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Assessing the Effect of Mg^{2+} ion on the Regulation of *Mycobacterium tuberculosis* PhoRP Two Component System through the Development of Mathematical Model

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Abstract: ***Mycobacterium tuberculosis is the third most infectious pathogen that causes tuberculosis and claims approximately 1.3 million lives each year across the globe. This pathogen sets up various stages of infection when it comes in contact with host macrophages. The activation and regulation of pathogenicity causing genes are carried out by PhoRP two component systems (TCS) being stimulated by Mg^{2+} ions in the surrounding medium of pathogen. Every TCS consists of two regulatory proteins, sensor kinase (PhoR) and response regulator (PhoP). In order to understand behavior of TCS under the effect of Mg^{2+} ions, a mathematical model was developed and validated from the existing data. The simulation of the model was carried out through MATLAB using RK-4 (Runga Kutta fourth order differential equation) method. Resultantly, behavior of TCS was found to be robust at all concentration of Mg^{2+} ions. The finding can be implicated at the time of development of drug against tuberculosis as to which gene/protein has the high sensitivity towards its stimuli.***

Keywords: Autophosphorylation, Response Regulator, Sensor kinase, Two-component system

1. Introduction

The bacterial two component system (TCS) is a kind of signal transduction system that senses environmental stimuli and responses accordingly. This system consists, mainly of two regultory proteins one of which functions as histidine kinase (HK) and other fucntions as response regultor (RR) in the course of signal cascade mechanism[1]. *Mycobacterium tuberculosis* possesses eleven two component systems controlling expression of those genes that are critically involved in the virulence, pathogenicity and survival[2]. Studies have demonstrated that PhoPR-TCS is one of the eleven TCSs peculiarly involved in the virulent activity of the pathogen[3]. PhoPR-TCS is a positive regultor of many genes which encodes gene for the biosynthesis of lipids like sulphatides(SL), diacyltrehalose (DAT) and polycyltrehalose (PAT[4]. These lipid components contribute to the virulency of *M. tuberculosis.* Studies have corroborated that *pks2* and *msl3* are responsible for the biosynthesis of SL, DAT and PAT respectively[5]. The expression of these lipid coding genes are regulated by PhoP in association with the autokinase activity of PhoR[6].

The mycobacterial PhoP protein closely resembles the PhoP of *Salmonella typhymurium* and has the similar effects over gene expression[7]. The only differnce between the two is stimulus in the surrounding medium and genes of interest being controlled by them. *Salmonella typhymurium* TCS responds to divalent cations, Ca^{2+} ions, pH and antimicrobial peptides as its enviromnetal stimuli. This system specifically responds to Mg^{2+} ions available in the surrounding medium of the pathogen[8,9]. PhoR protein respnoses to environmental stimuli and propagates signals. The phosphotransfer reaction between PhoQ* (activated form) and PhoP takes place, followed by the activation of PhoP[8]. In case of *Mycobacterial* PhoPR TCS, Mg^{2+} ions have not been substatilally proved to be stimulating factor for PhoR. Same as *Mycobacterium tuberculosis* TCS, *Bacilus subtilis* TCS senses phosphate as its environmental stimulus[10]. This TCS is activated when phosphate is limited in the medium, regulates, expression of 31phosphate utilizing genes[11].

In above all cases, cations or anions have been demonstrated to be stimulating factors except for *M. tuberculosis.* Some of the studies and wet lab analyses have corroborated that Mg^{2+} functions as the stimulating factor for PhoPR, but most of them completely abnegate these informations and sophisticate that stimulus has yet been unknown for this TCS. The analyses carried out by Shaun B. Walter have reveled stimulating roles of Mg^{2+} ions and their association with *M. tuberculosis* TCS. When Pathogen is allowed to grow in a Mg^{2+} deficient environment its growth is abolished to some extent and then restored back when more cations are added into the medium.

