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Universitas Scientiarum

Univ. Sci. 2013, Vol. 18 (2): 189-202
doi: 10.11144/Javeriana.SC18-2.sfts

ORIGINAL PAPER

Structural features of the two-component system LisR/LisK suggests multiple responses for the adaptation and survival of *Listeria monocytogenes*

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Abstract

Here, we characterized the structure of the two-component regulatory system, LisR/LisK, in *Listeria monocytogenes*. To predict the structure of both proteins and the relationship between them, we employed several bioinformatic tools and databases. Based on our results, LisK protein is embedded in the cell membrane and its modular composition (HAMP, histidine kinase and ATPase domains) is associated with its autophosphorylation (His-266). A stimulus-response likely determines the sequential signal propagation from the bacterial cell surface to its cytoplasmic components. According to our results, LisR is a cytoplasmic protein with a receptor domain (homologous to CheY) that comprises a phospho-acceptor residue (Asp-52) and a DNA-binding domain, which may allow the transmission of a specific transcriptional response. LisR/LisK has been experimentally characterized both biochemically and functionally in other *Bacilli* pathophysiology; our structure-function approach may facilitate the design of suitable inhibitors.

Keywords: *Listeria monocytogenes*; LisR/LisK; two-component regulatory systems; protein histidine kinase.

Edited by George Barreto ✉ & Alberto Acosta

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Received: 12-04-2013 **Accepted:** 30-07-2013
Published on line: 20-08-2013

Citation: Arenas N, Gutiérrez A, Sánchez M, Salazar LM, Reyes E. (2013) Structural features of the two-component system LisR/LisK suggests multiple responses for the adaptation and survival of Listeria monocytogenes. *Universitas Scientiarum* 18(2): 189-202 doi: 10.11144/Javeriana.SC18-2.sfts.

Funding: Universidad Nacional de Colombia - Universidad de Ciencias Aplicadas y Ambientales.

Electronic supplementary material: 1

SICI: 2027-1352(201305/08)18:2<189:SFOTTSLSMRFTAASOLM>2.0.TS;2-U

Introduction

Listeria monocytogenes is a ubiquitous pathogen that is well adapted to its environment; it can successfully infect animals and humans, primarily immunocompromised individuals, pregnant women and newborns; consumed in contaminated food, the pathogen can produce infection and even cause death (Freitag et al. 2009). While it is not the most prevalent foodborne bacterium, *L. monocytogenes* outbreaks have been recorded since 1981; it has significantly impacted human morbidity and mortality (annual incidence varies from 0.1 to 0.7 cases/100000 inhabitants) and has generated economic losses in high-income countries from

1996 to 2005 (Warriner & Namvar 2009). The bacilli´s ecological ubiquity, its stress tolerance and high virulence have made the implementation of guidelines to control listeriosis outbreaks difficult. Therefore, finding potential drugs and methods to allow the early diagnosis and control *L. monocytogenes* outbreaks is essential.

L. monocytogenes has effectively adapted to the selective pressures generated by industrial and manufacturing food preservation procedures. The preservation of its own homeostatic balance and bacterial colonization is determined by its response to these environments. Several of these external stimuli are decoded and processed by versatile signaling proteins, including two-component regulatory systems, transcription factors and phosphatases (Gandhi 2007). Because the *L. monocytogenes* genome encodes a wide array of signal transduction proteins, *L. monocytogenes* bacilli have been catalogued as "the multifaceted model" for bacterial signal transduction research (Hamon et al. 2006). The availability of the *L. monocytogenes* genome sequence has facilitated the development of new approaches to determine the function of gene products and their possible roles in pathogenesis (Glaser et al. 2001).

In this work, we focused on the LisR/LisK proteins of *L. monocytogenes*. The LisK gene encodes a histidine kinase sensor, and LisR encodes a transcriptional regulator; together, the genes comprise an essential two-component regulatory system. The inactivation of LisK results in the high attenuation of activity of *L. monocytogenes* strains and growth variations in acidic conditions; accordingly, the mutant displays impaired proliferation in BALB/c mouse spleens (Cotter et al. 1999). LisR/LisK mutants can grow at alkaline pH values; the proteins are deduced to support oxidative, ethanol stress and high salt concentrations. These mutants also display reduced tolerance to moderate concentrations of certain antibiotics, such as nisin and some cephalosporins (Cotter et al. 2002; Sleator and Hill 2005).

The molecular mechanisms used by *L. monocytogenes* to survive are uncertain and no crystal structures are obtainable for the LisR/LisK two-component system, in fact, key questions regarding the life-cycle biology and environmental responses mediated by LisR/LisK still remain unanswered (Vazquez-Boland et al. 2001). Therefore, studies on the structure and phylogenetic features of LisR/LisK proteins may promote a better understanding of their roles in functional stress and their response mechanisms so that they may be used in new pharmacological applications (Rowland et al. 2004). The purpose of this study is to provide insights into the unique structural features of the LisK sensor and the LisR chemoreceptor and to elucidate specific protein-protein interactions and the mechanism of phosphotransfer between them.

Materials and methods

Sequence screening and alignments: We obtained FASTA sequences for LisK (genome accession number LMO01378, GenBank accession code CAC99456.1, 483 residues long) and LisR (genome accession number LMO01377, GenBank accession number CAC99455.1, 226 residues long) from the ListiList (http://genolist.pasteur.fr/ListiList/index.html) and Uniprot (http://www.uniprot.org/) databases. We screened homolog sequences using the PSI-BLAST system (http://blast.ncbi.nlm.nih.gov/) and default parameters (Altschul et al. 1997) using the *L. monocytogenes* LisR/LisK sequences as queries. Very similar sequences were used to generate multiple sequence alignments using the ClustalW server (http://align.genome.jp/); because it was the best choice for homology analysis, we set the weight matrix to Blosum.

