A dynamic network analysis of the physiological state of foodborne pathogens: application to *Escherichia coli* during osmotic stress and comparison with *Salmonella Typhimurium*

Métris A.¹, George S.², Baranyi J.**

*Institute of Food Research, Norwich Research Park, NR4 7UA, United Kingdom.

**Corresponding author. Tel.: +44-1603-255-121; fax: +44-1603-507-723.
E-mail address: jozsef.baranyi@ifr.ac.uk

Abstract

To characterise the physiological state of cells during adaptation to osmotic stress, we decompose the dynamic regulatory network of *E. coli* into subgraphs. We then compare the results of *E. coli* and *Salmonella*. Beside the sigma factor associated with stress, the response involves global regulators that modify nucleoid conformation which has been shown to be different in the two bacteria. In *Salmonella*, some genes involved in osmotic stress are also linked to virulence regulation. We conclude that decomposition of regulatory networks into subgraphs as a function of environmental conditions may be a useful representation of the physiological state of bacteria.

Keywords: *Salmonella Typhimurium*; Systems Biology; Network Analysis; Gene Expression; Subgraphs

1. Introduction

Predictive microbiology for food safety owes its success to the reproducibility of the growth rate in given environmental conditions. Because the growth rate is an autonomous parameter which describes a whole population, it can be considered as changing continuously with environmental conditions. As a consequence, it is possible to predict the growth rate by interpolation in conditions where it was not necessarily measured.

Secondary models were initially developed to describe the effect of environmental conditions on the growth rate. Although attempts have been made to describe the lag time with the same approach, much of the uncertainty of the
prediction of lag time remains because it depends not only on environmental conditions, but also on the physiological state of the bacteria themselves. Lag time, survival and probability of growth are all affected by the physiological state of bacteria and may vary from strain to strain. Secondary models are developed independently for different strains or species and the parameters of the model do not relate to the genes of the bacteria nor their interaction.

Including information at the molecular level is not trivial. Firstly, entities are diverse (genes, proteins or metabolites) and how to integrate them remains a challenge. Measurements tend to give relative rather than absolute values and tend to be qualitative rather than quantitative. In these conditions, unless specific subsystems are considered, analytical mathematical methods are not feasible. A bacterial cell may in fact be viewed as a complex system, typically represented in the form of graphs. We propose that a useful description of the physiological states of food-borne pathogens may be obtained if genomic information and condition-dependent data are represented in combination. For instance, metabolic flows can be mapped to metabolic networks or expression data to regulatory network. In this presentation, we will give an example of the latter: a dynamic network analysis of the physiological state of Escherichia coli and Salmonella during osmotic stress.

2. Bacterial regulatory networks

2.1 Definition and properties of transcriptional networks

In order to modify their behaviour when subjected to stress, bacteria have to respond promptly to stimuli. One way they achieve this, is altering the transcription of genes. Transcription factors (TFs) are proteins which mediate these changes by binding to DNA in the promoter region of genes and either enhancing or repressing their transcription. Modulators of the transcription include subunits of the RNA polymerase such as sigma factors and small RNAs. A transcriptional regulatory network is a representation of the interactions between TFs and their target genes, TGs, which can be analysed in the context of network theory.

The transcriptional network of the model organism Escherichia coli K12 has been studied for many years and can be found in RegulonDB (http://www.ccg.unam.mx/en/projects/collado/regulondb). Its topology, as for other organisms, is scale-free. This means that there are a few regulators, the global regulators, which regulate a large number of TGs while most TFs regulate only a small number of TGs. Global regulators include, among others, carbon usage regulators such as the catabolite gene activator protein, Crp, environmental sensors such as the cold shock protein, CspA and nucleoid associated proteins such as the Histone-like nucleoid structuring protein, H-NS, the factor for inversion stimulation, Fis and the integration host factor, IHF. Transcriptional networks are also hierarchical; they can be organised in pyramid shape layers according to the number of TGs that TFs regulate. Finally, they are modular: a set of connected nodes work together to transform a signal into a function. Analysing these modules during adaptation may lead to a better understanding of bacterial states.

2.2 Decomposition into subgraphs

Not all TFs are expressed at any time, so to describe the dynamics of transcription it has been proposed to decompose the transcriptional networks into subgraphs where transcription is active. The traditional division in operons, which stems from chromosomal organisation, may not be the best to describe the kinetics of transcription because more than one operon may be regulated by the same TF. Other subdivisions include regulons defined by all the TGs and TFs regulated by one given TF, orignons that originate from a distinct class of sensors or network motifs. Motifs are subgraphs which have been shown to be over-represented in transcriptional networks of E. coli and yeast. Different motifs result in different dynamic behaviour. For instance, when a single TF activates many TGs, the TF allows a co-ordinated regulation of all TGs. Feed forward loops are formed of a TG which is regulated by two TFs. Two specific types are over-represented in E. coli: one has the effect of damping any fluctuation in the signal while the other produces a pulse in the expression of the TG. Alternatively, the TGs are activated by a combination of TFs, these are typical of stress response modules.
3. Application to the dynamics of *E. coli* and *Salmonella* under osmotic stress

3.1 Regulation pattern during the lag time of *E. coli* under osmotic stress

During lag time, the immediate response to osmotic stress induced by addition of NaCl is production of chaperones as a response to the stress on the membrane and the induction of transport of ions like potassium glutamate. The production of chaperones may be regulated by a multistep protease system that senses disruptions in membrane proteins and/or the production of σ^32, a sigma factor with a positive autoregulation. Exchange of ions includes accumulation of potassium glutamate as water diffuses out^10. In a second phase, osmotic genes such as those corresponding to the osmoprotectant trehalose (OtsA and OtsB) are expressed as a result in change of expression of σ^3. σ^3 is an example of a dense overlapping regulon, i.e. it regulates or modulates the regulation of a set of TGs with a broad range of functions. Potassium glutamate is also required for activation^11 for the induction of osmB and osmY, two proteins that are expressed transiently at the beginning of the lag time with osmotic stress induced by NaCl^12.

Most of the proteins expressed during the early lag are under the regulation of the global regulators such as those modifying nucleotide structures, for example, Fis, HNS and the IHF. The other notable global regulator involved is the oxidative stress regulator, OxyR and corresponding oxidative stress proteins. Finally, at the end of lag and beginning of exponential phase, a change of metabolism is observed with *E. coli* co-regulated by global regulators such as Fur and Crp-CAMP and a switch of metabolism from aerobic to anaerobic at a threshold NaCl concentration^13.

3.2 Comparison of *Salmonella Typhimurium* and *E. coli*

The transcription profile of *Salmonella* follows a pattern similar to that of *E. coli* but differs for specific genes, some linked to virulence and antibiotic resistance^14. This may be because osmotic stress is a cue used by *Salmonella* in the intestinal lumen. Another difference in regulation may also come from DNA supercoiling; the response to osmotic stress is different in *E. coli* and *Salmonella*^15.

4. Conclusion

We conclude that, in the same way as growth rate can be predicted from environmental conditions, decomposition of regulatory networks into subgraphs may be useful representations of the physiological state of bacteria, and that genomic capability represented by a potential network needs to be taken into account along with environmental conditions. We found that adaptation to osmotic stress brings a global regulatory re-organisation during the lag time, driven mainly by the dense overlapping regulon under σ^3. To do this analysis, new gene expression data measured by micro-array and data from the literature on proteomics were combined with the regulatory network, but other sources of –omics data and levels of metabolism may also be combined to obtain dynamic networks.

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References