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MODEL FOR GROWTH AND AI-2 TYPE QUORUM SENSING OF *SALMONELLA* TYPHIMURIUM SL1344

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Abstract: The last decades, the research on cell-cell communication or quorum sensing has been quite intense. Bacterial communication allows bacteria to coordinate their behavior and to act as one entity. Quorum sensing controls microbiological functions of medical, agricultural and industrial importance and a better understanding of the underlying mechanisms and the conditions under which the signaling occurs, offers possibilities for new applications. In this paper the production of the AI-2 signaling molecule by *Salmonella* Typhimurium is investigated in a controlled environment (bioreactor). The experimental results are applied to build a macroscopic model that describes the evolution of the AI-2 production and uptake by *Salmonella* Typhimurium in relation to the growth kinetics and biomass concentration.

Keywords: *Salmonella* Typhimurium, bioreactor, quorum sensing, overflow metabolism, macroscopic model

1. INTRODUCTION

Bacteria communicate through production, release and detection (sensing) of signaling molecules. This cell-cell communication or quorum sensing allows a bacterial population to coordinate its behavior by regulation of gene expression and it is involved in a lot of important microbiological functions like virulence, survival and host-pathogen interactions (Xavier and Bassler, 2003). Therefore, a better understanding of the underlying mechanisms of cell-cell communication offers perspectives for potential application in fields like medicine and agriculture.

Different bacterial species use different molecules to communicate. Among the different quorum sensing systems, the only one shared by Gram-positive and Gram-negative bacteria involves the production of autoinducer-2 or AI-2. LuxS, the AI-2 synthase, is widely spread, suggesting that AI-2 is a common language for interspecies communication (Xavier and Bassler, 2003).

Quorum sensing related experiments are most commonly performed on small scale (in the range of mL or even μL) which makes it hard to control or frequently sample the evolution of bacterial communication in time. In contrast, in this study experiments are performed in the controlled environment of a bioreactor. A macroscopic, simple structured model is developed for AI-2 type quorum sensing by *Salmonella* Typhimurium SL1344. This dynamic model embeds information about growth, metabolism and AI-2 signaling.

This paper is organized as follows. First, the materials and methods are introduced in Section 2. Section 3 covers the main part of this paper. In this section firstly the results of the bioreactor experiments are discussed, followed by the formulation of the mathematical model and the implementation of this model. The last section 4 summarizes the main results and conclusions.

2. MATERIALS AND METHODS

2.1 Bioreactor experiments

Experiments were performed in a computer controlled BioFlo 3000 benchtop bioreactor (New Brunswick Scientific, USA) with an autoclavable vessel of 1.25 to 5L working volume. PID cascade controllers ensure that the fermentation temperature is kept constant at 37°C, pH at 6 and the dissolved oxygen concentration at 30%. Feeding strategies were realized using a Masterflex peristaltic pump. Culture media samples were removed at regular time intervals. Cell density was obtained through measurement of optical density (OD) at 595 nm (Genesis 10S, Thermo Spectronic). Galactose and acetate concentrations were measured by gas chromatography.

A preculture containing *Salmonella* Typhimurium SL1344 was grown overnight in plain LB-medium (10 g/L NaCl (VWR, prolabo), 10 g/L tryptone (LAB M) and 5 g/L yeast extract (LAB M)) at a temperature of 37°C. This preculture was transferred in a volume ratio of 1/1000 in the reactor vessel containing LB medium supplemented with galactose (VWR, prolabo) as carbon source.

2.2 AI-2 activity bioluminescence assay

AI-2 was measured in the AI-2 bioluminescence assay as described previously by Surette and Bassler (1998). Light production was measured using a CCD camera (Berthold Night Owl, PerkinElmer Life Science) or a luminescence reader (Fluoroskan Ascent FL, Labsystems). Sterile AB medium served as negative control. Cell-free AB broth of an overnight culture of the acyl-homoserine lactone-defective *V. harveyi* BB152 was used as the AI-2-producing positive control. Results are reported as a percentage of the induction level produced by the positive control. Assays were performed at least in triplicate.