Domain search and homology modeling: We performed the domain assignment using the SMART (http://smart.embl-heidelberg.de/) database search engine (Letunic et al. 2008) together with the Pfam database of domains and protein families (http://pfam.sanger.ac.uk/) (Finn et al. 2006) and NCBI's Conserved Domain Database (CDD) (Marchler-Bauer et al. 2009). Domain schematic representation was performed using DOG, the domain illustrator program (Ren et al. 2009). We assessed membrane

association using a consensus of transmembrane topology prediction tools: TopPred (http://mobyle.pasteur.fr/cgi-bin/portal.py?form=toppred), TMPred (http://www.ch.embnet.org/software/TMPRED_form.html) and TMHMM (http://www.cbs.dtu.dk/services/TMHMM/) (Hofmann & Stoffel 1993; Claros & Heijne 1994; Krogh et al. 2001). Using the PSORT server (http://psort.hgc.jp/), we established the subcellular location was (Gardy et al. 2003) and determined potential phosphorylation sites using the KinasePhos server (http://kinasephos.mbc.nctu.edu.tw/index.php) (Huang et al. 2005). To estimate possible interactions and partner proteins, we used the String 8.0 database and web-tool (Search Tool for the Retrieval of Interacting Genes/Proteins; http://string-db.org/) to then assess the involvement of *L. monocytogenes* LisR/LisK in metabolic pathways or cellular processes (Jensen et al. 2009).

A LisK fragment (residue range 226 to 479) incorporating its intracellular domains was modeled based on the crystal structure of the entire cytoplasmic portion of a *Thermotoga maritima* sensor histidine-kinase protein (pdb code 2C2A) solved at 1.9 Å resolution (Marina et al. 2005) using the Swiss Model server (http://swissmodel.expasy.org/) (Arnold et al. 2006). PrrA was selected as a good template for homology modeling because the X-ray crystal structure of the response regulatory protein PrrA (resolution 1.77 Å) from *Mycobacterium tuberculosis* (pdb code 1YS6) (Nowak et al. 2006) shares 41% sequence identity with LisR (residue range from 2 to 225). We assessed the overall stereo-chemical quality of the models using a Ramachandran-plot through RAMPAGE analysis (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) (Lovell et al. 2003). Using the Chimera program, we visualized the model and generated the drawings (Pettersen et al. 2004). For further analysis we used the Profunc server (http://www.ebi.ac.uk/thornton-srv/databases/profunc/) (Laskowski et al. 2005) to examine the three-dimensional models to find other LisR functions. Lastly, we placed the models in the protein PMDB model database (Castrignano et al. 2006).

Phylogenetic analyses: We used two approaches to examine the evolutionary relationship between LisR and LisK proteins. First, we calculated phylogenetic trees using MEGA4.1 (Tamura et al. 2007) using the neighbor-joining (NJ) method (Saitou & Nei 1987). We inferred the bootstrap consensus tree from 5000 replicates and used it to represent the evolutionary history of the two-component system. The evolutionary distances were computed using the Poisson correction method (Nei & Kumar 2000).

In the second approach, we analyzed the same alignments using ProtTest (Abascal et al. 2005) to determine the protein evolution model that was the best fit for the LisR/LisK sequences. Using Phyml 3.0.1 (Guindon & Gascuel 2003) with 1000 bootstrap repeats, we carried out the phylogenetic reconstruction and visualized the phylogenetic tree using the NJplot software program (Perrière & Gouy 1996); the best tree topology is shown in the results section.

Results

Domain assessment and subcellular location suggest that LisK functions as an environmental receptor and LisR functions as a signal transducer: Our LisK transmembrane helix calculation denotes two primary helices, expected to form distinct hydrophobic domains, embedded within the membrane bilayer. The first helix is located at the N-terminus and spans the membrane (from residues 17-37, designed TM-1) from the cytosolic side to the extracellular side, trailed by a large loop that defines a periplasmic-sensing region (shaped by 139 residues, unrecognized as a domain in the databases). LisK is additionally membrane-anchored by its second transmembrane helix (from amino acids 177-197, TM-2) (**Figure 1a**). This topology indicates that LisK is like a polytopic membrane protein; its functional domains are arranged on the cytoplasmic face of the membrane toward the C-terminal end.

The domain prediction of LisK established the following three functional domains: HAMP (residues 201-255; E-value: 7.04e-32), histidine-kinase (residues 256-323; E-value: 5.82e-15) and ATPase (residues 367-479; E-value: 7.04e-32). According to the Pfam database, these domains

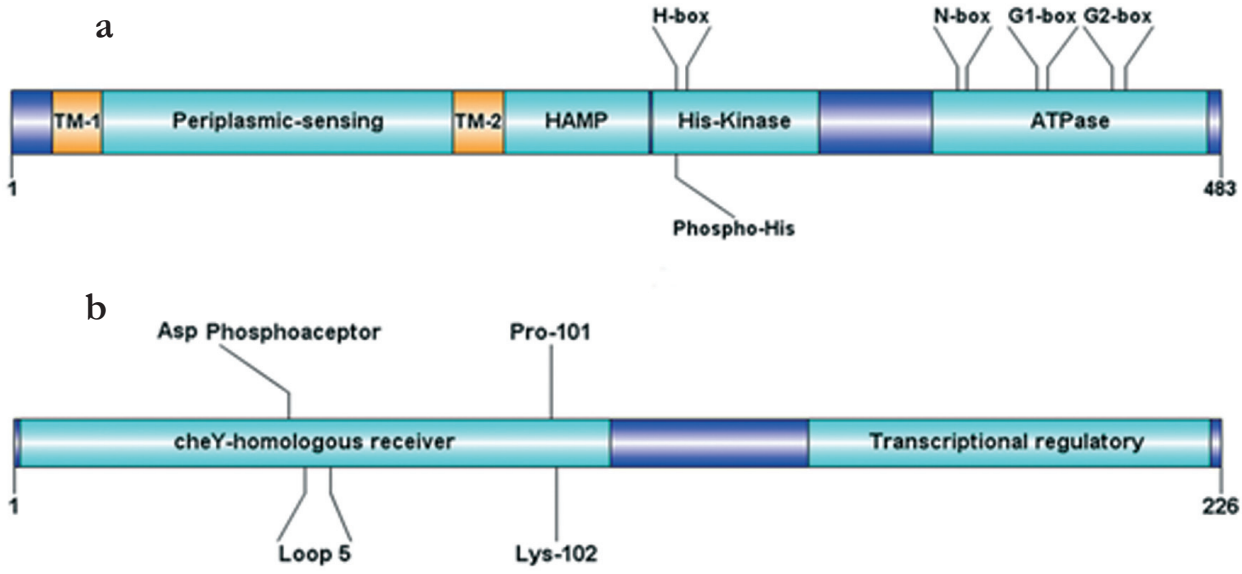

Fig. 1. a. Domain composition of the LisK sensor protein. The following domains were identified: HAMP (found on histidine kinases, adenylyl cyclases, methyl-accepting chemotaxis proteins and phosphatases), histidine kinase and ATPase; H, N, G1 and G2 boxes and the histidine kinase-265 residue are also depicted. b. Schematic representation of the LisR regulator response. The CheY homologous receptor and transcriptional regulatory domains are distributed at the extreme N- and C-termini, respectively. The regions containing signatures of the receptor module are aspartic acid-54 (phospho-acceptor residue), loop-5, proline-101 and lysine-102.