In *Salmonella typhimurium* PhoPQ-TCS PhoP, primarily, activates P-type ATPase transporter encoding genes. ATPase imports divalent cations into cell[12]. When concentration of Mg^{2+} ions in the cell is excessed, the cytoplasmic domain of PhoQ gets automatically dephosphorylated and phosphates are replaced by Mg^{2+} ions causing a change in confromation of PhoQ followed by abolishmnet of signal transduction. Thus gene activation process is completely stopped and no Mg^{2+} ion transportation takes place afterward[13]. Mg^{2+} ions aid in strenthening and stablishing the cell wall of the bacterium[14]. Moreover, the concentration of Mg^{2+} ions in the cell determines the transcriptions of corresponding regulon. No data suggest the substantial intimacy of Mg^{2+} with *M. tuberculosis* PhoPR-TCS but significant effect of this cation over growth of the bacterium drag us towards a

novel set of hypothesis. Here we propose that Mg^{2+} stimulates the *M. tuberculosis* PhoPR-TCS and controls its regulation and indirectly affects the expression of gene.

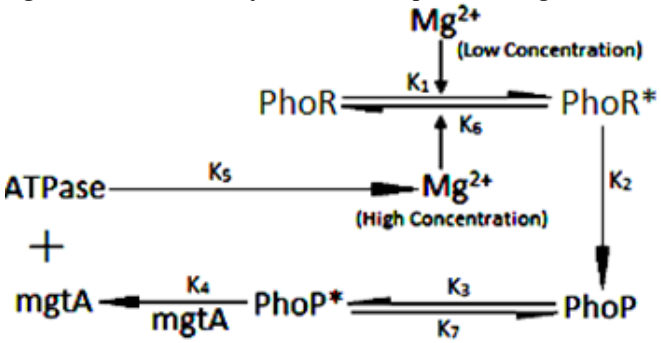

Figure 1: General presentation of the model, depicting the feedback mechanism of the system

2. Model Description

It is signal transduction process where each species are collide with certain species to forward the signal to other species. All species participating in the reactions had certain half life and some initial concentration in the cell on the basis of which the rate of reactions was calculated. Initially, PhoR is autophosphorylated (PhoR*), a phosphate group was automatically attached to the conserved histidine residue of protein in the absence of Mg^{2+} ions at the rate of $K_1 = 0.021\ s^{-1}$[15][16]. PhoR* transfererd phospahate to PhoP, cognate response regulator, to the conserved aspartate residue via phosphotransfer reaction and made PhoP activate (PhoP*) at a reaction rate of $K_2 = 0.002723\ M^{-1}\ s^{-1}$[16]. By nature, PhoP* is a positive regulator i.e., never found to suppress the expression of any gene, hence involved in the process of activation of genes only. The activation of *mgtA* was carried out by PhoP* at a rate of $K_3 = 0.0000395\ M^{-1}\ s^{-1}$[17]. In this reaction, expression of *mgtA* had been controlled by Phop* which is dependent of autokinase activity of PhoR* and phosphotransfer reaction, implied that both proteins are under the control of Mg^{2+} concentration. The moderate concentration of Mg^{2+} was maintained by ATPase transporter in order to ful fill the need of pathogen[18]. ATPase transported Mg^{2+} into cell thereby increasing its intracellular concentration. Bacterium utilized Mg^{2+} ions in cell wall modifiation (cation aided stablishment and LPS modifications) and metabolism[19]. The transportation of Mg^{2+} ions took place at the pace of $K_4 = 0.000039\ s^{-1}$, as calculated from the half life of PhoR*, much lower than the rate of its activation or interaction stimulated by ions[20]. The excess amount of Mg^{2+} in the cell bound with PhoR* by replacing phosphate group from histidine residue and thus a complex of [PhoR*-Mg^{2+}] was formed at the rate of $K_5 = 0.001408\ M^{-1}\ s^{-1}$, indicating that dephosphorylation of PhoR* was a time taking process being accomplished in two steps[12][21]. The complex of [PhoR*-Mg^{2+}] turned PhoR* in its deactivated form (PhoR) and the phosphoryl group was completely replaced by Mg^{2+} at the rate of $K_6 = 0.0014189\ M^{-1}\ s^{-1}$ [12][22]. Simultaneously, PhoP* was deactivated as PhoR* restored back into PhoR and activity of PhoP* was abolished at a rate of $K_7 = 0.0012\ M^{-1}\ s^{-1}$[12].

