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Interplay of two quorum sensing regulation systems of *Vibrio fischeri*

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Abstract

Many bacteria developed a possibility to recognise aspects of their environment or to communicate with each other by chemical signals. An important strategy is the so-called quorum sensing (QS), a regulatory mechanism for the gene expression, where the bacteria measure their own cell density by means of this signalling pathway. One of the best-studied species using QS is the marine luminescent bacterium *Vibrio fischeri* which is considered here as a model organism.

The two main regulatory pathways (*lux* and *ain*) are combined to a regulation system, the dynamics is modelled by an ODE system. This system is analysed thoroughly, considering stationary states, dynamical behaviour and the possible biological meaning of it. The influence of different parameter values on the behaviour is examined, the same basic system is able to reflect the peculiarities of different bacteria strains (respectively, their mutants).

Keywords: Quorum sensing; ODE system; Stationary states; Model validation; Qualitative behaviour

1. Introduction

The quorum sensing (QS) concept was originally introduced as a supposed regulatory mechanism by which bacteria control the expression of genes in response to their cell density (Fuqua and Greenberg, 2002). The bacteria produce a chemical signalling substance (autoinducer) which is released into the environment, i.e. the extracellular autoinducer concentration depends on the present cell density. Furthermore, the bacteria sense the environmental concentration of the autoinducer. If a certain threshold concentration is exceeded, then the bacteria change their behaviour by gene transcription induction. This mechanism is believed to enable concerted actions such as attacking self-defending tissues, suppressing competing species by antibiotica release, or defending against immune systems, or mutually beneficial actions like bioluminescence in light organs. An alternative interpretation of the purpose of autoinducer release suggests that bacteria sense the diffusible space around a single cell which would be important to minimise the unproductive loss of released substances (e.g. exoenzymes or siderophores) by diffusion (Redfield, 2002). Unifying these different aspects leads to an interpretation of the autoinducer regulation called "Efficiency Sensing" by which the release of autoinducer into the environment is regarded as a proxy for estimating the possible release of more costly substances as diffusible extracellular effectors (Hense et al., 2007). There are many examples of bacteria with such a regulation system (see e.g. Fuqua and Greenberg, 2002; Gray and Garey, 2001; Miller and Bassler, 2001; Waters and Bassler, 2005; Whitehead et al., 2001). In *Vibrio fischeri* and other Gram-negative bacteria, the signal molecules of the acyl homoserine lactone (AHL) type are produced by AHL synthases (e.g. members of the so-called LuxI family). The response to local concentrations of AHLs is mediated by transcriptional activators (e.g. proteins of the so-called LuxR family) (Greenberg, 1997; Hastings and Greenberg, 1999). The marine bioluminescent bacterium *V. fischeri* is present in the open sea but also grows in the light organs of squids and fishes. In these organs the bacteria reach a certain density and start to produce proteins which are

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responsible for the emission of photons (on a visible level). As diffusion limitation probably plays a minor role in these organs, it seems justified to use here the "classical" term QS. *V. fischeri* is a paradigmatic, well-studied example of a QS-regulated bacterium, since it can be cultivated *in vitro* and *in vivo* and the QS-regulated reaction (bioluminescence) can be analysed easily. It was the first species where AHL (3OC6HSL) and the *luxI* and *luxR* genes were identified (Eberhard et al., 1981; Engebrecht and Silverman, 1984). Therefore, it is well-suited as a model organism for the understanding of QS regulation.

The *lux* operon in *V. fischeri* contains the genes which code for the bacterial light producing system (*luxCDABE*). The proteins LuxA and LuxB form the light-generating enzyme luciferase (Stabb, 2005). The products of the two genes *luxI*, which is also located in the *lux* operon, and *luxR* regulate the operon. The genes *luxI* and *luxR* seem to represent the main regulation system for luminescence in *V. fischeri*, at least at the late (high cell density) bacterial colonisation state (Lupp and Ruby, 2005).

