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CONFERENCE PROCEEDINGS

Quorum-Sensing Regulation in Soil Pseudomonads

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Abstract—228 strains of soil and rhizosphere pseudomonads isolated in different geographic zones were screened, with the use of two tester systems, for the capacity to produce *N*-acyl-homoserine lactones (AHLs), which are autoinducers involved in quorum-sensing (QS) regulation. AHL production was found in 11.4% of the strains investigated. In five *Pseudomonas chlororaphis* strains shown to be active AHL producers and chosen for further study, PCR identified two QS systems that involved the *phzI, phzR, csaI*, and *csaR* genes; this finding suggests the conservative nature of these regulation systems in *P. chlororaphis.* Strain *P. chlororaphis* 449, chosen as a model object and studied in greater detail, produced three AHL species including *N*-butanoyl-homoserine lactone and *N*-hexanoyl-homoserine lactone. This strain produced three types of phenazine antibiotics, as well as siderophores and cyanide; it also exhibited antagonistic properties toward a wide spectrum of phytopathogenic fungi. The *phzI* and *csaI* genes, coding for synthases of AHLs of two types, were cloned and sequenced; mutants with knocked-out *phzI* and *csaI* genes were obtained. With the use of transposon mutagenesis and the gene substitution method, mutations were obtained in the global expression regulator genes *gacS,* coding for the GacA–GacS regulation system kinase, and *rpoS*, coding for the sigma S subunit of RNA polymerase. The effect of these mutations on the AHL synthesis and on the regulation of various metabolic processes in *P. chlororaphis* was studied.

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Regulation of gene expression by a mechanism dependent on the density of the bacterial population (quorum sensing, QS) plays a significant role in the control of a large number of processes occurring in the cells of gram-negative bacteria. Regulatory systems of this type are involved in the interactions of bacteria with higher organisms during pathogenesis or symbiosis and in the regulation of the expression of genes related to antibiotic and enzyme biosynthesis, bioluminescence, conjugation, etc. [1–9]. The QS systems of gram-negative bacteria obligatorily involve low-molecular-weight autoinducers, namely, *N*-acyl-homoserine lactones (AHL). These signal molecules have been shown to be produced by many soil and rhizosphere bacteria (pseudomonads in particular) whose metabolism is tightly associated with plants. However, only for a few bacteria have the QS systems and their role in the regulation of the bacterial metabolism been thoroughly studied.

Our studies aim at the investigation of QS regulation in bacteria of the genus *Pseudomonas*. The main lines of research are (1) identification and study of the genes related to QS systems; (2) investigation of the regulation of the functioning of QS systems, of the interaction of QS systems with other systems of global regulation of gene expression, and of the hierarchy of regulation systems; and (3) investigation of the role of QS in the regulation of various processes occurring in cells.

At the first stage of our work, we screened various *Pseudomonas* species, isolated from soil or rhizosphere in different geographic zones of Russia and the republics of the former Soviet Union (collection of T.A. Sorokina, Institute of Molecular Genetics, Russian Academy of Sciences), for the capacity to produce AHLs. Determination of AHLs was performed with the use of two tester systems. The first system employed the mutant strain *Chromobacterium violaceum* CV026, which, due to the insertion of the Tn5 transposon into the gene of AHL synthase, produces neither *N*-hexanoyl-homoserine lactone nor the purple–blue pigment violacein whose synthesis is dependent on it. In the presence of exogenous AHL, the synthesis of the pigment is restored [10]. The second test system employed strain *Agrobacterium tumefaciens* NTL4/pZLR4,

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which produces β-galactosidase in the presence of exogenous AHLs [11]. The combined use of these two test systems, which allows most of the currently known AHLs to be revealed [12], resulted in the detection of AHL synthesis in 26 of the 228 strains of soil and rhizosphere pseudomonads screened by us. This capacity was most frequent and pronounced in *P. chlororaphis* (= *P. aureofaciens*) and was less frequent in *P. putida, P. fluorescens, P. lemonieri*, and *P. geniculata* (ranked in order of decreasing frequency of AHL production) [13].