Here, question arise about the reveresible phosphatase activity of PhoR*. How does it promote the removal of Pi from PhoP* when concentration of Mg^{2+} ions in the cell reaches higher? It has been proposed that dephosphorylation of phospho-PhoP involves the reversal of phosphate transfer from aspartate residue in PhoP back to histidine residue in PhoR [7][12]. From In vitro study, it has not yet been cleared that PhoR has pleiotropic role in signal transduction mechanism possessing autokinase activity and phosphatase activity as well. From the description of the model presented (Fig:1) it has been cleared that PhoR-PhoP two component system is a self regulatory system, essential for the expression of specific genes to withstand the environmental perturbation and fulfill the need of prolonged survivalof pahtogen.

Table 1: Molecular species with their respective description and notation used in the simulation, (*mgtA*, ATPase coding gene has been constant throughout the system).

Sl.	Molecular Species	Cellular State	Notation
1.	PhoR	Unphosphorylated sensor kinase	X1
2.	PhoR*	Phosphorylated sensor kinase	X2
3.	PhoP	Unphosphorylated/ inactivated response regulator	X3
4.	PhoP*	Phosphorylated/ activated response regulator	X4
5.	ATPase	Mg^{2+} transporter	X5
6.	Mg^{2+}	Inducer of PhoR	X6
7.	PhoR*Mg^{2+}	Complex of inducer and activated kinase	X7

Table 2: The rate of reaction and reaction constants of the species

Sl.	*Rate constant*	*Reaction Name*	*Rate constant*
1.	K_1	Autophosphorylation of PhoR	$0.021\ M^{-1}\ s^{-1}$
2.	K_2	Phosphotransfer of PhoP	$0.002723\ M^{-1}\ s^{-1}$
3.	K_3	Expression of ATPase activation of *mgTA*	$0.0000395\ M^{-1}\ s^{-1}$
4.	K_4	Transportaion of Mg^{2+} by ATPase	$0.000039\ M^{-1}\ s^{-1}$
5.	K_5	Formation of Mg^{2+}-PhoR* complex	$0.001408\ M^{-1}\ s^{-1}$
6.	K_6	Deactivation of PhoR* into PhoR	$0.0014189\ M^{-1}\ s^{-1}$
7.	K_7	Deactivation of PhoP* into PhoP	$0.021\ M^{-1}\ s^{-1}$

Table 3: Reaction channals and mathematical model inferred from proposed mathematical model

Sl.	Reaction Channals	Mathematical Model
1.	$x_1 \xrightarrow{k_1} x_2$	$\frac{dx_1}{dt} = -k_1[x_1] + k_6[x_7]$
2.	$x_3 + x_2 \xrightarrow{k_2} x_4$	$\frac{dx_2}{dt} = k_1[x_2] - k_2[x_3][x_2] - k_5[x_6][x_2]$
3.	$x_4 + mgtA \xrightarrow{k_3} x_5 + mgtA$	$\frac{dx_3}{dt} = -k_2[x_3][x_2] + k_7[x_7][x_4]$
4.	$x_5 \xrightarrow{k_4} x_6 + x_5$	$\frac{dx_4}{dt} = k_2[x_3][x_2] - k_3[x_4]MgtA - k_7[x_7][x_4]$
5.	$x_6 + x_2 \xrightarrow{k_5} x_7$	$\frac{dx_5}{dt} = k_3[x_4]MgtA$
6.	$x_7 \xrightarrow{k_6} x_1$	$\frac{dx_6}{dt} = k_4[x_5] - k_5[x_6][x_2]$
7.	$x_7 + x_4 \xrightarrow{k_7} x_3$	$\frac{dx_7}{dt} = k_5[x_6][x_2] - k_6[x_7] - k_7[x_7][x_4]$

3. Results

The proposed mathematical model was subjected under MATLAB programming using RK-4(Runga Kutta fourth order differential equation) that generated different simulations at different concentrations of Mg^{2+} ions(Fig 2a., 2b., 2c., and 2d.).