In many cases there is more than one QS system present in a cell, e.g. in *Pseudomonas aeruginosa* (Fagerlind et al., 2003) or in *Rhizobium spp.* (Wisniewski-Dye and Downie, 2002). Kuo et al. (1994) investigated mutants of *V. fischeri*, which are defective in *luxI*. The mutants showed some *lux* operon transcription, with a synthase locus distinct from *luxI* (Dunlap, 1992). This observation lead to the discovery of a second autoinducer system involved in the regulation of the luminescence (Kuo et al., 1994, 1996), called *ain* system. Here, the AHL synthase AinS produces another AHL (C8HSL).

A putative third system was detected in the sequenced genome of *V. fischeri*. It is based on LuxS, which synthesises the so-called autoinducer 2 (AI-2) (Lupp and Ruby, 2004; Miller and Bassler, 2001). AI-2, a furanosyl borate diester, appears in many Gram-negative and Gram-positive bacteria species and has been proposed to be a medium for interspecies communication (Xavier and Bassler, 2003).

The LuxS system seems to influence both the regulation of luminescence and the colonisation competence, but the quantitative contribution to luminescence is small compared to the *ain* system (Lupp and Ruby, 2004), so we will neglect it here.

Autoinducer systems seem to occur in many bacterial species and may play a role in pathogenic or symbiotic bacteria. Therefore, an understanding of these regulatory systems may be beneficial in medicine and agriculture. The example of *V. fischeri* shows that several autoinducer systems can interact. Such interaction of two or more regulatory systems can hardly be understood on a merely intuitive level and requires some mathematical analysis. Since the experimental data for *V. fischeri* are by far the most detailed, we base our modelling approach on this species.

The goal of this paper is to understand the interplay of the two regulation systems, *lux* and *ain*, with regard to stationary states and dynamic behaviour.

In Section 2 a complete and detailed pathway for the *lux* and the *ain* systems is built based on the available experimental data for these systems. This regulatory pathway is described by a system of ordinary differential equations. We show in Section 3 that a large number of stationary states may appear, enabling the bacteria to adapt their behaviour to different environmental conditions.

The dynamical behaviour of the complete system is investigated numerically. In particular, the behaviour of different mutants is compared to that of the wildtype. Indeed, the fact that the "model mutants" behave similarly as the observed experimental (biological) mutants, gives credence to the present modelling approach. Even though the experimental mutants represented by different strains of *V. fischeri* behave quite different; their behaviour can be simulated by changing few parameters.

2. The intracellular pathway and the corresponding model

The signalling pathway is shown in Fig. 1. In the *lux* subsystem, LuxI produces 3OC6HSL, which diffuses in and out of the cells (Kaplan and Greenberg, 1985). The receptor LuxR and 3OC6HSL form LuxR-3OC6HSL complexes which associate further to polymers. After binding of the polymer to the *lux* operon, it positively regulates the gene transcription of *luxI*. As LuxI (in contrast to the LuxR) is encoded on the *lux* operon, the system contains a positive feedback. The operon constitutively produces the autoinducer in low amounts. If the cell density increases, the positive feedback loop is induced, resulting in an increased autoinducer production per cell.

According to Lupp and Ruby (2005), the transcription of *ainS* and the concentration of C8HSL are also regulated by a positive feedback loop, which consists of an inactivation of LuxO (due to a phosphorelay cascade, activated by C8HSL), and an inactivation of the *litR* transcription by LuxO. LitR, in turn, regulates positively the gene transcription of *ainS*. Since LitR belongs to the TetR family, for which the formation of a homodimer is known (Ramos et al., 2005), dimers are probably the active form of LitR.

According to Kuo et al. (1996), in absence of 3OC6HSL the *ain* system can activate the *lux* operon transcription (on a lower level than in combination with the *lux* system). Since a *luxI ainS* mutant (defective in both *luxI* and *ainS*) did not exhibit any luminescence and none of the known autoinducers was detected, it is assumed that *V. fischeri* does not produce any further luminescence regulating autoinducers. A comparison between an *ainS* mutant and the wildtype revealed that the *ain* system seems to inhibit the luminescence induction, i.e. the *ainS*-defective mutant induced the luminescence at a lower population density and increased it more rapidly, compared to the wildtype. This behaviour can be explained by assuming that the two autoinducers 3OC6HSL and C8HSL compete in forming a complex with LuxR, where the complex C8HSL-LuxR has