Oceanological and Hydrobiological Studies
Vol. XXXIV, No. 2

Institute of Oceanography (55-61) University of Gdańsk
2005

Research Article

A NOVEL *VIRBIO HRAVEYI* MUTANT WITH ALTERED QUORUM SENSING REGULATION

AGATA CZYŻ[1], RYSZARD ZIELKE[2] AND GRZEGORZ WĘGRZYN[2,3,*]

[1] Laboratory of Molecular Biology (affiliated with the University of Gdańsk),
Institute of Biochemistry and Biophysics,
Polish Academy of Sciences,
Kładki 24, 80-822 Gdańsk, Poland
[2] Department of Molecular Biology, University of Gdańsk,
Kładki 24, 80-822 Gdańsk, Poland
[3] Institute of Oceanology, Polish Academy of Sciences,
Św. Wojciecha 5, 81-347 Gdynia, Poland

**Corresponding author e-mail: wegrzyn@biotech.univ.gda.pl*

Key words: quorum sensing, marine bacteria, bioluminescence

Abstract

The expression of genes involved in the luminescence (*lux* genes) of many light-emitting bacteria, including the marine bacterium *Vibrio harveyi*, is regulated by a phenomenon called quorum sensing. The expression of the *lux* genes, and thus the efficiency of light emission, depends on the concentration of cells in the environment. Bacterial luminescence is effective when cells occur at high density, whereas light emission is negligible in diluted cultures. Quorum sensing regulation is a complex process which requires the functioning of many genes. The current paper describes the recently isolated novel *V. harveyi* mutant, which now appears to be impaired

in quorum sensing. The mutant produces autoinducers normally, but it is partially defective in responding to these molecules, thus its quorum sensing reaction is delayed relative to wild-type bacteria.

INTRODUCTION

Quorum sensing is a bacterial regulatory mechanism resulting in the dependence of the efficiency of a given cellular reaction on the concentration of cells in the environment (for reviews see Swift *et al.* 1998, Winans and Bassler 2002). Various functions are regulated by quorum sensing, including the pathogenicity of some microbes and the luminescence of marine bacteria. The existence of this mechanism means that the regulated process is effective when cells occur at a high density, but it is negligible in diluted cultures.

Vibrio harveyi is a luminescent, marine bacterium. The reaction of its luminescence is catalyzed by luciferase, an enzyme composed of subunits α and β that are encoded by the *luxA* and *luxB* genes, respectively (Belas *et al.* 1982). The substrates for the luciferase are long-chain aldehydes and $FMNH_2$. The genes coding for luciferase subunits are included in the *luxCDABEGH* operon. Genes *luxC, luxD*, and *luxE* code for proteins that form a complex of fatty acid reductase, whereas the products of genes *luxG* and *luxH* are responsible for the synthesis of reduced flavine (Meighen 1994). Apart from these genes, *V. harveyi* contains several additional genes involved in the regulation of bioluminescence. There are regulatory genes *luxR, luxO*, and *luxU* , genes coding for two autoinducer synthetases (*luxL* and *luxM*, coding for the synthetase of the autoinducer AI-1, and *luxS*, coding the synthetase of the autoinducer AI-2), and genes coding sensors of autoindurcers: *luxN* (sensor of AI-1) and *luxP* and *luxQ* (sensor of AI-2) (Bassler *et al.* 1994, Freeman and Bassler 1999). The *luxR* gene product is a *luxCDABEGH* operon activator (Chatterjee *et al.* 1996, Miyamoto *et al.* 1996). The negative regulator of this operon is the LuxO protein (Bassler *et al.* 1994). The sensory proteins LuxN and luxPQ are responsible for detecting autoinducers (AI-1 and AI-2, respectively). Subsequent signal transduction, mediated by the LuxU protein and based on phosphorylation and dephosphorylation reactions, leads to the inactivation of LuxO (Freeman and Bassler 1999, Freeman *et al.* 2000) and the stimulation of the expression of the *luxCDABEGH* operon. This leads to the efficient production of luciferase and other enzymes necessary for the luminescence reaction. Such regulation ensures that luminescence is very efficient when cells produce large amounts of autoinducers, which occurs only at high cell density due to a positive feedback mechanism based on the stimulation of autoinducer synthesis by neighboring bacteria that release the same type of autoinducer outside cells.

