bacteria belonging to various genera. A number of studies have been developed in an attempt to understand the exact mechanism involved in signal transduction during the biosynthesis of secondary metabolites. The complete sequencing of the genome of some actinomycete species has allowed understanding the biochemical activities of some components and conserved proteins that participate in these specialized systems. However, there are still questions about the functioning of signal transduction and its relationship to osmotic stress or any other environmental stimulus, and its involvement in cell differentiation. An example of the above is the PhoR-PhoP system in *Streptomyces avermitilis* showing that PhoP acts as a repressor in the biosynthesis of avermectin while regulating genes involved in morphological differentiation [25]. Another open question in the analysis of the functioning of two-component systems is how a specific group of residues is able to differentiate between one histidine kinase and another? On the other hand, the study of sigma factors and their coupling to the system also represents a challenge, since their participation in signal transduction and in the regulation of specific pathways cannot be generalized [49, 48, 13]. Despite these drawbacks and its complexity, the prospects for the study of two-component systems are promising, since better analytical tools are becoming available that will contribute to advance and understand the sophisticated regulation system. Several investigations focused on TCSs in actinomycetes are relevant due to the pharmaceutical and industrial impacts. A deep understanding will allow proposing strategies to disrupt communication and increase the biosynthesis of secondary metabolites for obtaining overproducing strains to benefit the industry.

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## References

1. KHAN ST. *Streptomyces* associated with a marine sponge *Haliclona* sp biosynthetic gene for secondary metabolites and products. *Environ Microbiol Black Sci Pub.* 13:391-403. (2011)
2. CHATER, K.F. Regulation of sporulation in *Streptomyces coelicolor*A3(2): a checkpoint multiplex? *Curr Opin Microbiol*, 4: 667–673. (2001)
3. OMURA S, IKEDA H, ISHIKAWA J, HANAMOTO A, TAKAHASHI C, SHINOSE M, TAKAHASHI Y, HORIKAWA H, NAKASAWA H, OSONOE T, KIKUCHI H, SHIBA T, SAKAKI Y, HATTORI M. Genome sequence of an industrial microorganism *Streptomyces avermitilis*: deducing the ability of producing secondary metabolites. *Proc Natl Acad Sci.* 98:12215–20. (2001)
4. PATZER SI, VOLKMAR B. Gene cluster involved in the biosynthesis of griseobactin, a catechol-peptide siderophore of *Streptomyces* sp. ATCC 700974. *J Bacteriol.* 192:426–35 (2010).
5. BRAÑA A. F., FIEDLER H. P., NAVA H., GONZÁLEZ V., SARMIENTO-VIZCAÍNO A., MOLINA A., ACUÑA J. L., GARCÍA L.A., BLANCO G. Two *Streptomyces* species producing antibiotic, antitumor, and anti-inflammatory compounds are widespread among intertidal macroalgae and deep-sea coral reef invertebrates from the central cantabrian sea. *Microb. Ecol.* 69:512–524 (2015)
6. SEVCIKOVA, B., AND KORMANEC, J. Differential production of two antibiotics of *Streptomyces coelicolor*A3(2), actinorhodin and undecylprodigiosin, upon salt stress conditions. *Arch Microbiol* 181: 384–389 (2004)
7. STOCK, A.M. ROBINSON, V.L. GOUDREAU, P. N. Two-component signal transduction. *Annu. Rev. Biochem.* 69, 183–215 (2000)