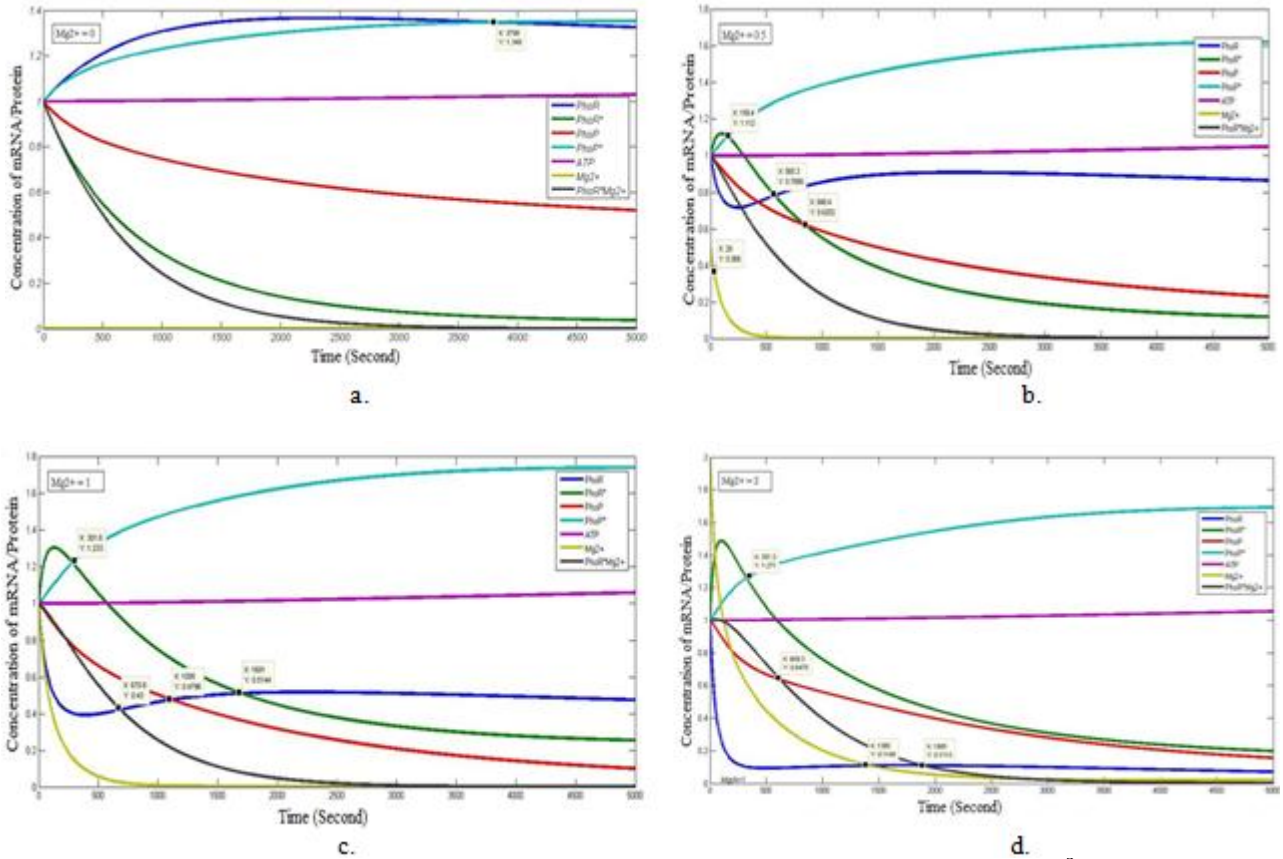

Figure 2: Simulation results (a, b, c & d) of the proposed model at different concentration of Mg^{+2} ion, PhoRP Two Component system of Mycobacterium tuberculsosi

The first simulation (fig-2a) was generated at zero concentration of Mg^{+2} ions. At this concentration of ion, both components (PhoR and PhoP) showed a significant variation of activation. At 1.348 mol/L concentration, both proetins were synchronized but a little deviation in PhoR was observed, followed by its stability, at later stage. It has been noticed that signal can not be transduced without activation of PhoR which strictly requires an optimal amount to be maintained. The amount of PhoR was more than PhoR* that did not synchronize with the amount of PhoP

and PhoP*. This was accounted mainly due to the the different nature and function of both of the proteins. The activation and signal tranduction through forming the complex of PhoR*PhoP was also hampered in absence of the ions, comparable with the optimal concentration of mRNA/protein. Oveall, the simulation revealed a normal physiological condition of the signaling process of mycobacterial two component system. The result confirmed that at zero concentration of the ions, PhoR* and PhoP* were not completely ceased out of the system.