The AI-2 bioluminescence assay is the only measurement technique available for AI-2. This technique provides, however, only a qualitative result (Turovskiy and Chikindas, 2006).

2.3 Mathematical tools

Numerical integration is performed with the NAG routine (Numerical Algorithms Group) `D02EJF` in Fortran and the `ode23s` routine in matlab (The Mathworks Inc., Natick). Absolute and relative tolerances of the integration routines are set to a very small value (i.e., 10^{-8}) to ensure correct simulations.

3. RESULTS AND DISCUSSION

3.1 Qualitative discussion of experimental data

Results of the AI-2 bio-assay are expressed relative to a positive control (see Section 2.2). As a result of variability at the level of the positive control, the AI-2 bio-assay is a non-quantitative measurement. Hence, AI-2 concentration is only interpreted at a qualitative level. In the further discussion, measured AI-2 values are rescaled with respect to the highest measurement in an experiment. Therefore, no AI-2 units are appointed. Another disadvantage of the AI-2 bio-assay, is the high noise level of the measurement. Conclusions concerning the qualitative AI-2 profile are therefore, in most cases, based on several replicate measurements.

Glucose was not added to the medium as substrate because it interferes with the AI-2 bio-assay (De Keersmaecker and Vanderleyden, 2003). Galactose was tested in the bio-assay and did not influence the AI-2 measurement and was, therefore, added to the medium as substrate. Employing galactose as carbon source instead of glucose induces the same growth and substrate profile (data not shown).

Batch as well as continuous bioreactor experiments are performed. The results of those experiments are discussed in the following sections.

3.1.1. Batch experiments Figure 1 presents the results of two batch experiments with different initial substrate concentrations. A list of symbols can be found in Table 1.

The AI-2 profile for the batch experiment is similar to the one described by Surette and Bassler

Table 1. List of symbols.

C_X	[OD]	cell density	σ_{C_S}	[g/(OD·L)·h^{-1}]	specific consumption rate for C_S
C_S	[g/L]	galactose concentration	$\sigma_{m,S}$	[g/(OD·L)·h^{-1}]	coefficient consumption C_S
C_I	[g/L]	storage carbohydrate	m	[g/(OD·L)·h^{-1}]	maintenance factor
C_A	[g/L]	acetate concentration	$Y_{X,A}$	[(OD ·L)/g]	yield coefficient of C_X on C_A
$C_{X,max}$	[OD]	maximum cell density on C_I	σ_{C_I}	[g/(OD·L)·h^{-1}]	specific consumption rate for C_I
μ_{C_I}	[h^{-1}]	specific growth rate on C_I	$Y_{X,I}$	[(OD ·L)/g]	yield coefficient of C_X on C_I
$\mu_{m,I}$	[h^{-1}]	coefficient growth on C_I	$Y_{A,X}$	[g/(OD·L)]	yield coefficient for C_A production
μ_{C_A}	[h^{-1}]	specific growth rate on C_A	σ_{C_A}	[g/(OD·L)·h^{-1}]	specific consumption rate for C_A
$\mu_{m,A}$	[h^{-1}]	coefficient growth on C_A	U	[L/h]	feed rate
V	[L]	volume			

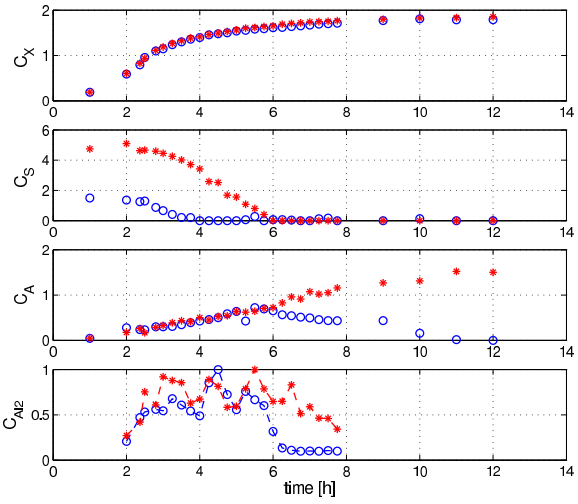

Fig. 1. Experimental data of two batch experiments with an initial substrate concentration of 5 g/L (*) and 1.5 g/l (o).

