ISSN 0891-4168, Molecular Genetics, Microbiology and Virology, 2014, Vol. 29, No. 4, pp. 167–171. © Allerton Press, Inc., 2014.
Original Russian Text © V.A. Plyuta, A.A. Popova, O.A. Koksharova, A.E. Kuznetsov, I.A. Khmel, 2014, published in Molekulyarnaya Genetika, Mikrobiologiya i Virusologiya, 2014, No. 4, pp. 10–13.

EXPERIMENTAL WORKS

The Ability of Natural Ketones to Interact with Bacterial Quorum Sensing Systems

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Received February 20, 2014

Abstract—The effect of natural ketones emitted by bacteria (2-nonanone, 2-heptanone, and 2-undecanone) on the functioning of the Quorum Sensing (QS) systems was studied. In this work, three lux-reporter strains containing the components of the LasI/LasR, RhlI/RhlR, and LuxI/LuxR QS systems were used as biosensors for N-acyl-homoserine lactones. It was shown that, at concentrations of ketones that exhibited little or no bacterial action, the ketones could modulate the QS response by suppressing the expression of the *lux* operon to a greater extent than the cell viability of these strains.

Keywords: ketones, Quorum Sensing, *lux* biosensors
DOI: 10.3103/S0891416814040077

INTRODUCTION

Many microorganisms synthesize volatiles, including volatile organic compounds (VOCs), which are able to suppress bacterial and fungal growth and have a stimulating or inhibiting effect on plants [1–3]. Bacterial VOC producers include soil or rhizosphere strains of various *Pseudomonas* and *Serratia* genera [4–6], human opportunistic pathogen *P. aeruginosa* [7, 8], and marine bacteria [9]. VOC production has been proposed to be a factor in competitive activities between microorganisms. Moreover, VOCs may play a significant role in communication between organisms (infochemicals) [10, 11]. VOCs can be considered as important components participating in interactions of microorganisms and higher organisms in the environment. As many cell processes in bacteria are regulated by Quorum Sensing (QS) systems, it is of interest to study if VOCs affect these systems, which function as global regulators of bacterial gene expression [12].

We have shown that volatiles emitted by various soil and rhizosphere *Pseudomonas* and *Serratia* strains (total pool) can suppress various forms of QS communication in bacteria that use N-acyl-homoserine lactones (AHLs) as signaling molecules, thus performing the Quorum Quenching (QQ) effect. The presence of VOCs significantly suppressed AHL synthesis. The inhibiting effect on the QS response was mediated by dimethyl disulfide (DMDS), which is the major volatile produced by *Serratia plymuthica* IC1270. It has been shown using RT-PCR that the total pool of volatiles emitted by *Pseudomonas chlororaphis* 449, *P. fluorescens* B-4117, and *S. plymuthica* IC1270 and DMDS alone suppress the gene expression of AHL synthases *phzI* and *csaI* in *P. chloraphis* 449. 1-Undecene, which is the main VOC produced by *P. fluorescens* B-4117, did not cause this effect [13, 14]. Modulation of the QS response (decrease or increase) by VOCs emitted by marine bacteria has been shown in [9] using two AHL biosensors.

Bacterial VOCs often contain ketones [7, 8, 10]. The purpose of this work is to study the effect of three separate natural ketones (2-nonanone, 2-heptanone, and 2-undecanone; Fig. 1) on the functioning of QS signaling in bacteria. Work has been performed using three *lux*-reporter *Escherichia coli* strains responding to various exogenous AHL. We have obtained the data showing that ketones produced by bacteria are able to interact with QS systems that incorporate AHL as a signaling molecule and suppress their functioning.

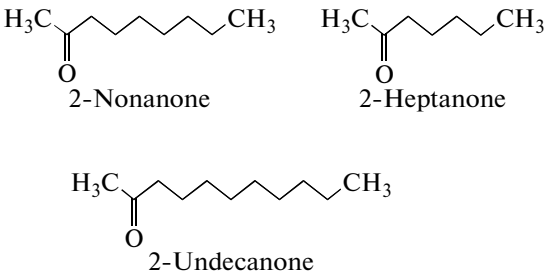

Fig. 1. Ketones used in the work.