have been described in modular sensor kinases and are involved in signal transduction in two-component systems. LisK showed remarkable topological similarity with other bacterial histidine kinases, such as the sensor proteins, PhoQ, CheA and EnvZ (Bilwes et al. 1999; Stock, Robinson et al. 2000; Teng et al. 2002). According to the KinasePhos prediction, the histidine-kinase domain contains a histidine phosphoryl-residue (residue 266, score 0.972) that may confer the phosphorelay step to the response regulator (Figure 1a).

LisR likely interacts with LisK as a cytoplasmic protein; this was determined using the PSORT analysis (score of 7.5; cytoplasmic proteins range 1-10) and its biological process information in the SwissProt protein database. We predicted a lack of transmembrane helices, signal peptides as well as other consensus sequences that would suggest membrane association or transportation; in fact, the Smart analysis suggests a strong biochemical function that indicates a transcriptional response association. LisR is composed of two distinct domains, an N-terminal receptor or CheY-homologous domain (positions 2 to 112; E-value: 6.09e-42) and a C-terminal DNA-binding domain (transcriptional regulation, positions 149 to 224; E-value: 1.50e-23) (Figure 1b). Because protein activation requires phosphorylation of the receptor domain, Asp-52 is likely involved, according to our phoB sequence comparison. Additionally, the Profunc results showed that the C-terminal effector domain contains DNA binding sites and indicates sequence similarity with other response regulators consisting of CheY-like receptors and winged-helix DNA-binding domains (Martínez-Hackert & Stock 1997).

LisK structure is consistent with a two-component regulatory transducer: We generated consistent structural and functional descriptions for LisK histidine kinase; despite unexceptional sequence similarities, we predicted an accurate structural model and associated homologous function with the template protein (**Figure 2a**). Using the Swiss Model alignment mode implemented in the server (PMDB ID:

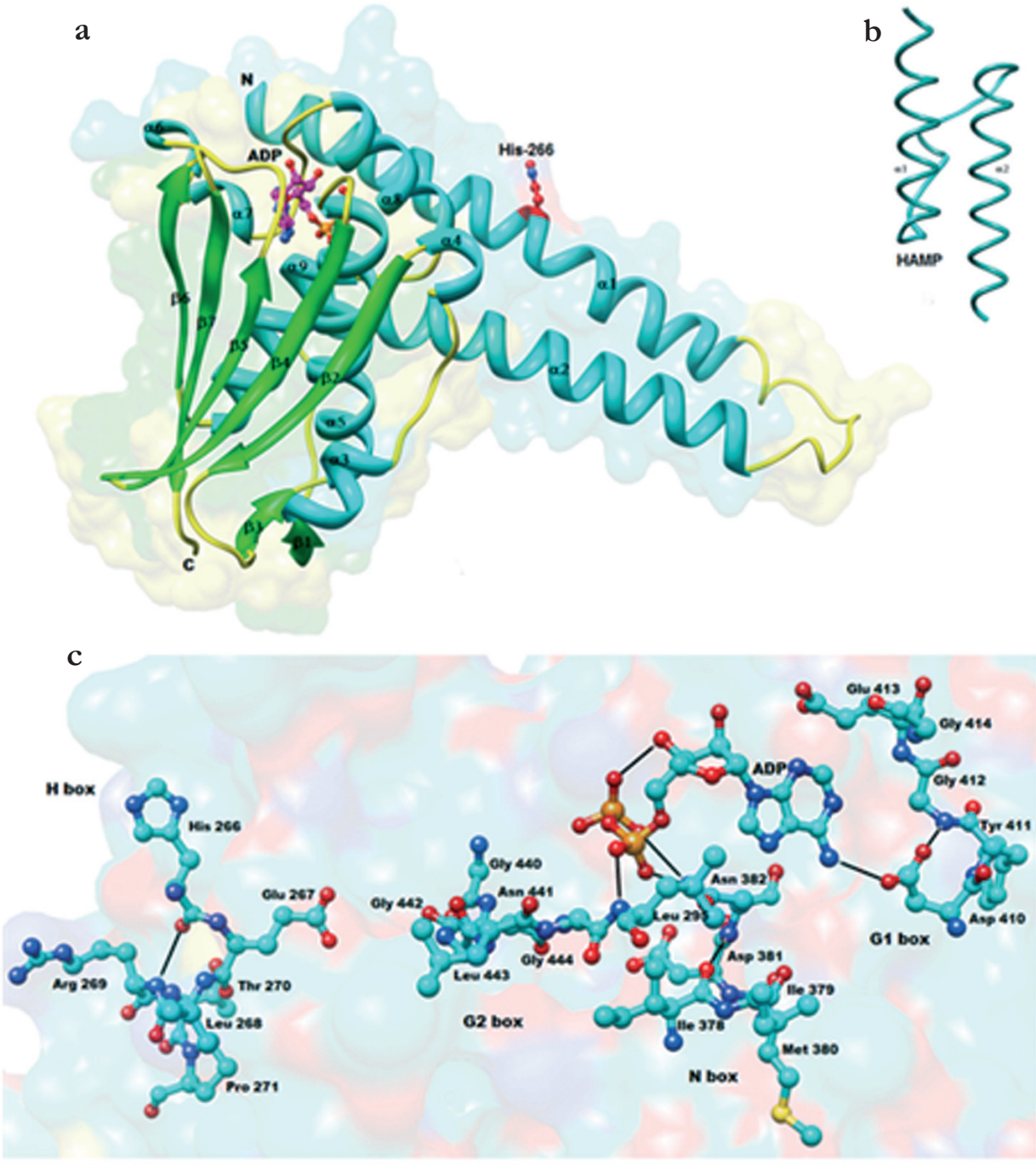

Fig. 2. a. Ribbon diagrams of the LisK (ADP complexed) are depicted; α-helices are shown in cyan, strands in green and the remaining backbone in yellow. Phosphohistidine 266 and ADP are represented by balls and sticks and are colored by heteroatom as red and magenta, respectively. b. The HAMP domain homodimer is represented in depth cue by ribbons rounded in light sea-green. c. Representation of the ADP-bound catalytic site focusing on the H-bond network (depicted as black lines) in the catalytic site. The model was created by labeling and drawing selected residues from the kinase boxes and ADP as ball-and-stick molecules (carbon, grey; nitrogen, blue; oxygen, red; phosphorus, green). The LisK backbone is shown as cyan ribbons.

PM0076533), we modeled a three-dimensional structure of *L. monocytogenes* LisK. The quality and stereochemistry assessment performed using the RAMPAGE server showed that the main-chain conformations for 98.8% of the amino acid residues were within the favored or allowed

zones of the Ramachandran plot, and 1.2% of the residues were in the outlier region; this result suggests that the model had good-quality molecular geometry.

Because the LisK structural model had no HAMP domain, we modeled it separately based on the homodimer structure of *Archaeoglobus fulgidus* (PM0076535 based on pdb code 2ASW) (Inouye 2006). The predicted HAMP model is composed of two large alpha helices (helices alpha-1and alpha-2) separated by a flexible loop; the parallel helices comprise each monomer and primarily contain hydrophobic residues, which are likely involved in packing interactions within two wrapped cores (Figure 2b). In addition, a possible conformational change may occur with the presence of a repulsive or attractive environment; this would afford movements to the left or right. As described previously, this rotation may be triggered by a specific recognition stimulus in the membrane-bound, periplasmic-sensing region (Hulko et al. 2006).

We also observed that the histidine-kinase domain contains phosphohistidine-265 located in the center of the extended and flexible alpha-1 helix. According to our predicted model, the conserved histidine kinase core is positioned in the parallel helix-helix dimerization region and connected to the ATP-binding catalytic domain (Figure 2a); therefore, homodimerization forms a four-helix bundle. By performing a CDD comparison, we predicted that the following residues might stimulate this homodimerization process: Phe-260, Ala-264, Leu-268, Pro-271, Met-275, His-278, Ser-296, Ser-300, Glu-303, Met-307, Leu-310 and Met-314.

Consensus-sequence comparisons support the delineation of the ATP-binding pocket which comprises the conserved H (266-His-Glu-Leu-Arg-Thr-Pro-271), N (378-Ile-Ile-Met-Asp-Asn-382), G1 (410-Asp-Tyr-Gly-Glu-Gly-414) and G2-kinase boxes (440-Gly-Asn-Gly-Leu-Gly-Leu-445) but not the conserved F-box (Figure 2c); this indicates that LisK is a class I histidine kinase (Szurmant et al. 2007).

The sequence comparison also showed that a DXG motif (410-Asp-Tyr-Gly-412 included in Box G1), formally named D-box, is placed into the G1 box; together with Asn-382, the D-box likely matches the metallic ions required for phosphorylation. This phenomenon has been observed in other glycine-rich-motif-containing kinases whose backbones may form hydrogen bonds with ATP phosphoryl groups, and was also established by our model (Figure 2c). This catalytic domain contains a disordered ATP-binding loop between alpha helix-6 and beta sheet-5; this has been perceived as a sensory-exposed region, which may interact directly or indirectly with the ADP ligand and may become a flexible lid.

LisR protein participates as transcriptional regulator and response effector protein: The predicted model comprises two compactly folded domains connected by a flexible linker (PMDB model code PM0076534). The LisR monomer contains a helix-turn-helix motif found in many DNA-binding proteins; the motif is formed by 8 alpha helices and 10 beta-sheets and a coiled-regions layer surrounds it. Its active site is N-terminally located and looped by an acidic pocket in which Asp-52 is the phospho-receptor residue. The activation of this residue may induce a conformational change, which would expose its hydrophobic surface and basic face, leading to increased DNA affinity (**Figure 3**). We inferred the presence and position of loop 5 (55-Leu-Pro-His-Leu-Asn-Gly-60), which is located near the active site and is highly solvent-accessible, as well as the small motif (101-Lys-Pro-Phe-103), which is located on long loop 9. This loop may be implicated in the activation process through proline-102, which orients the side chain of lysine-101 toward the active site to form a salt bridge with the putative phosphorylation site. These findings are consistent with previous mechanisms reported for homologous proteins; however, additional residues involved in the activation process, such as conserved Asn, Ser and Val, were not identified.

A comparison of the *Escherichia coli* LisR and PhoB structures (1GXP, chains A and B) (Blanco et al. 2002) shows sequence similarity (32.67%), structural similarity (99%, E-value 7.08E-08) and backbone RMSD (2.01 Å); this prediction also identifies residues that are likely to be implicated in