Five strains of *P. chlororaphis*, isolated from the rhizosphere of various plants in different geographic zones, proved to be active AHL producers and were chosen for further study. Earlier, two QS regulation systems were found in strain *P. chlororaphis* 30-84: the PhzI–PhzR system, involved in the regulation of the synthesis of phenazine pigments (antibiotics); and the CsaI–CsaR system [14]. By using PCR amplification, we demonstrated the presence of the genes of both of these systems (*phzI, phzR, csaI*, and *csaR*) in cells of each of the five *P. chlororaphis* strains that we studied. This suggests evolutionary conservatism of the QS systems in *P. chlororaphis.*

Strain *P. chlororaphis* 449, isolated from maize rhizosphere in Kiev oblast, Ukraine, was chosen as a model object for further analysis of QS systems, of the genetic control of their functioning, and of their role in the regulation of the processes occurring in cells. The properties of strain 449 were studied in detail. It was shown to exhibit antagonistic activity against a broad spectrum of phytopathogenic fungi. The strain was found to produce three phenazine antibiotics, which are the main factors accounting for its inhibitory effect on phytopathogenic fungi. By using thin-layer chromatography and a biotest with *C. violaceum* CV026, we found that *P. chlororaphis* 449 cells produce AHLs of three types: *N*-hexanoyl-homoserine lactone, *N*-butanoyl-homoserine lactone, and one more minor compound. Strain 449 cells produced cyanide and siderophores, which may contribute to its antagonistic activity. In this strain, we found exoprotease, lipase, phosphatase, polygalacturonase, and pectin methylesterase activities.

Then, we performed cloning of genes belonging to QS systems, namely, the *phzI* and *csaI* genes, coding for the synthases of AHLs of two types. Chromosomal DNA of *P. chlororaphis* was digested with several restriction endonucleases and ligated, after which *Escherichia coli* XL1 cells were transformed with the ligated mixture. With the use of primers specific to the *phzI* and *csaI* genes, PCR analysis of the cloned fragments was performed, and clones yielding PCR products of appropriate size were selected. The nucleotide sequences of the *phzI* and *csaI* genes were determined (584 and 659 bp, respectively). Their comparison with nucleotide sequences available in databanks revealed significant homology with the genes coding for AHL synthases of *P. chlororaphis* 30-84 (93 and 92% identity, respectively) and *P. fluorescens* 2-79 (83 and 81% identity). In order to perform an in-depth study of the role of these two QS systems in the regulation of the processes occurring in cells, mutants with knocked out *phzI* and *csaI* genes were obtained by us by using the gene replacement method [15]. To do this, we obtained PCR-amplified fragments of the *phzI* and *csaI* genes and cloned them in plasmid pEx18Tc in *E. coli* cells. Then, using the restriction–ligation method, we introduced casettes that contained kanamycin- or gentamycin-resistance genes into the fragments cloned; as a result, the nucleotide sequences in the *phzI* and *csaI* genes were interrupted. The recombinant plasmids were transferred from *E. coli* S17-1 cells to *P. chlororaphis* cells. The acquired plasmids could not be stably maintained in *P. chlororaphis* cells and were eliminated; however, the cloned fragments of *phzI* and *csaI* genes (which contained insertions) were incorporated into the *P. chlororaphis* chromosome due to recombination followed by selection in kanamycin- or gentamycin-containing media. As a result, the wild-type *phzI* and *csaI* genes contained in the chromosome of the parent strain *P. chlororaphis* 449 were replaced by mutant genes. We also obtained a double mutant *phzI csaI*, in which neither of the QS systems was functional.

By the same method, we obtained a mutation in the *rpoS* gene, coding for RNA polymerase sigma S subunit, which is a global regulator and a key factor in the regulation of the expression of genes that are active during cell transition to the stationary growth phase and upon various stresses [16, 17]. In addition, by means of transposon-induced mutagenesis [18], we obtained a mutation in the *gacS* gene, which codes for the sensor kinase involved in the GacA–GacS global regulatory system [19]. This mutant strain did not synthesize AHL or phenazine antibiotics and was characterized by a suppressed swarming capacity.

Currently, we are investigating the effect of the above-described mutations on AHL synthesis and on the regulation of various metabolic processes occurring in *P. chlororaphis* cells and performing experiments aimed at obtaining mutations in the genes coding for other global regulators of gene expression.

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