(1998) for erlenmeyer experiments. AI-2 is produced during the exponential growth phase and disappears at the start of the stationary phase. This results in a peak of AI-2 concentration at the beginning of the stationary phase.

First, the preferential substrate, i.e., galactose, is consumed. During growth on galactose, acetate is produced as by-product (overflow metabolism). Once all galactose is consumed, a metabolic switch takes place and *Salmonella* starts consuming acetate. Although there is no significant difference at the level of cell density between the two batch experiments, the AI-2 profiles exhibit distinct pattern since the time instant at which the maximum AI-2 concentration is observed, differs. AI-2 is produced during exponential growth until all galactose is consumed. After depletion of galactose, the general trend in AI-2 concentration in the medium is decreasing. Even though growth proceeds by consuming the produced acetate. These observations suggest that growth on a preferential substrate, i.e., in this case galactose, is necessary for AI-2 production. Because of the non-quantitative nature of the AI-2 bio-assay, it is not possible to infer whether there is also a difference in the amount of AI-2 produced for the experiments with different levels of galactose available.

3.1.2. Continuous experiments For the continuous experiment, a feeding solution containing LB supplemented with 10 g/L galactose was added to the reactor. Medium was removed from the reactor at the same rate, so the reactor volume (5 L) remained constant during the experiment. The results from the continuous experiment are displayed in Figure 2.

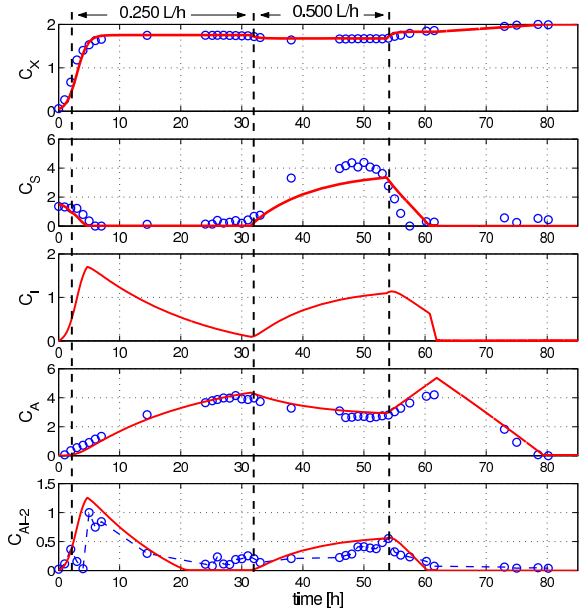

Fig. 2. Experimental data and model simulations for a continuous experiment ($C_S(0)$=1.5 g/L). Equations are provided in Table 2 and completed with Equation (1). The parameter values are listed in Table 3. The parameter values for AI-2 production/uptake are Y_{AI2}= 1.300 and $\sigma_{m,AI2}$ =0.2500.

When a continuous culture reaches a steady state, the specific growth rate equals the dilution rate. By selecting the appropriate dilution rate and giving the system time to evolve to an equilibrium, the specific growth rate can be set to a desired value. First a dilution rate of 0.050 h^{-1} is applied. After steady state has been reached, the dilution rate is switched to a higher value, namely 0.100 h^{-1}.

In contrast to a batch experiment, this feeding strategy allows the study of AI-2 produc-

Table 2. Model equations for growth and overflow metabolism. See Table 1 for a list of symbols.