Simulation showed a great change with the change in the concentration of ions from 0 to 0.5 (fig-2b). Both PhoR and PhoR* increased gradually from their previous concentration and synchronized at a concentration of 0.7886 mol/L and then became constant. The sudden fluctuation in the level of PhoR* caused by increasing concentration of ions affected the PhoP* positively, making the protein to increase higher in concentration and retaintion in the system as well. The increase and decrease in both type of proteins is dependent of their interaction and transfer of inorganic phospahte molecule being affected by concentration/level of Mg^{2+} ion present in the system. After all, the singnal transduction in TCS has to be achieved with opmital amount of PhoP*, set as more than 1.0 mol/L concentration of mRNA/protein. PhoP, as stated earlier, is the response regulator of TCS. It has to be converted into PhoP* through phosphotransfer reaction of PhoR*. In the simulation, at this level of $Mg^{2+,}$ PhoP* achieved its goal that of reaching to an optimal amount in order to activate the genes required to carry out necessary change in the pathogen. Moreover, the increased level of PhoP* coincided with eventuality of the signal transduction in TCS. Autoactivation of PhoR into PhoR* was followed by activation of PhoP into PhoP*. PhoP* and PhoR* synchronized by 301.6 seconds with a concentration of 1.233 mol/L. From this point of synchronization, PhoR* rapidly decreased down and became constant by 1681 seconds with decreased concentration of 0.5144 mol/L. The complex of PhoR*Mg^{2+} was shown with decreased amount because binding of Mg^{2+} with PhoR deactivates PhoR* in the system. Mg^{2+} showed the similar trend as that of PhoR*Mg^{2+} complex, because it is utilized by the system at fast rate to meet the requirements of the pahtogen.

Other simulation results depicted the same pattern of fluctuations in the concentration of proteins (fig-2c and 2d). The only difference which could be observed, was the effect of increased Mg^{2+} ions over the rapid response of PhoR*. Higher the concentration, fig-4; 1 mol/L, faster the response. The period of decreasing for PhoR* after activation had been lasting more with the increased concentration of ions (fig-2d). Other cmponenets like ATP, PhoR*Mg^{2+} complex and PhoP remained same throughout the system and did nto show any significant change/fluctuation in their concentration over the varied concentration of Mg^{2+} ions.

4. Discussion

Mycobacterium uberculosis requires Mg^{2+} ions to grow, modify and strenthen its cell that make hefty protective environment against immune system of the host [21]. The amount of Mg^{2+} ions is crucial in terms of regulating the TCS because its concentration decides activation/deactivation of genes through signal cascade maechanism. [22]. The TCS at different concentration of the ions showed different behaviour. The response time by both (PhoR and PhoP) represented the eventuality of the system. PhoR autophosphorylated first follwed by the phosphorylation (activation) of PhoP. The activation of PhoP into phoP* was concerned with the activation of necessary gene in order to bring out vital changes, mostly that of bacterial growth and modification of its cell wall. PhoP* was shown to be the most crucial among all as it had to finally activate (via transfer of inorganic phosphate) the genes of interest. Significantly, PhoP* remained high in concentration in the system even after the decreased level of the sensorr kinase. The concentration of PhP* did not seem to be changed or affected by the presense or absence of Mg^{2+} ions. From 0-1 (fig-2a, 2b and 2c) unit of concentration of Mg^{2+} PhoP* showed noticable increase in being static, whereas at 2 unit of concentration, it showed gradual decrease in becoming constant. PhoR* was also shifted accordingly which seemingly acted first and directly affected by PhoP. Eventually, higher the PhoP* lesser the PhoR*, because PhoP* preferentially activates *mgtA* that causes rapid influx of Mg^{2+} ions. After being utilized, the remainig ions were bound to sensor kinase and deactivate PhoR*, hence signal cascade is terminated. The time required by both of the components was managed very well in the system in the course of signal transduction. Consistency in activation of the these components corroborated that a little amount of PhoP* was always maintained in the system making it robust in ion starving condition. It was also confirmed that ions had both postive and negative effect over TCS. The fluctuation in level of the components confirmed that PhoR was activated for a short period of time and synchronized with PhoP* in no time. The result represented the sensitivity of ions towrds PhoR in the cellular environment of *Mycobacterium tubebeculosis* that how rapidly TCS managed to meet the gene activation. The optimal amount of PhoP at all concentration of ions strengthened the hypothesis that genes are activated and expressed as long as pathogen survive in the defensive environment of host.