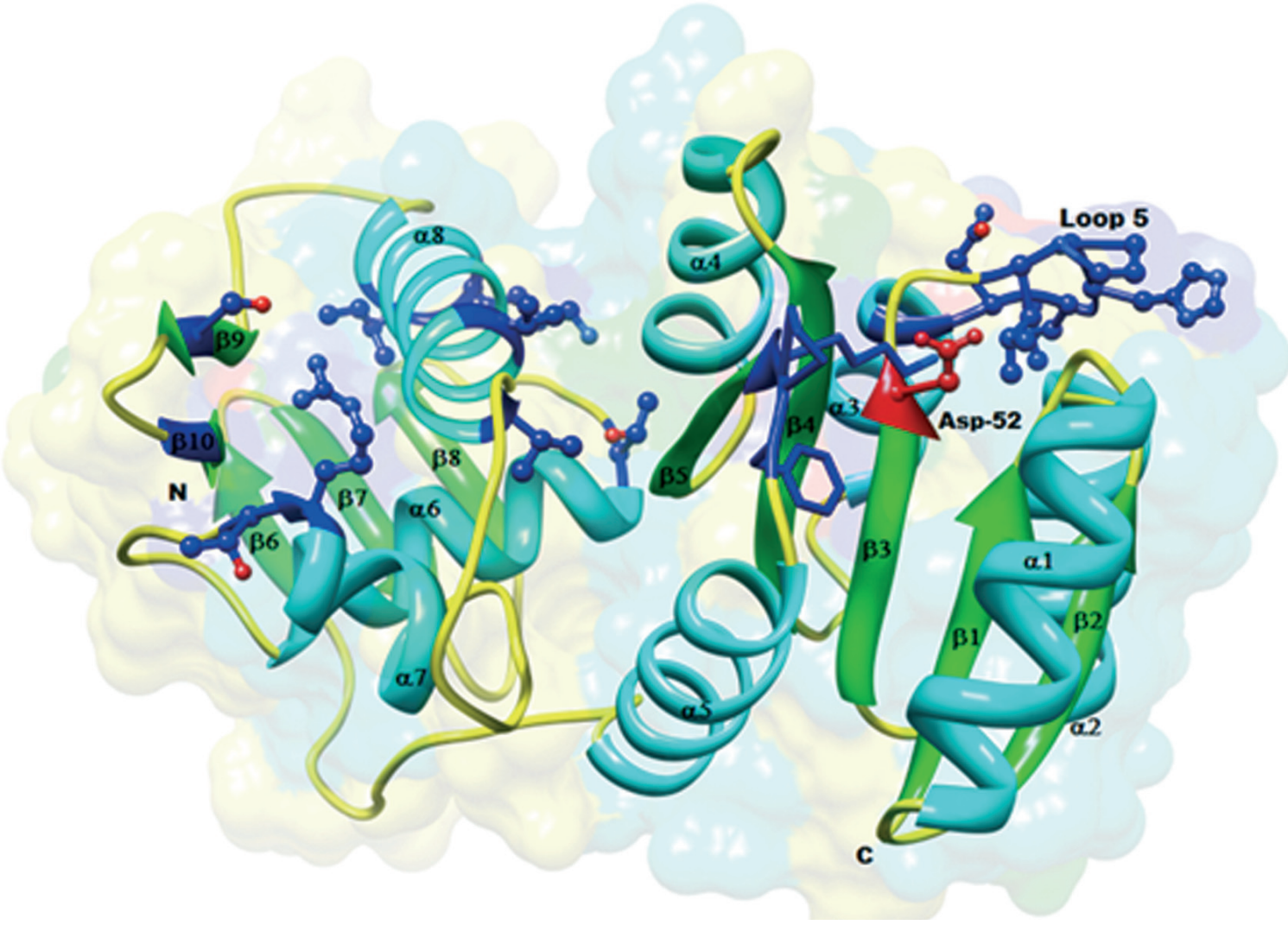

Fig. 3. Graphical representation of the LisR three-dimensional structure. The stereo view is colored by its secondary structure as follows: red α-helices, green β-sheets and yellow for the remaining backbone. Select residues in loop-5, Pro-101 and Lys-102 from the cheY homologue domain and DNA-binding residues are sketched in blue.

DNA binding (Thr-155, Thr-174, Arg-175, Val-193, Val-196, Lys-204, Ile-205, Thr-216 and Gly-221) (**Figure 4a**). The DNA-binding domain displayed the proper grooves required to accommodate the substrate without steric hindrance; this enables positioning of the nucleic acid target near the catalytic pocket (Figure 4b). LisR clearly represents a putative bacterial secondary signaling molecule that causes several responses at the transcriptional level (Parkinson and Kofoid 1992).

Using a Ramachandran plot we performed an evaluation of the model and obtained a good-quality model for LisR, which indicated that 90.1% of the residues were present in the favored region, 6.8% in the allowed region and 3.2% in the outlier region.

Phylogenetic implications for LisK/LisR two-component systems suggest a virulence linkage to related organisms: Ancient gene duplications (supported by high basal bootstrap values) and deep bifurcations characterize LisR evolution; this may indicate that a birth-and-death model under strong natural selection operated on these proteins. Both LisR evolutionary strategies (see Materials and Methods) displayed parsimony; both strategies required fewer steps to reproduce