Differential equations:

$$\frac{dC_X}{dt} = \left(\mu_{C_I} \cdot (1 - \frac{C_X}{C_{X,max}}) + \mu_{C_A}\right) \cdot C_X - D \cdot C_X$$
$$\frac{dC_S}{dt} = -\sigma_{C_S} \cdot C_X + D \cdot (C_{S,in} - C_S)$$
$$\frac{dC_I}{dt} = \sigma_{C_S} \cdot C_X - \sigma_{C_I} \cdot C_X - D \cdot C_I$$
$$\frac{dC_A}{dt} = Y_{AX} \cdot \mu_{C_I} \cdot C_X - \sigma_{C_A} \cdot C_X - D \cdot C_A$$

($D = \frac{U}{V}$ = dilution rate)

Specific rates:

$$\mu_{C_I} = \mu_{m,I} \cdot \frac{C_I}{C_I + \epsilon} \qquad \sigma_{C_A} = \frac{\mu_{C_A}}{Y_{XA}}$$
$$\mu_{C_A} = \mu_{m,A} \cdot \frac{C_A}{C_A + \epsilon} \cdot (1 - \frac{C_I}{C_I + \epsilon}) \qquad \sigma_{C_I} = \frac{\mu_{C_I}}{Y_{XI}}$$
$$\sigma_{C_S} = (\sigma_{m,S} \cdot (1 - \frac{C_X}{C_{X,max}}) + m) \cdot \frac{C_S}{C_S + \epsilon}$$

($\epsilon = 10^{-3}$)

tion/uptake at a specific growth rate for an extended period of time. A steady state is reached for all measured variables, including the signaling molecule AI-2. These results are in accordance with the results reported by DeLisa *et al.* (2001) for *Escherichia coli*. AI-2 concentration is maintained during the feeding phase. Once the feeding is stopped, the specific growth rate drops to zero and the AI-2 concentration decreases below the detection limit. These observations support the hypothesis that AI-2 production and maintenance are strongly correlated with the specific growth rate (substrate metabolism). To produce and maintain a high AI-2 level, the population should be actively growing. A high cell density (quorum) is not sufficient. Once growth ceases, AI-2 disappears from the medium, although cell density remains high. Hence, this observation proves that AI-2 quorum sensing is related to the metabolic status of cells and not (solely) to cell density.

3.2 Mathematical model formulation

3.2.1. Growth and overflow metabolism Batch experiments with different initial substrate concentration revealed that galactose is preferentially used for growth but is not a limiting factor. As can be seen in Figure 1, experiments with different substrate availability lead to the same growth profile. This can be interpreted as follows. Galactose does not act as growth-limiting substrate. In the growth medium, different candidate substrates are available but galactose is consumed preferentially. The dynamics of the bioreactor experiments are described by mass balance type equations (Bastin and Dochain, 1990). The simple structured model proposed for growth and overflow metabolism is presented in Table 2.

Growth is modeled in two phases. Substrate (C_S, i.e., galactose) is internalized as storage carbohydrate (C_I) and used for both growth (first growth phase) and acetate formation (C_A) (overflow metabolism). The first growth phase is modeled by a logistic equation. Once all the internalized substrate or storage carbohydrate is consumed, the bacterial population starts growing on acetate (second growth phase). The switch from one substrate (i.e., galactose) to another (i.e., acetate) is implemented in the model by Monod-type equations with a very low Monod constant (ϵ).

3.2.2. Mathematical model for AI-2 production and uptake The measured AI-2 concentration in the medium is the result of an equilibrium between production and uptake.

AI-2 is synthesized by LuxS and subsequently released. The results of the performed bioreactor experiments suggest that production of the AI-2 signaling molecule is influenced by the substrate metabolism (see Section 3.1).

The only AI-2 regulated genes identified yet, are the genes of the *lsrACDBFGE* or LuxS regulated operon, which encode for an ABC-transporter for uptake of AI-2 (Taga *et al.*, 2003). Basal expression of the Lsr transporter allows AI-2 to enter the cytoplasm. The internalized AI-2 is phosphorylated and induces the transcription of the *lsr* operon by inactivation of LsrR (repressor of the *lsr* operon). So, AI-2 regulates its own uptake.