5. Conclusion

The regulation of TCS is affected by Mg^{2+} ions to all possible extent which was shown by fluctuations in the level of PhoP and PhoR proteins. The ions have both positive and negative effect over TCS. The result showed that important genes are activated even after ions are switched off from surrounding medium. So, targeting of ions influx and efflux would be of no use in terms of development of drug aginst the pathogen. With some other aspect it can be further tested for more simulations with varying concentration of ions. Since, TCS regulates those genes which are directly involved in pathogenecity and survival of *Mycobaterium tuberculosis*, understanding the nature and behaiour of individual protein will provide an insight into finding of novel drug target against tuberculosis. The simulation in this work represented the mechanism of gene regulation and its sensitivity twords stimulus and provided the understading about how to deal with when targetting a molecule/protein for any other two component system of the pathogen.

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References

[1] T. C. Zahrt and V. Deretic, "Mycobacterium tuberculosis signal transduction system required for persistent infections," Proc. Natl. Acad. Sci., vol. 98, no. 22, pp. 12706–12711, Oct. 2001.
[2] S. Malhotra, V. J. Cook, J. N. Wolfe, P. Tang, K. Elwood, and M. K. Sharma, "A mutation in Mycobacterium tuberculosis rpoB gene confers rifampin resistance in three HIV-TB cases," Tuberculosis, vol. 90, no. 2, pp. 152–157, Mar. 2010.
[3] E. Pérez, S. Samper, Y. Bordas, C. Guilhot, B. Gicquel, and C. Martín, "An essential role for phoP in Mycobacterium tuberculosis virulence," Mol. Microbiol., vol. 41, no. 1, pp. 179–187, Jul. 2001.
[4] J. Gonzalo Asensio, C. Maia, N. L. Ferrer, N. Barilone, F. Laval, C. Y. Soto, N. Winter, M. Daffé, B. Gicquel, C. Martín, and M. Jackson, "The virulence-associated two-component PhoP-PhoR system controls the biosynthesis of polyketide-derived lipids in Mycobacterium tuberculosis," J. Biol. Chem., vol. 281, no. 3, pp. 1313–1316, Jan. 2006.
[5] S. B. Walters, E. Dubnau, I. Kolesnikova, F. Laval, M. Daffe, and I. Smith, "The Mycobacterium tuberculosis PhoPR two-component system regulates genes essential for virulence and complex lipid biosynthesis," Mol. Microbiol., vol. 60, no. 2, pp. 312–330, Apr. 2006.
[6] M.-L. Chesne-Seck, N. Barilone, F. Boudou, J. Gonzalo Asensio, P. E. Kolattukudy, C. Martín, S. T. Cole, B. Gicquel, D. N. Gopaul, and M. Jackson, "A point mutation in the two-component regulator PhoP-PhoR accounts for the absence of polyketide-derived acyltrehaloses but not that of phthiocerol dimycocerosates in Mycobacterium tuberculosis H37Ra," J. Bacteriol., vol. 190, no. 4, pp. 1329–1334, Feb. 2008.
[7] E. A. Groisman, "The pleiotropic two-component regulatory system PhoP-PhoQ," J. Bacteriol., vol. 183, no. 6, pp. 1835–1842, Mar. 2001.
[8] J. B. McPhee, M. Bains, G. Winsor, S. Lewenza, A. Kwasnicka, M. D. Brazas, F. S. L. Brinkman, and R. E. W. Hancock, "Contribution of the PhoP-PhoQ and PmrA-PmrB two-component regulatory systems to Mg2+-induced gene regulation in Pseudomonas aeruginosa," J. Bacteriol., vol. 188, no. 11, pp. 3995–4006, Jun. 2006.