8. HOCH, J.A. AND SILHAVY, T.J. *Two-Component Signal Transduction*, ASM Press. Washington DC., 1995.
9. RODRÍGUEZ H., RICO S., DÍAZ M., AND SANTAMARÍA R.I., Two-component systems in *Streptomyces*: key regulators of antibiotic complex pathways. *Microbial Cell Factories* 12:127. (2013)

10. KIM, D. & FORST, S. Genomic analysis of the histidine kinase family in bacteria and archaea. *Microbiology* 147, 1197–1212. (2001)
11. BENTLEY, S.D., CHATER, K.F., CERDENO-TARRAGA, A.M., CHALLIS, G.L., THOMSON, N.R., JAMES, K.D., HARRIS DE, QUAIL MA, KIESER H, HARPER D, BATEMAN A, BROWN S, CHANDRA G, CHEN CW, COLLINS M, CRONIN A, FRASER A, GOBLE A, HIDALGO J, HORNSBY T, HOWARTH S, HUANG CH, KIESER T, LARKE L, MURPHY L, OLIVER K, O'NEIL S, RABBINOWITSCH E, RAJANDREAM MA, RUTHERFORD K, RUTTER S, SEEGER K, SAUNDERS D, SHARP S, SQUARES R, SQUARES S, TAYLOR K, WARREN T, WIETZORREK A, WOODWARD J, BARRELL BG, PARKHILL J, HOPWOOD DA. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2). *Nature* 417: 141–147. (2002)
12. BISHOP, A., FIELDING, S., DYSON, P., AND HERRON, P. Systematic insertional mutagenesis of a streptomycete genome: a link between osmoadaptation and antibiotic production. *Genome Res* 14: 893–900. (2004)
13. FERNÁNDEZ. L, BISHOP A, PARKES L, DEL SOL. R, SALERNO. P, SEVCIKOVA. B, MAZURAKOVA. V, KORMANEC. J AND DYSON. P. Osmoregulation in *Streptomyces coelicolor*: modulation of SigB activity by OsaC. *Molecular Microbiology*, 71(5): 1250–1262. (2009).
14. RONG GAO AND ANN M. STOCK. Biological Insights from Structures of Two-Component Proteins. *Annu Rev Microbiol.* 63: 133–154. (2009).
15. CHEUNG J. AND HENDRICKSON W. Sensor domains of two-component regulatory systems. *Current Opinion in Microbiology.* 13:116–123. (2010)
16. FINN RD, TATE J, MISTRY J, COGGILL PC, SAMMUT SJ, HOTZ HR, CERIC G, FORSLUND K, EDDY SR, SONNHAMMER ELL, BATEMAN A. The Pfam protein families database. *Nucleic Acids Res.* 36:D281–8. (2008)
17. MARINA A, WALDBURGER CD, HENDRICKSON WA. Structure of the entire cytoplasmic portion of a sensor histidine-kinase protein. *EMBO J.* 24:4247–59. (2005)
18. HUTCHINGS MATTHEW I, HOSKISSON PAUL A, CHANDRA GOVIND AND BUTTNER MARK J. Sensing and responding to diverse extracellular signals? Analysis of the sensor kinases and response regulators of *Streptomyces coelicolor* A3(2). *Microbiology.* 150, 2795–2806. (2004)
19. RABBIND SINGH A, SENTHAMARA KANNAN PARANTHAMAN, THANGAVELA CHITRA, DANDA RAVIKANTH, PANDIAN S. KARUTHA AND DHARMALINGAM KUPPAMUTHU. ChiShistidine kinase negatively regulates the production of chitinase ChiC in *Streptomyces peucetius*. *Microbiol Res.* (2013)
20. RODRÍGUEZ H., RICO S., YEPES A., FRANCO-ECHEVERRÍA E., ANTORAZ S., SANTAMARÍA R. I., DÍAZ M. 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.* Vol.6 (2015)
21. PARK AND JI-MIN. Identification of a novel unpaired histidine sensor kinase affecting secondary metabolism and morphological differentiation in *Streptomyces acidiscabies* ATCC 49003. *Folia Microbiologica.* Volume 60, Issue 4, pp 279-287. (2015)
22. RONG GAO AND ANN M. STOCK. Biological Insights from Structures of Two-Component Proteins. *Annu Rev Microbiol.* 63: 133–154. (2009)
23. O'CONNOR, T. J., KANELLIS, P., NODWELL, J. R. The ramC gene is required for morphogenesis in *Streptomyces coelicolor* and expressed in a cell type-specific manner under the direct control of RamR. *MolMicrobiol* 45, 45–57. (2002)
24. AL-BASSAM MM, BIBB MJ, BUSH MJ, CHANDRA G, BUTTNER MJ. Response Regulator Heterodimer Formation Controls a Key Stage in *Streptomyces* Development. *PLoS Genet* 10(8): e1004554. (2014).