Although many genes involved in quorum sensing have been identified, it seems that there are other factors, as yet unidentified, that participate in this regulatory mechanism (Winans and Bassler 2002). The authors have recently isolated a *V. harveyi* mutant (a strain named BB7X) which, apart from other phenotypes, revealed decreased luminescence, and, in particular, delayed the reappearance of luminescence after the dilution of bacterial culture, which strongly suggested impairment in quorum sensing regulation (Czyż *et al.* 2001). The mutant was described as bearing an insertion in the *cgtA* gene, coding for an evolutionary conserved small GTP-binding protein (Czyż et al. 2001). The aim of the current investigation was to determine at which step quorum sensing is affected in this mutant.

MATERIALS AND METHODS

Bacterial strains

V. harveyi wild-type strain, BB7 (Belas *et al.* 1982), and its derivative, BB7X, described previously as *cgtA*::Tn*5*TpMCS mutant (Czyż et al. 2001) were used.

Monitoring the quorum sensing response

Bacterial cultures were grown to high cell density (to allow efficient luminescence) in BOSS medium (Klein *et al.* 1998). The cultures were diluted 10,000-fold in fresh medium and cultivation was continued. Samples were withdrawn at time intervals and luminescence was estimated using a luminometer (Sirius, Berthold Detection Systems). In the same samples, the number of bacterial cells was estimated by plating. Relative luminescence was calculated as luminometer impulses per cell. In some experiments, a supernatant from a dense culture (0.25 volume of the recipient culture) was added at the time of low-efficient luminescence.

RESULTS AND DISCUSSION

Due to quorum sensing regulation, *V. harveyi* cells growing at high cell density emit light efficiently (for a recent review, see Winans and Bassler 2002). After the dilution of a dense *V. harveyi* culture, the luminescence reaction becomes less and less efficient. However, as the culture grows, cell concentration increases and the cells start to produce autoinducer again, which results in the reappearance of efficient luminescence.

Such a classical decrease and increase of luminescence was observed previously in cultures of wild-type (strain BB7) bacteria (Czyż *et al.* 2001). This

phenomenon was also observed by the current authors in cultures of the BB7X mutant strain; however, a significantly higher cell density had to be achieved to stimulate luminescence (Czyż *et al.* 2001). These results indicated that the quorum sensing regulation might be impaired in the mutant. If so, there are three possible mechanisms of such impairment: (i) BB7X produces autoinducer(s) less efficiently; (ii) autoinducers synthesized by BB7X are less active; (iii) this mutant is partially defective in transducing the signal. To answer the question of which of these three possible mechanisms operates, the luminescence of diluted cultures of BB7 and BB7X strains was monitored after the addition of supernatants of centrifuged dense cultures of one of these strains (all possible combinations were studied). Since supernatants of dense cultures contain autoinducers, they provoke efficient luminescence when added to diluted cultures.

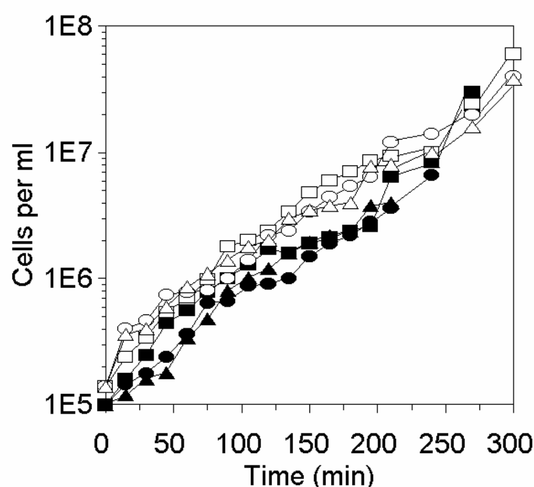

Fig. 1. Growth of *Virbio harveyi* wild-type (BB7, closed symbols) and *cgtA* (BB7X, open symbols) strains in BOSS medium without any treatment (squares) and after the addition of 0.25 volume of supernatant from a dense culture of BB7 (circles) or BB7X (triangles)

The growth curves of the diluted cultures of both strains, either with or without added supernatant, were similar (Fig. 1). Therefore, any observed differences in luminescence efficiency could not have been caused by putative changes in bacterial growth rates caused by the addition of supernatants.

