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

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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.

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

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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[1]. 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 ingrate them remains a challenge[2]. 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[3] 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 *E. 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[4]. 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 **i**nversion **s**timulation, Fis and the integration host factor, IHF. Transcriptional networks are also hierarchical[5]; 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[6]. 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, origons that originate from a distinct class of sensors[7] or network motifs[8]. 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 coordinated regulation of all TGs. Feed forward loops are formed of a TG which is regulated by two TFs. Two specific types are overrepresented 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[9]. Alternatively, the TGs are activated by a combination of TFs, these are typical of stress response modules[8].