25. YANG, KUI, QI, LI-HUA, ZHANG, MEI, HOU, XIAN-FENG, PAN, HAI-XUE, TANG, GONG-LI, WANG, WEI, YUAN, HUA. The SARP Family Regulator Txn9 and Two-Component Response Regulator Txn11 are Key Activators for Trioxacarcin Biosynthesis in *Streptomyces bottropensis*. *Current Microbiology.* Volume 71, Issue 4, pp 458-464. (2015)
26. YOO, YOUNGJI, HWANG, JAE-YEON, SHIN, HEA-LUYUNG, CUI, HEQING, LEE, JINWON, YOON, YEOJOON. Characterization of negative regulatory genes for the biosynthesis of rapamycin in *Streptomyces rapamycinicus* and its application for improved production. *Journal of Industrial Microbiology & Biotechnology.* Volume 42, Issue 1, pp 125-135. (2015)
27. ARAVIND, L. & PONTING, C. P. The GAF domain: an evolutionary link between diverse phototransducing proteins. *Trends BiochemSci* 22, 458–459. (1997)



28. GU YZ, HOGENESCH JB, BRADFIELD CA. The PAS Superfamily: Sensors of Environmental and Developmental Signals. Annual Review of Pharmacology and Toxicology, Vol. 40, pp. 519-561. (2000)
29. Genome Project of *Streptomyces avermitilis*. NITE Incorporated Administrative Agency. National Institute of Technology and Evaluation. Japan. <http://www.ls.kitasato-u.ac.jp>
30. FERNÁNDEZ. L, BISHOP A, PARKES L, DEL SOL. R, SALERNO. P, SEVCIKOVA. B, MAZURAKOVA. V, KORMANEC. J AND DYSON. P. Osmoregulation in *Streptomyces coelicolor*: modulation of SigB activity by OsaC. Molecular Microbiology, 71(5): 1250–1262. (2009).
31. ANTÓN N, SANTOS-ABERTURAS J, MENDES M, GUERRA S, MARTÍN J, APARICIO. J. PimM, a PAS domain positive regulator of pimaricin biosynthesis in *Streptomyces natalensis*. Microbiology 153(9): 3174-3183 (2007).
32. BRODY, M. S., VIJAY, K. & PRICE, C. W. Catalytic function of an  $\alpha/\beta$  hydrolase is required for energy stress activation of the  $\sigma$ B transcription factor in *Bacillus subtilis*. J Bacteriol .183, 6422–6428. (2001).
33. AKBAR, S., GAIDENKO, T. A., KANG, C. M., O'REILLY, M., DEVINE, K. M. & PRICE, C. W. New family of regulators in the environmental signaling pathway which activates the general stress transcription factor sB of *Bacillus subtilis*. J Bacteriol. 183, 1329–1338. (2001).