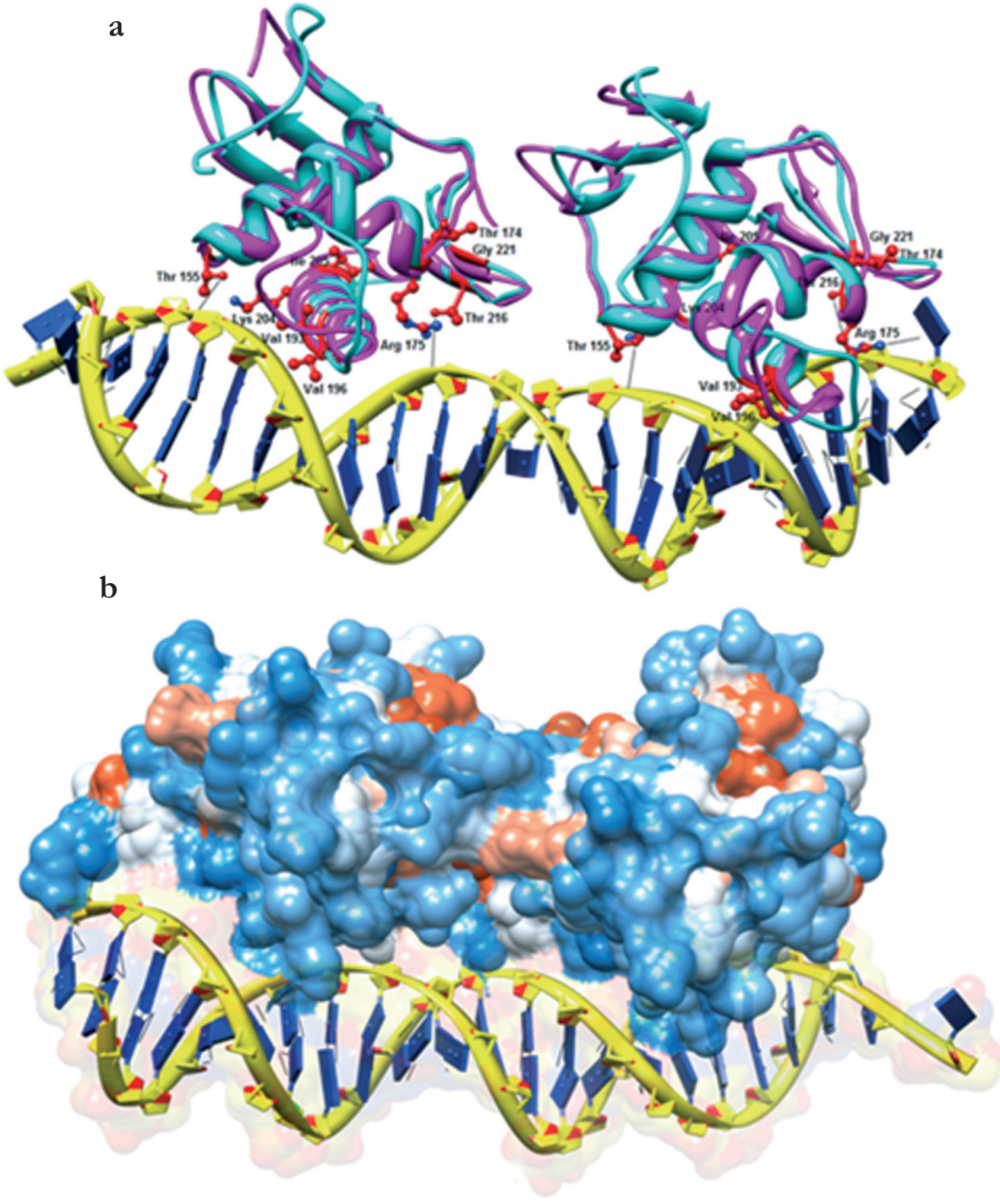

Fig. 4 a. Structural overview of the LisR-DNA complex. Ribbon diagrams of the superimposition of LisR (cyan) and the PhoB-DNA complex (magenta) (RMSD 2.01 Å) are shown. The amino acids contacting the DNA grooves from the LisR homodimer are colored red and are represented as balls and sticks. b. The LisR homodimer is represented by a hydrophobic surface tethered with a likely DNA target. The electrostatic contacts are colored blue for basic and red for acidic contacts. The double-stranded DNA is colored yellow for the phosphate-sugar backbone and blue for the nitrogenous bases.

the topology with good bootstrap values (the summary tree is shown in (**Figure 5a**).

We tested natural selection using several approaches; the results suggested that the dS

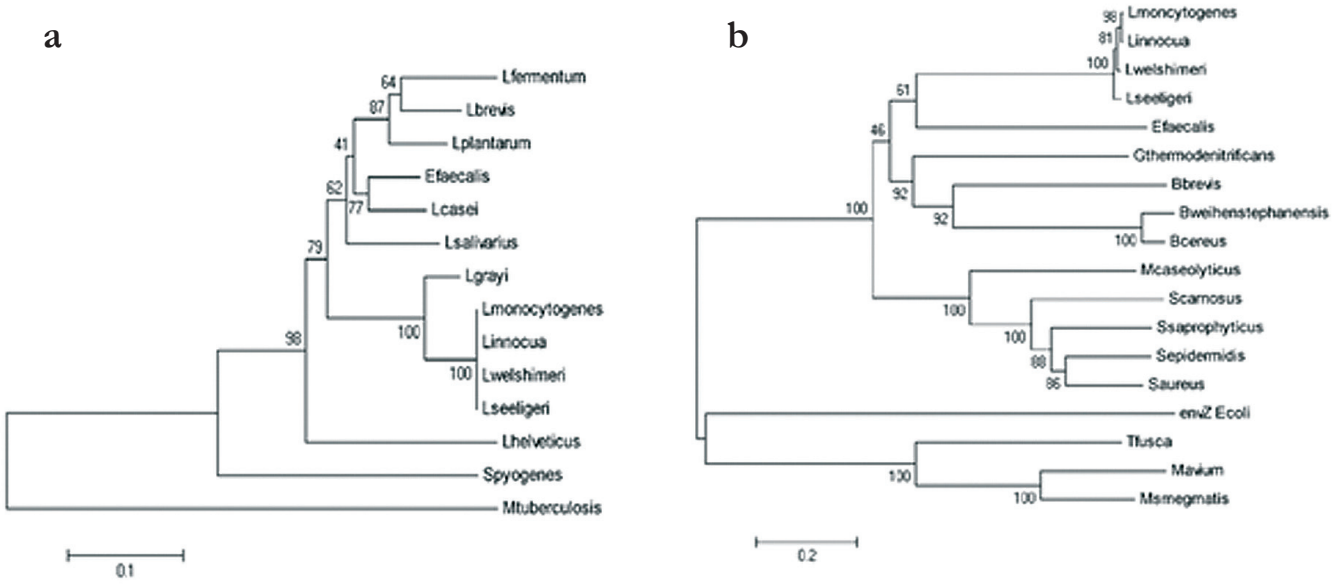

Fig. 5. a, b. Phylogenetic summary trees developed using the MEGA 4.0 software program and Phyml 3.0.1. Fourteen LisR and 18 LisK protein sequences were aligned separately in Muscle. The alignments were used to construct a tree using the NJ, the LG+G and the Poisson correction methods using 5,000 bootstrap repetitions for statistical robustness. Only the nodes with values of more than 50% statistical significance have been shown..

level was higher than the dN level (**Table 1**). The statistical significance detected a 0.000 probability in the Z-test for purifying selection. The Tajima test D-value was 0.823535, and dS/dN was 2.4055 according to the SNAP server. These values suggest that a genetic birth-and-death model subjected to strong purifying selection best fit LisR protein evolution.

