Stable and oscillatory signals from unstirred bacterial cultures

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A complex kinetic behavior of the bioluminescence was observed after transition of the *lux* gene fused *Alcaligenes eutrophus* from stirred to unstirred conditions. It is suggested that the bioluminescence signal carries information about the cell density dependent ("quorum sensing") expression of the *lux* genes and also suffers modulation by the structural changes, which predetermines the subsequent film formation. Dissipative structurization of the dense culture was assumed to explain the oscillatory responses observable bellow a certain critical temperature which is between 22 and 24 °C.

Key words: self-organization, quorum sensing, oscillations, *lux*-genes

INTRODUCTION

Intercellular communications and concerted multicellular activities are now generally accepted to be common among bacteria [1]. Currently it is well documented that small "quorum sensing" signal molecules are used to "switch" the expression of a wide variety of genes [1–3]. To date, quorum-sensing circuits have been identified in a wide range of bacteria where they regulate various functions including bioluminescence and biofilm formation [3]. The past decade has also witnessed discovery of bacterial self-organization, another phenomenon in the cooperative behavior of bacteria [4]. A self-organized culture exhibits the macroscopic nonuniformity of the concentration of bacterial populations. Moreover, spatial patterns produced by autoaggregated bacteria can be strikingly ordered [1, 4, 5].

A recent example of the concerted behavior of cells is oscillatory bioluminescence in unstirred cultures of the *lux* genes harboring bacteria [6–9]. It is notable that oscillatory bioluminescence as well as bacterial self-organization are clearly population-density-dependent processes. The aim of the present work was to exemplify the complexity of the bioluminescence kinetics observable at high cell densities and to derive arguments on the possible relationship among self-organization, oscillatory responses and quorum sensing in cultures of the *lux*-gene fused *A eutrophus*.

EXPERIMENTAL

A. eutrophus (AE1239) containing the *luxCDABE* genes of *Vibrio fisheri* placed under the control of copper inducible promoter were grown in LB medium until the critical concentration of about 0.15 mg/ml [6]. This concentration of biomass predetermines the nonlinear dependence of bioluminescence on cell density [6]. Induction of the culture was provided by addition of 10 mM $CuSO_4$ stock solution in LB medium to a final concentration of 2 mM. The incubation of the culture (10 ml) for several hours under stirring was followed by sampling and monitoring the bioluminescence kinetics. Glass test tubes were used as reaction vials. The volume of samples was 0.6 ml. A fiber optic luminometer [8, 9] was used for the monitoring of bioluminescence intensity. The procedures of incubation and signal monitoring were carried out at stable ambient temperatures.

RESULTS AND DISCUSSION

A complex transitional kinetic behavior of light emission is exemplified in Fig. 1. There are several notable features of the observed kinetics. First, the maximal bioluminescence signals exhibit a fast exponential growth (straight lines in the semi-log plane). Second, the signals adapted to unstirred conditions exhibit relative stability. Third, an imperceptible increase of the ambient temperature causes transition from oscillatory to non-oscillatory kinetics.

The exponential growth of the bioluminescence signal may indicate a quorum-sensing regulation of the *lux* gene expression [2, 3]. Thus, it is most probable that "quorum sensing" circuits are switched on at chosen experimental conditions. As noted abo-

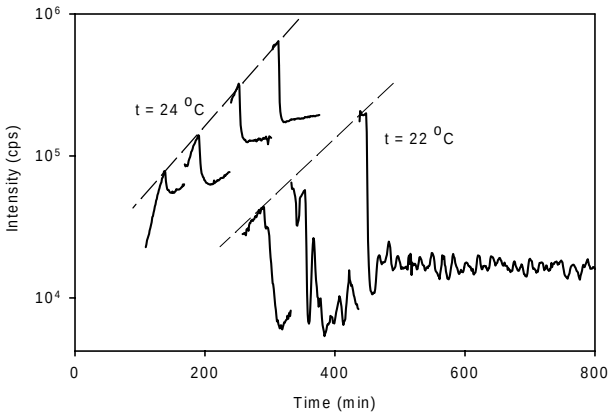

Fig. 1. Kinetic behavior of light emission from *A. eutrophus* adapting to unstirred conditions at different ambient temperatures

ve, these circuits may regulate film formation. We have observed a corresponding phase separation (deposits) at the late stages of prolonged experiments, typically when the concentration of the biomass reached 0.5 mg/ml. Thus, it is believable that the quorum sensing regulates the structure of the unstirred culture as well as the bioluminescence reaction.

To date, oscillatory responses from the cultures of *A. eutrophus* were observed at temperatures close to 20 °C [6, 7]. Our attempts to initiate oscillations at higher temperatures were unsuccessful (an example is shown in Fig. 1). Consequently, the temperature along with cell density is a crucial parameter predetermining the spatial-temporal order in an unstirred culture. Considering available information on the bioluminescence kinetics, we can estimate the site of the oscillatory luminescence phenomenon in the schematic H-shaped phase diagram (Fig. 2). The oscillatory phase may be attributed to the prolonged autoagregation of the culture, also known as bacterial self-organization [1, 4, 5]. This phase also represents the particular dissipative structures which, according to I. Prigogine's theory, may spontaneously occur in the dissipative non-linear open systems [10].

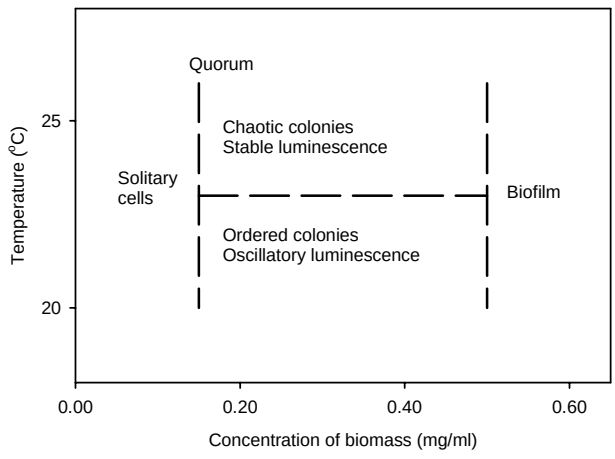

Fig. 2. Morphological phases of *A. eutrophus* achievable under unstirred conditions. H-shaped lines represent crude estimates of the particular complex curves in the imaginary phase diagram

In conclusion, *lux*-gene engineered cells seem to be suitable for investigation of cell density dependent processes in unstirred cultures. Indeed, the bioluminescence signal carries information on the appearance of the "quorum sensing" and also suffers modulation by the soft structural changes such as formation of the dissipative structures.

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STABILŪS IR OSCILIUOJANTYS SIGNALAI IŠ NEMAIŠOMŲ BAKTERIJŲ KULTŪRŲ

S a n t r a u k a

Pakitusios *Lux* genais žymėtų *Alcaligenes eutrophus* inkubavimo sąlygos nutraukus kultūros maišymą atskleidė sudėtingus adaptacinius bioliuminescencijos signalus. Stebima kinetika aiškinama nuo ląstelių tankio priklausoma *lux* genų ekspresija ir struktūriniais pokyčiais tankiose kultūrose. Nustatyta, kad savitvarkėms disipatyvioms struktūroms priskirti osciliuojantys signalai gali būti stebimi esant žemesnei už krizinę temperatūrai, t. y. tarp 22 ir 24°C.