Bacterial strains used in the work

Strain	Characteristics	Origin or reference
Escherichia coli DH5α	F-endA1 hsdR17($rk^- mk^+$) supE44 thi-1 recAl gyrA96 relAl φ80dlacZ ΔM15 λ	Collection of the Institute of Molecular Genetics, Russian Academy of Sciences, Moscow
E. coli DH5α/pSB401	*E. coli* DH5α, pSB401: 1uxR$^+$1uxI::1uxCDABE, Tet^{R}p15A origin	[15]
E. coli JLD271	K-12 Δ1acX74 sdiA271::Cam	[17]
E. coli JLD271/pAL101	*E. coli* JLD271, pAL101: rhlR+rhlI::luxCDABE; Tet^{R}pl5A origin	[17]
E. coli JLD271/pAL105	*E. coli* JLD271, pAL 105: lasR+ luxI::luxCDABE; Tet^{R}p15A origin	[17]

MATERIALS AND METHODS

Bacteria, Culturing Conditions

Bacterial strains used in the work are shown in the table. Bacteria were grown in liquid LB medium or LA medium (Sigma-Aldrich, United States) at 30°C. Antibiotics were added into media in concentrations of 20 μg/mL of tetracycline hydrochloride (AppliChem, Germany) and 25 μg/mL of chloramphenicol (Sigma, United States). 2-Nonanone (>99%), 2-heptanone (>99%), and 2-undecanone (98%) were obtained from Sigma-Aldrich Chimie GmbH (Steinheim, Germany).

The Effect of Ketones on Biosensors

Three biosensors containing the *lux* operon as a reporter were used in the work. Biosensor cells were grown for 17–18 h at 30°C in LB medium with corresponding antibiotics (see table). Biosensor cultures were diluted ten times with LB without antibiotics. Ketones were added directly into the liquid culture. AHL was added into an ethyl acetate volume not exceeding 2 μL per 2 mL of the culture; larger amounts of ethyl acetate suppressed *E. coli* growth. Tubes were closed with parafilm in order to prevent emission of volatile ketones; cultures were grown at 30°C.

Bioluminescence of sensors containing specified concentrations of ketones and corresponding AHL standard and the control (contains AHL standard but does not contain ketones) was measured in the course of growth of cultures (usually after 4 or 6 h of growth). Measurements were carried out using a Modulus microplate Multimode Reader (Turner BioSystems, United States). Cells obtained from corresponding culture dilutions were then plated into LA medium. The number of CFU/mL was determined in the control and in samples with various concentrations of ketones. Each experiment was performed at least three times.

RESULTS AND DISCUSSION

Three ketones synthesized by bacteria and having various chain lengths but the same position of the ketone group in the chain were used in this work (Fig. 1).

We preliminarily determined the effect of the concentration of added AHLs that are recognized by the used biosensors and the time of incubation with AHLs on induction of the QS response, i.e., bioluminescence intensity. It was revealed that the optimum time of taking measurements is 6 h after culturing with AHLs when luminescence is high but still has not reached a plateau. Then it was necessary to determine the effect of the studied ketones on cell viability; it was reasonable to use ketone concentrations that did not suppress, or slightly suppressed, cell growth in order to distinguish between these two effects. It is clear that the death of the majority of sensor cells will be accompanied by a sharp decrease in bioluminescence which is determined by the *lux* operon due to the death of bacteria. The results of the performed experiments are shown in Figs. 2–4.

When an *E. coli* JLD271/pAL105 reporter strain containing the components of the QS system of *Pseudomonas aeruginosa* (the gene of the receptor regulatory protein LasR that interacts with AHLs and the gene of AHL synthase LasI containing the insertion of *lux* reporter) N-3-oxo-dodecanoyl-homoserine lactone (3-oxo-C12-HL), which is specific to this QS system, was added into the medium [12]. At final concentrations of AHL of 0.25 μg/mL and 2-nonanone of 1 μM, the number of cells grown for 6 h of the experiment was ~100% as in the control in the absence of ketones; at the same time, bioluminescence decreased on average up to 46% of the control (see Fig. 2); i.e., the QS response was more sensitive to 2-nonanone than cell viability. At a 2-nonanone concentration of 2 μM, the number of living cells decreased up to 62% and bioluminescence decayed up to 34% of the con-