It was found that the addition of a supernatant from a dense culture of BB7 to a diluted culture of this strain resulted in a rapid increase in luminescence, as expected (Fig. 2). The reaction was very similar when a supernatant from BB7X dense culture was added to the diluted BB7 culture. This indicates that strain

BB7X can produce active autoinducers in amounts sufficient for normal quorum sensing regulation. Additionally, no differences were observed between the luminescence of diluted cultures of BB7X when the effects of adding supernatants from BB7 and BB7X dense cultures were compared (Fig. 2). However, the comparison of the luminescence induction in the BB7 and BB7X cultures indicated that the response of the latter strain to the addition of supernatants from dense cultures was significantly delayed relative to the former (Fig. 2). Therefore, the authors concluded that the intracellular transduction of the signal provided by autoinducer molecules is impaired in the mutant.

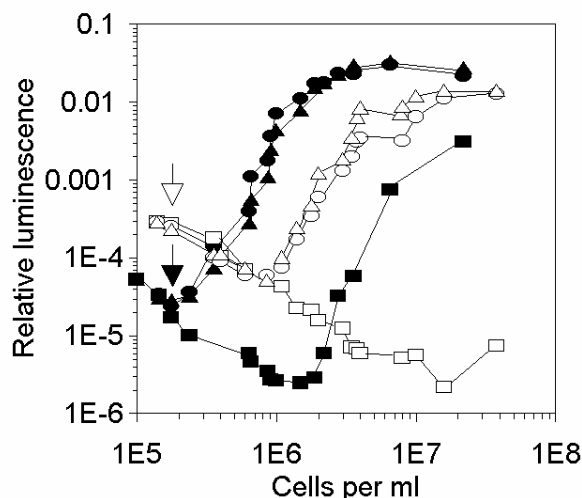

Fig. 2. Relative luminescence of *Virbio harveyi* wild-type (BB7, closed symbols) and *cgtA* (BB7X, open symbols) strains in BOSS medium without any treatment (squares) and after the addition of 0.25 volume of supernatant from a dense culture of BB7 (circles) or BB7X (triangles). Dense cultures were diluted 10,000 times at time zero, and supernatants from dense cultures were added to the investigated cultures at the time indicated by arrows

It remains to be elucidated what the molecular mechanism is for the impaired response of the BB7X mutant to autoinducer molecules. Since BB7X was described as bearing an insertion in the *cgtA* gene, and this gene codes for a GTP-binding protein, one could speculate that the CgtA protein is involved in signal transduction reactions leading to autoinducer-mediated gene expression stimulation. In fact, homologues of CgtA from other bacteria (*e.g.*, *Bacillus subtilis* and *Escherichia coli*) were proposed to play roles in various cellular processes that may involve signal transduction, including the regulation of sporulation initiation (Vidwans *et al.* 1995), the control of DNA replication

(Kok *et al.* 1994, Ulanowska *et al.* 2003), chromosome partitioning (Kobayashi *et al*. 2001; Dutkiewicz *et al.* 2002), the regulation of DNA repair (Zielke *et al.* 2003), the stress-dependent activation of transcription factor σ^B (Scott and Haldenwang, 1999), and the functions of ribosomes (Scott *et al.* 2000). Conversely, it seems that many effects observed in various mutants in homologous of the *cgtA* gene may be indirect. Thus, although in the current study the stage of the impairment of quorum sensing in the BB7X strain was determined, further studies are required to understand the specific function of *cgtA* in the quorum sensing regulation.

ACKNOWLEDGMENT

This work was supported by the Polish Ministry of Science and Information Technology (project grant no. 6 P04B 022 20 to A.C.) and the Institute of Oceanology, Polish Academy of Sciences (task grant no. IV.3.1. to G.W.).