[9] S. Silver and M. Walderhaug, "Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria," Microbiol. Rev., vol. 56, no. 1, pp. 195–228, Mar. 1992.
[10] M. Cimino, C. Thomas, A. Namouchi, S. Dubrac, B. Gicquel, and D. N. Gopaul, "Identification of DNA binding motifs of the Mycobacterium tuberculosis PhoP/PhoR two-component signal transduction system," PloS One, vol. 7, no. 8, p. e42876, 2012.
[11] C. Johnston, D. A. Pegues, C. J. Hueck, A. Lee, and S. I. Miller, "Transcriptional activation of Salmonella typhimurium invasion genes by a member of the phosphorylated response-regulator superfamily," Mol. Microbiol., vol. 22, no. 4, pp. 715–727, Nov. 1996.
[12] M. E. Castelli, E. García Véscovi, and F. C. Soncini, "The phosphatase activity is the target for Mg2+ regulation of the sensor protein PhoQ in Salmonella," J. Biol. Chem., vol. 275, no. 30, pp. 22948–22954, Jul. 2000.
[13] M. D. Snavely, C. G. Miller, and M. E. Maguire, "The mgtB Mg2+ transport locus of Salmonella typhimurium encodes a P-type ATPase," J. Biol. Chem., vol. 266, no. 2, pp. 815–823, Jan. 1991.
[14] J.-P. Lavigne, D. O'callaghan, and A.-B. Blanc-Potard, "Requirement of MgtC for Brucella suis intramacrophage growth: a potential mechanism shared by Salmonella enterica and Mycobacterium tuberculosis for adaptation to a low-Mg2+ environment," Infect. Immun., vol. 73, no. 5, pp. 3160–3163, May 2005.
[15] C. D. Waldburger and R. T. Sauer, "Signal detection by the PhoQ sensor-transmitter. Characterization of the sensor domain and a response-impaired mutant that identifies ligand-binding determinants," J. Biol. Chem., vol. 271, no. 43, pp. 26630–26636, Oct. 1996.
[16] J. Barchiesi, M. E. Castelli, F. C. Soncini, and E. G. Véscovi, "mgtA Expression is induced by rob overexpression and mediates a Salmonella enterica resistance phenotype," J. Bacteriol., vol. 190, no. 14, pp. 4951–4958, Jul. 2008.
[17] L. F. Kox, M. M. Wösten, and E. A. Groisman, "A small protein that mediates the activation of a two-component system by another two-component system," EMBO J., vol. 19, no. 8, pp. 1861–1872, Apr. 2000.
[18] F. Garcia-del Portillo, J. W. Foster, and B. B. Finlay, "Role of acid tolerance response genes in Salmonella typhimurium virulence," Infect. Immun., vol. 61, no. 10, pp. 4489–4492, Oct. 1993.
[19] J.-P. Lavigne, D. O'callaghan, and A.-B. Blanc-Potard, "Requirement of MgtC for Brucella suis intramacrophage growth: a potential mechanism shared by Salmonella enterica and Mycobacterium tuberculosis for adaptation to a low-Mg2+ environment," Infect. Immun., vol. 73, no. 5, pp. 3160–3163, May 2005.
[20] D. Agranoff and S. Krishna, "Metal ion transport and regulation in Mycobacterium tuberculosis," Front. Biosci. J. Virtual Libr., vol. 9, pp. 2996–3006, Sep. 2004.
[21] D. Agranoff and S. Krishna, "Metal ion transport and regulation in Mycobacterium tuberculosis," Front. Biosci. J. Virtual Libr., vol. 9, pp. 2996–3006, Sep. 2004.
[22] D. K. Saini, "DevR-DevS is a bona fide two-component system of Mycobacterium tuberculosis that is hypoxia-responsive in the absence of the DNA-binding domain of DevR," Microbiology, vol. 150, no. 4, pp. 865–875, Apr. 2004.