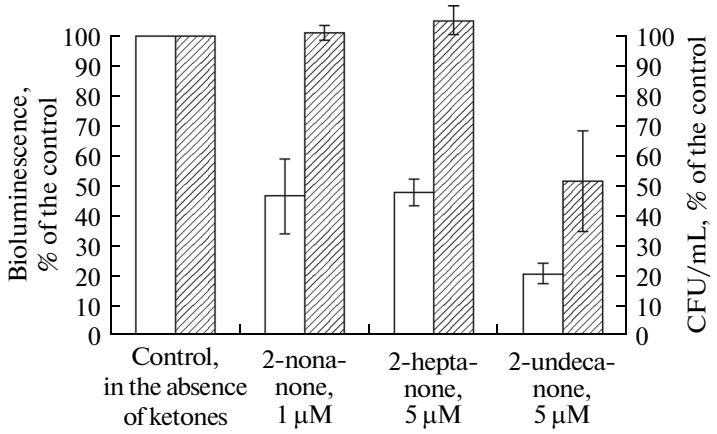

Fig. 2. Effect of the ketones on bioluminescence of *E. coli* JLD271/pAL105 biosensor.
Light columns show the level of bioluminescence of the reporter strain (relative units) as percentage of the control; hatching designates CFU/mL of the reporter strain as percentage of the control. As the control, a sample containing 3-oxo-C12-HL in the absence of ketones was used.

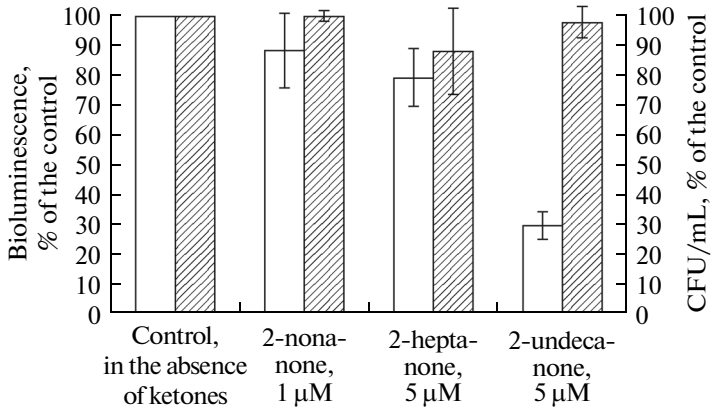

Fig. 3. Effect of the ketones on bioluminescence of *E. coli* JLD271/pAL101 biosensor.
Designations are the same as in Fig. 2. As the control, a sample containing C4-HL in the absence of ketones was used.

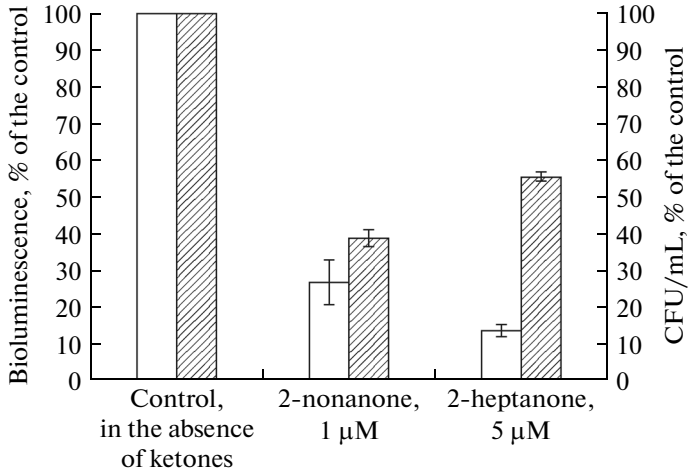

Fig. 4. Effect of the ketones on bioluminescence of *E. coli* DH5α/pSB401 biosensor.
Designations are the same as in Figs. 2 and 3. As the control, a sample containing C8-HL in the absence of ketones was used.

trol. Higher concentrations of 2-nonanone caused total death of the biosensor.

A similar situation was observed under the action of 2-heptanone (5 μM): the absence of suppression of cell viability and a decrease in luminescence of the reporter strain up to 47% as compared to the control without 2-heptanone. We observed a different effect using the same concentration of 2-undecanone: the biosensor was more sensitive to it, and the number of CFU/mL decreased up to 50% of the control. However, in this case, bioluminescence was also inhibited to a greater extent, up to 20% of the control. Consequently, suppression of bioluminescence under the action of three studied ketones on the *E. coli* JLD271/pAL105 biosensor was significantly higher than the effect of the ketones on cell viability (see Fig. 2).