REFERENCES

Bassler B. L., Wright M., Silverman M. R., 1994, *Sequence and function of luxO, a negative regulator of luminescence in Vibrio harveyi*, Mol. Microbiol., 12, 403-412

Belas R., Mileham A., Cohn D., Hilmen M., Simon M., Silverman M., 1982, *Bacterial luminescence: isolation and expression of the luciferase genes from Vibrio harveyi*, Science, 218, 791-793

Chatterjee J., Miyamoto M., Meighen E.A., 1996, *Autoregulation of luxR: the Vibrio harveyi lux-operon activator functions as a represor*, Mol. Microbiol., 20, 415-425

Czyż A., Zielke R., Konopa G., Węgrzyn G., 2001, *A Vibrio harveyi insertional mutant in the cgtA (obg, yhbZ) gene, whose homologues are present in diverse organisms ranging from bacteria to humans and are essential genes in many bacterial species*, Microbiology, 147, 183-191

Dutkiewicz R., Słomińska M., Węgrzyn G., Czyż A. (2002*) Overexpression of the cgtA (yhbZ, obgE) gene, coding for an essential GTP-binding protein, impairs the regulation of chromosomalfunctions in Escherichia coli*. Curr. Microbiol., 45, 440–445

Freeman J. A., Bassler B. L., 1999, *A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in Vibrio harveyi*, Mol. Microbiol., 31, 665-677

Freeman J. A. Lilley B. N., Bassler B. L., 2000, *A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in Vibrio harveyi*, Mol. Microbiol., 35, 139-149

Klein G., Żmijewski M., Krzewska J., Czeczatka M., Lipińska, B., 1998, *Cloning and characterization of the dnaK heat shock operon of the marine bacterium Vibrio harveyi*, Mol. Gen. Genet., 259, 179-189

Kobayashi G., Moriya S., Wada C., 2001, *Deficiency of essential GTP-binding protein ObgE in Escherichia coli inhibits chromosome partition,* Mol. Microbiol., 41, 1037–1051

Kok J., Trach K. A., Hoch J. A., 1994, *Effects on Bacillus subtilis of a conditional lethal mutation in the essential GTP-binding protein Obg.* J. Bacteriol., 176, 7155–7160

Meighen E. A., 1994, *Genetics of bacterial bioluminescence*, Annu. Rev. Genet., 28, 117-139

Miyamoto C. M., Chatterjee J., Swartzman E., Szittner R., Meighen E. A., 1996, *The role of lux autoinducer in regulating luminescence in Vibrio harveyi; control of luxR expression*, Mol. Microbiol., 19, 767-775

Scott J. M., Haldenwang W. G., 1999, *Obg, an essential GTP binding protein of Bacillus subtilis, is necessary for stress activation of transcription factor* σ^B. J. Bacteriol., 181, 4653–4660

Scott J. M., Ju J., Mitchell T., Haldenwang W. G., 2000, *The Bacillus subtilis GTP binding protein Obg and regulators of the* σ^B *stress response transcription factor cofractionate with ribosomes*. J. Bacteriol., 182, 2771–2777

Swift S., Throup J., Bycroft B., Willimas P., Stewart G., 1998, *Quorum sensing: bacterial cell-cell signaling from bioluminescence to pathogenicity*, [in:] *Molecular Microbiology,* Busby S. J. W., Thomas C. M., Brown N. L. (eds.), Springer-Verlag, Berlin-Heidelberg, 185-207

Ulanowska K., Sikora A., Węgrzyn G., Czyż A., 2003, *Role of the cgtA gene function in DNA replication of extrachromosomal elements in Escherichia coli.* Plasmid, 50, 45-52

Vidwans S. J., Ireton K., Grossman A. D., 1995, *Possible role for the essential GTP-binding protein Obg in regulating the initiation of sporulation in Bacillus subtilis*. J. Bacteriol., 177, 3308–3311.

Winans S. C., Bassler B. L., 2002, *Mob psychology*, J. Bacteriol., 184, 873-883

Zielke R., Sikora A., Dutkiewicz R., Węgrzyn G., Czyż A., 2003, *Involvement of the cgtA gene function in stimulation of DNA repair in Escherichia coli and Vibrio harveyi*, Microbiology, 149, 1763-1770