34. AVILA-PEREZ, M., HELLINGWERF, K. J., KORT, R. Blue light activates the sB-dependent stress response of *Bacillus subtilis* via YtvA. J Bacteriol. 188, 6411–6414. (2006)
35. GRUBER, T. M., GROSS, C. A. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu Rev Microbiol 57, 441–466. (2003)
36. OTANI H, HIGO A, NANAMIYA H, HORINOUCI S, OHNISHI Y. An alternative sigma factor governs the principal sigma factor in *Streptomyces griseus*. MolMicrobiol. 87(6):1223-36. (2013)
37. HECKER, M., PANE' -FARRE', J. & VOELKER, U. SigB-dependent general stress response in *Bacillus subtilis* and related gram-positive bacteria. Annu Rev Microbiol. 61, 215–236. (2007)
38. PRICE, C. W. General stress response. In *Bacillus subtilis* and its Closest Relatives: from Genes to Cells, pp. 369–384. Edited by A. L. Sonenshein, J. A. Hoch & R. Losick. Washington, DC: American Society for Microbiology. (2002)
39. MITTENHUBER, G. A phylogenomic study of the general stress response sigma factor sB of *Bacillus subtilis* and its regulatory proteins. J MolMicrobiolBiotechnol 4, 427–452. (2002)
40. CHO, Y. H., LEE, E. J., AHN, B. E. & ROE, J. H. SigB, an RNA polymerase sigma factor required for osmoprotection and proper differentiation of *Streptomyces coelicolor*. MolMicrobiol 42, 205–214. (2001).
41. LEE, E. J., KAROONUTHAISIRI, N., KIM, H. S., PARK, J. H., CHA, C. J., KAO, C. M. & ROE, J. H. A master regulator  $\sigma$ B governs osmotic and oxidative response as well as differentiation via a network of sigma factors in *Streptomyces coelicolor*. MolMicrobiol 57, 1252–1264. (2005)
42. VIOLLIER, P. H., KELEMEN, G. H., DALE, G. E., NGUYEN, K. T., BUTTNER, M. J. & THOMPSON, C. J.. Specialized osmotic stress response systems involve multiple SigB-like sigma factors in *Streptomyces coelicolor*. MolMicrobiol. 47, 699–714. (2003)
43. KELEMEN, G.H., VIOLLIER, P.H., TENOR, J., MARRI, L., BUTTNER, M.J., AND THOMPSON, C.J. A connection between stress and development in the multicellular prokaryote *Streptomyces coelicolor* A3(2). MolMicrobiol 40: 804–814. (2001)
44. SEVCIKOVA, B., BENADA, O., KOFRONOVA, O., AND KORMANEC, J. Stress-response sigma factor sigma (H) is essential for morphological differentiation of *Streptomyces coelicolor*A3(2). Arch Microbiol 177: 98–106. (2001)
45. WEI ZH, WU H, BAI L, DENG Z, ZHONG JJ. Temperature shift-induced reactive oxygen species enhanced validamycin A production in fermentation of *Streptomyces hygroscopicus* 5008. Bioprocess Biosyst Eng. 35(8):1309-16. (2012).
46. SHUAI LUO, DI SUN, JIANYA ZHU, ZHI CHEN, YING WEN, JILUN LI. An extracytoplasmic function sigma factor,  $\sigma$ 25, differentially regulates avermectin and oligomycin biosynthesis in *Streptomyces avermitilis*. Applied Microbiology and Biotechnology. 98, 7097-7112. (2014).
47. LIU, SHUI-PING, YU, PIN, YUAN, PENG-HUI, ZHOU, ZHEN-XING, BU, QING-TING, MAO, XU-MING, LI, YONG-QUAN. Sigma factor WhiGch positively regulates natamycin production in *Streptomyces chattanoogensis*L10. Applied Microbiology and Biotechnology. Volume 99, Issue 6, pp 2715-2726. (2015)
48. DE BEEN M, FRANCKE. C, SIEZEN. R AND ABEE. T. Novel sB regulation modules of Gram-positive bacteria involve the use of complex hybrid histidine kinases. Microbiology, 157: 3–12. (2011).
49. AIT-OUAZZOU. A, MAÑAS. P, CONDÓN. S, PAGÁN. R AND GARCÍA. D. Role of general stress-response alternative sigma factors  $\sigma$ S (RpoS) and  $\sigma$ B (SigB) in bacterial heat resistance as a function of treatment medium pH. International Journal of Food Microbiology 153: 358–364. (2012).