The second *lux* biosensor used was *E. coli* JLD271/pAL101 constructed on the basis of the components of the second AHL-using QS system of *P. aeruginosa*, RhlI/RhlR. N-butanoyl-homoserine lactone (C4-HL) is a specific AHL for this *P. aeruginosa* system [12]. It was added to the culture in a concentration of 0.25 μg/mL. 2-Nonanone and 2-heptanone used in the same concentrations as in the case on the *E. coli* JLD271/pAL105 biosensor did not affect significantly cell viability and luminescence. However, the addition of 5 μM of 2-undecanone into the culture was accompanied by a luminescence decrease up to 30% as compared to the control. Sensor viability remained the same as in the control (see Fig. 3). Consequently, in the case of this QS system, only 2-undecanone out of three tested ketones influenced the QS response significantly. An increase in ketone concentrations resulted in abrupt decay in cell viability.

The third reporter strain used in the study of the effect of ketones on the functioning of QS systems was a *E. coli* DH5α/pSB401 *lux*-biosensor obtained on the basis of the QS system LuxI/LuxR from *Vibrio fischeri*. This sensor contains the gene of the *lux*R receptor and the gene of *lux*I AHL synthase with the insertion of *lux* operon; it is the most sensitive to AHLs C6-HL–C-8-HL including AHLs containing oxo-groups [15]. As an AHL standard, N-octanoyl-homoserine lactone (C8-HL) was added into the culture in a concentration of 0.25 μg/mL. We used the same concentrations of the ketones as in the cases of biosensors based on LasI/LasR and RhlI/RhlR QS systems. The presence of 2-nonanone caused a decrease in biosensor luminescence up to 28% of the control level; however, cell viability under its action also decreased up to ~40% of the control. The effect of 2-heptanone on bioluminescence was more pronounced: it caused a decrease of up to 14% of the control without 2-heptanone at a decrease in viability of up to 55% of the control (see Fig. 4). An increase in 2-nonanone and 2-heptanone concentrations up to more than 2 and 5 μM, respectively, was accompanied by total death of biosensor cells. The *E. coli* DH5α/pSB401 biosensor turned out to be extremely sensitive to 2-undecanone: practically all cells died when this ketone was added to the culture. The reason for the difference in sensitivity to 2-undecanone as compared to other biosensors is not clear. Consequently, under the action of two ketones (2-nonanone and 2-heptanone), we observed the same dependence as in the cases of other sensors—a decrease in the QS response to a greater extent than in cell viability.

From the results of this work, we can conclude that natural volatile ketones are able to modulate gene expression of various bacterial QS systems using various AHLs as signaling molecules and to a lesser extent affect the viability of biosensor cells. Suppression of the expression of the reporter *lux* operon is probably caused by inhibition of operon transcription from the promoter of the AHL synthase gene. These effects depend on the viability of biosensors and, as we showed, are better seen under conditions in which ketones slightly suppress, or do not suppress, cell growth. Moreover, biosensors containing the components of different QS systems differed in sensitivity to ketones. Stimulation of the expression of the reporter *lux* operon by ketones was not found in any case. We did not determine if ketones affected AHLs directly. However, it was shown in previous work [13] that there was no direct inactivation of AHLs in the case of the QQ effect of the total VOC pool emitted by *P. chlororaphis* that contained significant amounts of 2-nonanone and 2-undecanone.

Synthesis of VOCs is a frequent property of many bacteria [16]. QS systems are known to participate in regulation of many cellular processes including the control of antagonistic properties of bacteria, their ability to colonize specific ecological niches, and virulence. We can assume that suppression of QS regulation by produced VOCs is another side of bacterial competition, along with the inhibitory effect of VOCs on their growth and viability. This property can be important for bacterial interaction in soil and plant rhizosphere and protection of plants from pathogenic bacteria. It is quite possible that VOCs also play a significant role in the interactions of human microflora.

ACKNOWLEDGMENTS

This work was partially supported by the Russian Foundation for Basic Research, grant no. 12-04-00636.

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Translated by O. Maloletkina