Based on the experimental observations described above and information obtained from literature, following equation is proposed to describe the AI-2 production and uptake.

$$\frac{dC_{AI2}}{dt} = \underbrace{\frac{\sigma_{C_S}}{Y_{AI2}} \cdot C_X}_{production} - \underbrace{C_X \cdot \sigma_{m,AI2} \cdot \frac{C_{AI2}}{C_{AI2}+\epsilon}}_{uptake} - D \cdot C_{AI2} \quad (1)$$

with $\epsilon = 10^{-3}$ and C_{AI2} [-] the AI-2 concentration, $\sigma_{m,AI2}$ [(1/OD)·h^{-1}] the coefficient for the specific consumption rate of AI-2 and Y_{AI2} [(g/L)$^{-1}$] is the yield coefficient for AI-2 production. The Monod-term with C_{AI2} in Equation (1) reflects the fact that AI-2 is needed to suspend the repression of LsrR on the AI-2 transporter and by doing so, to regulate its own uptake.

3.2.3. Model simulations Simulations were compared with experimental data. Results are displayed in Figures 3, 4 and 2. For the equations describing growth and overflow metabolism (see Table 2), the same parameter values are employed for all the simulations. For the description of AI-2, parameters had to be adapted for each data set. The need for different parameters is a result of the non-quantitative nature of the AI-2 measurement. The parameter values are listed in Table 3.

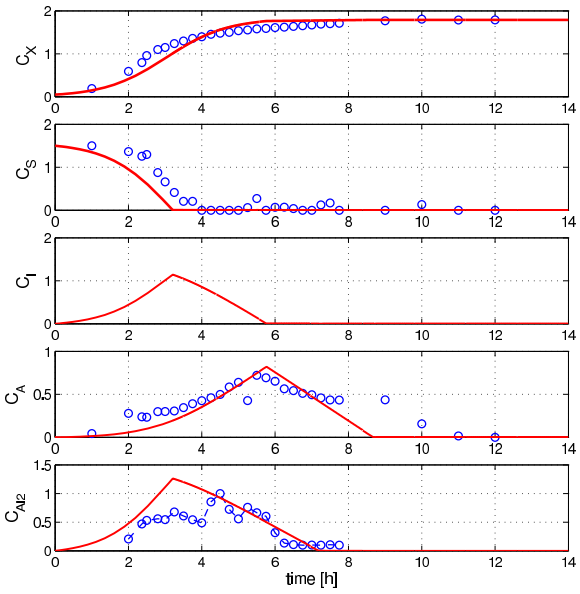

Fig. 3. Experimental data and model simulations for a batch experiment ($C_S(0)$=1.5 g/L). Equations are provided in Table 2 and completed with Equation (1). The parameter values are listed in Table 3. The parameter values for AI-2 production/uptake are Y_{AI2}= 1.000 and $\sigma_{m,AI2}$ =0.2000.

Though the proposed model still needs some refinement, it provides a good description for both batch and continuous data. The most prominent difference between data and prediction are at the