Table 1. The Tajima test statistic was estimated using MEGA4. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The following abbreviations were used: m = number of sites, S = Number of segregating sites, ps = S/m, Θ = ps/a1 and π = nucleotide diversity. D is the Tajima test statistic (see chapter 12 of ref. 3 for equations). The Z-test was estimated in the overall population using MEGA4. All positions containing gaps and missing data were eliminated from the dataset. The SNAP server was used with a standard bacterial genetic code using nucleotide sequences, and two sequences were eliminated for the populations.

Test	**Parameters**					
Tajima	**M**	**S**	**Ps**	**Θ**	**Π**	**D**
LisR	14	150	0,678733	0,213429	0,252598	0,823535
LisK	18	371	0,958656	0,278715	0,670011	5,984902
Z-test	**Neutral/stat**	**Positive/stat**	**Purifying/stat**			
LisR	0,000/-5,746	1,000/-5,725	0,000/5,699			
LisK	-	-	-			
SNAP	**dS**	**dN**	**dS/dN**	**ps/pn**		
LisR	1,6937	0,7665	2,4055	1,5532		
LisK	-	-	-	-		

The combination of the birth-and-death model and purifying selection has led to the formation of polymorphisms adapted to specific niches and species; this suggests that LisR genes have existed in the bacterial genome for millions of years and that LisR was a determinant of natural selection and thereby could have participated by inducing the divergence of these types of bacterial species. Both evolutionary strategies (see Materials and Methods) appeared to be very parsimonious for LisR; both required fewer steps to reproduce the topology with good bootstrap values (the summary tree is shown in Figure 5a).

We manually edited the LisK alignments because of the high frequency of low-complexity regions in the proteins; this process modified the entire gene structure and prevented us from developing deeper dS/dN analyses, but it facilitated the Tajima test (REF). LisK evolution is determined by an ancient gene duplication followed by several bifurcation processes, which generated two principal clades; each clade displays a separate evolutionary history. The Tajima test D-value was 5.984902, which may indicate that a genetic birth-and-death evolutionary model subjected to very strong purifying selection explicates LisK evolution.

The members of the LisR/LisK complex display the same biological evolutionary strategy; both enzymes possibly require archetypal timeframes for data mining, which is necessary to explore the co-evolution hypothesis (Figure 5b) and to explain the evolutionary history of this complex in depth (Table 1).

Discussion

LisK/LisR two-component regulatory system orchestrates multicellular responses for *L. monocytogenes*: Our results suggest that LisK contains a homodimeric extracytoplasmic-sensing domain in which a stoichiometric reaction (ATP + protein L-histidine = ADP + protein N-phospho-L-histidine) most likely entails protein activation, which is dependent upon the formation of a two-helices homodimer. It is likely then that the HAMP domain positions the protein in proximity to other auxiliary domains following ligand binding, and interaction between them is most likely the initial step in the stabilization of the closed conformation. Therefore, the HAMP domains may have regulatory roles in the phosphorylation of homodimeric receptors and transmit conformational changes from the periplasmic stimuli-sensing region to the cytoplasmic signaling kinase (Szurmant et al. 2007).

The ligand-binding energy may align the monomers in a particular orientation and provide a complex network of contacts to regulate dimer affinity. Initially, the histidine residue undergoes autophosphorylation and transference of the phosphoryl group to the conserved His-266 in LisK; it then transfers the high-energy γ-phosphoryl group to the conserved Asp-54 of the receptor domain. Either the phosphorylation of LisR or the receptor domain results in homodimerization and exposes the DNA-binding site of the effector domain, enabling it to bind DNA and consequently regulate gene-specific transcription (Figure 4b).

Many of these systems are enmeshed in various processes of a signaling cascade involving multiple proteins or they may contribute to several simultaneous responses (Viadas et al. 2010). Therefore, we verified possible protein interactions of LisR/LisK using the String tool. We predicted that at least 9 proteins associated with a transcriptional response (LisR, phoP, cesR, resD, rr26, lmo1060, lmo1172, lmo1507 and lmo2583) are down- or upregulated by LisK; this suggests promiscuous signal activation for several response effectors and cross-talk between these regulators (**suppl. 1**). Regulators are primarily involved in processes such as chemotaxis, osmoregulation, ion transport, pH tolerance, growth or the response of virulent *L. monocytogenes* strains to the host's cells and to environmental niches (**Figure 6**). For example, *htrA*, a gene linked to osmolarity tolerance and virulence regulation in *L. monocytogenes*, is now known to be transcriptionally controlled by LisR/LisK (Stack et al. 2005). Interestingly, network interactions include the PhoB and LisR proteins. This finding could explain dramatic changes in other related transcriptional regulators; experiments have revealed that PhoP is a masterful regulator of