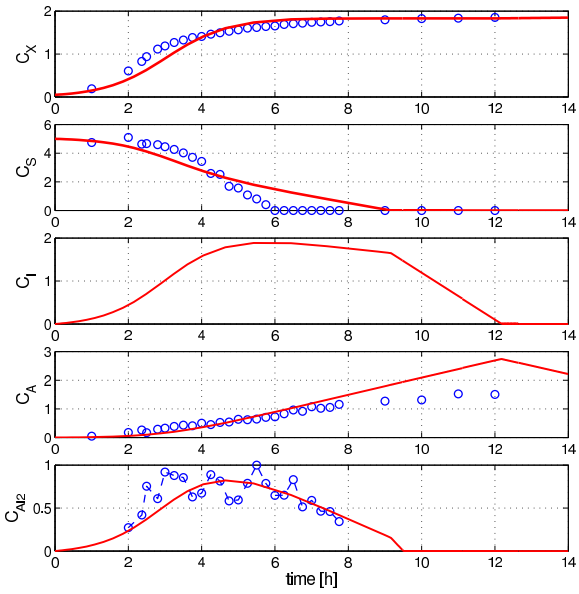

Fig. 4. Experimental data and model simulations for a batch experiment ($C_S(0)$=5 g/L). Equations are provided in Table 2 and completed with Equation (1). The parameter values are listed in Table 3. The parameter values for AI-2 production/uptake are Y_{AI2}= 1.700 and $\sigma_{m,AI2}$ =0.2500.

level of galactose consumption for the batch data. There seems to be a lag in galactose consumption, which is not included in the model. For high substrate concentration (see Figure 4), the predicted instantaneous consumption rate is lower than the observed one.

The model also provides a good description of the conditions were production is maintained or where a peak in AI-2 concentration is observed. The observed differences in AI-2 evolution between prediction and data are a consequence of the connection between AI-2 production and galactose consumption. Improving the description of galactose consumption, will also lead to an improved description of AI-2 activity.

Despite the shortcomings mentioned above, the proposed model provides a good description of growth and AI-2 activity of *Salmonella* Typhimurium for different modes of operation.

4. CONCLUSIONS

The experimental results confirm that AI-2 is produced during exponential growth, but only during growth on the preferential substrate (i.e., in this case galactose). A second growth phase in which the produced acetate is consumed, does not result into AI-2 production.

By feeding the reactor with a galactose solution in a continuous culture, the growth phase is

Table 3. Parameter values and initial concentration used for simulation.

$C_X(0)$	[OD]	0.05	m	[g/(OD · L)·h^{-1}]	0.2480
$C_{X,max}$	[OD]	1.830	$Y_{X,I}$	[(OD ·L)/g]	4.000
$\mu_{m,I}$	[h^{-1}]	1.200	$Y_{A,X}$	[g/(OD·L)]	$1.7602 \cdot 10^{1}$
$\mu_{m,A}$	[h^{-1}]	$0.5000 \cdot 10^{-2}$	$Y_{X,A}$	[(OD ·L)/g]	$0.3125 \cdot 10^{-1}$
$\sigma_{m,S}$	[g/(OD · L)·h^{-1}]	1.500			

prolonged keeping the specific growth rate at a user-defined value. During this persistent phase of metabolic activity, the AI-2 concentration in the growth medium is maintained. Once the feeding stops and all substrate is consumed, the AI-2 level in the medium drops below detection level. These results support the hypothesis that AI-2 production is strongly correlated with preferential substrate metabolism, rather than to biomass quorum only.

Walters and Sperandio (2006) report that most communication systems seem to be intrinsically involved in metabolism. Finding out which system is concerned in communication and which only in metabolism is an important, but not a trivial task. Our results of continuous experiments support the hypothesis that the AI-2 molecule is strongly correlated with metabolism. This could also mean that bacteria do not only communicate about quorum, but also about their environment and growth potential (Surette and Bassler, 1998).

The experimental observations together with knowledge from literature concerning the uptake mechanism for AI-2 (the *lsr* or LuxS regulated operon) are then combined in a macroscopic model that describes growth and AI-2 production and uptake of *Salmonella* Typhimurium SL1344. The predictions of the models are compared with experimental data for different modes of operation and deliver satisfying results.

The AI-2 bio-assay is, at this moment, the only measurement technique available for AI-2. This technique provides a qualitative profile rather than a quantitative measurement (Turovskiy and Chikindas, 2006). The lack of a quantitative measurement hampers the model building process. Nevertheless, the proposed model structure provides an accurate description of the AI-2 evolution profile for different operation modes. The model predicts accurately the conditions were production is maintained or where a peak in AI-2 concentration is observed. Due to the lack of an accurate quantitative measurement for the AI-2 molecule, parameter estimation is not an issue at this moment.

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