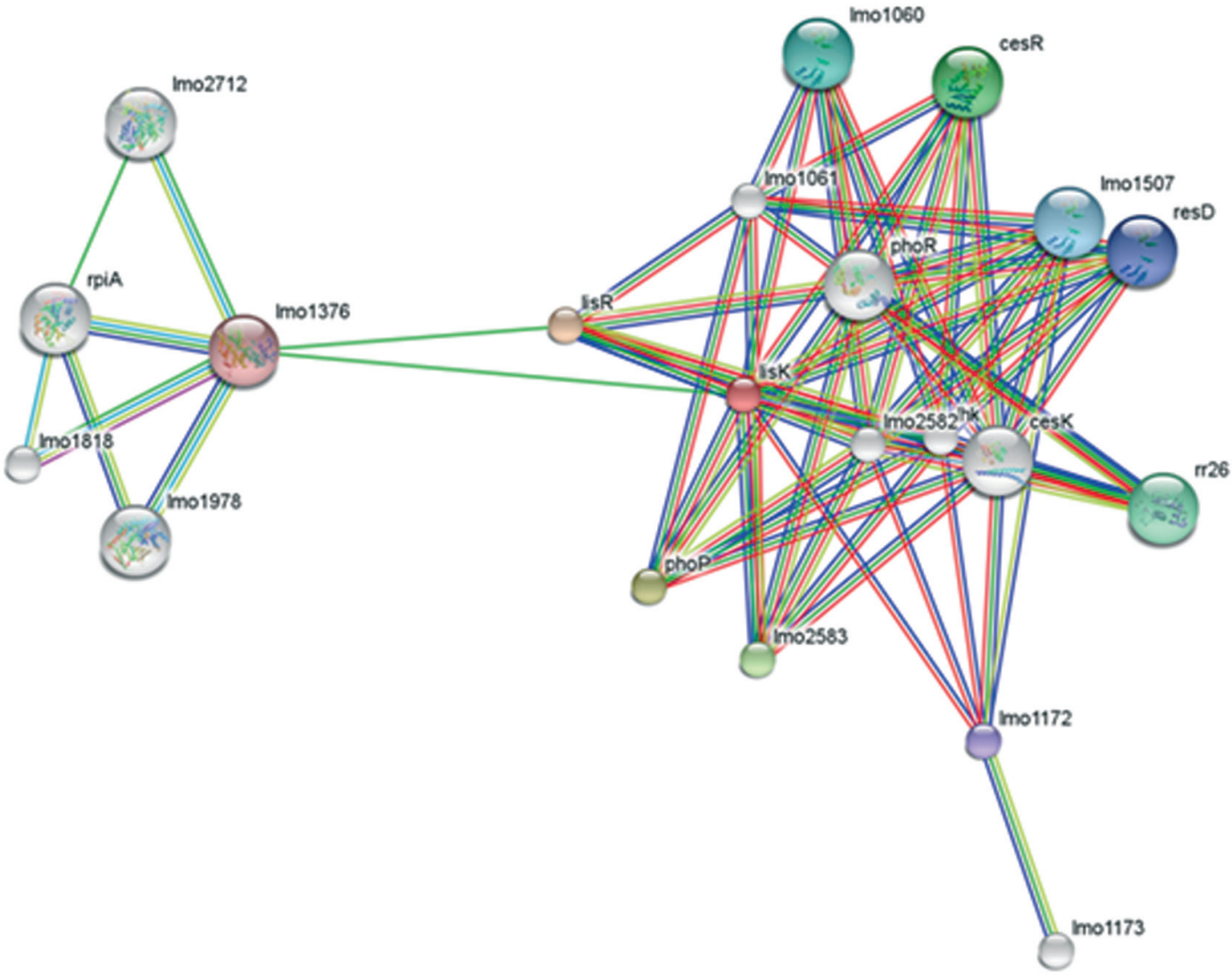

Fig. 6. Network interactions for the LisR/LisK two-component system. The nodes represent individual proteins and are connected with lines if they interact with one another. The classes of proteins and lines are colored differently as indicated by the STRING database. The protein names are shown according to their genome annotations.

persistence and virulence in human pathogens such as *Mycobacterium tuberculosis* (Gonzalo-Asensio et al. 2008).

Conclusion

Our results further explain the LisR/LisK two-component regulatory system and underscore its flexibility in switching between positive or negative feedback of the molecular device during *L. monocytogenes* adaptation and persistence during life-threatening conditions in host tissues or food-shortage conditions (Ray & Igoshin 2010). Moreover, the structural characterization of LisK and LisR performed here proposes that an integral two-component signal-transducing pair is the initial step to a pathway that affects *L. monocytogenes* physiology and pathogenesis. By understanding this step, we can begin to understand the full function of this system. Suitably, future studies on animal models of new LisR/LisK proteins, which inhibit listeriosis, should confirm the potential of this target as a treatment.

Acknowledgments

We acknowledge Professor Leonardo Lareo (R.I.P) for mentoring this research (RIP). This work was conducted using the computational resources provided by the Chemistry Department of the Universidad Nacional de Colombia.

Conflict of Interest

The authors declare that there are no competing interests.

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Rasgos estructurales de los componentes LisR/LisK sugieren múltiples respuestas para la adaptación y la supervivencia de *Listeria monocytogenes*

Resumen. Se caracterizó la estructura del sistema de regulación de dos componentes LisR/LisK de *Listeria monocytogenes*. Se emplearon herramientas bioinformáticas y bases de datos para predecir la estructura e interacciones de las dos proteínas y se modelaron. Los resultados predicen que la proteína LisK está embebida en la membrana celular y su composición modular (dominios HAMP, histidina kinasa and ATPasa) está asociada a su autofosforilación (His-266). Un efecto estímulo respuesta determina la propagación secuencial de la señal desde la membrana celular hacia componentes citoplasmáticos. A su vez, se predice que LisR es una proteína citosólica con un dominio de receptor (homólogo a cheY) que incluye el residuo fosfo-aceptor (Asp-52) y el dominio de unión a ADN, el cual puede permitir la transmisión de una respuesta específica a nivel transcripcional. Los componentes LisR/LisK han sido bioquímica y funcionalmente caracterizados experimentalmente en la patofisiología de otros bacilos. Es por ello, que la aproximación de los resultados basados en estructura-función podría facilitar el diseño de inhibidores específicos.

Palabras clave: *Listeria monocytogenes*; LisR/LisK; sistemas reguladores de dos componentes; proteina-histidina kinasa.

Características estruturais do sistema de dois componentes LisR/LisK as quais sugerem várias respostas para a adaptação e sobrevivência de *Listeria monocytogenes*

Resumo. O objetivo do estudo foi caracterizar estruturalmente o sistema de regulação de dois componentes LisR/LisK de Listeria monocytogenes. Foram utilizadas diversas ferramentas de bioinformática e bancos de dados para predizer a estrutura das duas proteínas, modelá-las e prever suas interações. Os resultados predizem que a proteína Lisk está incorporada na composição da membrana celular e sua composição modular (domínios HAMP, histidina quinase e ATPase) está associada com a sua autofosforilação (His-266). Um efeito de estímulo e resposta determina a propagação sequencial do sinal a partir da membrana celular em componentes citoplasmáticos. Os resultados predizem que LisR é uma proteína citosólica com um domínio recetor (homólogo a CheY) que inclui o resíduo fosfo-aceitador (Asp-52) e o domínio de ligação ao ADN, o que pode permitir a transmissão de uma resposta específica a nível transcricional. Como LisR/Lisk foi, química e funcionalmente, caracterizada experimentalmente na fisiopatologia de outros bacilos, esta abordagem baseada na estrutura-função pode facilitar a conceção de inibidores específicos.

Palavras-chave: *Listeria monocytogenes*; LisR/LisK; sistema de regulação de dois componentes; proteína-histidina kinasa.