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The effect of Introduction of the Heterologous Gene Encoding the N-acyl-homoserine lactonase (*aiiA*) on the Properties of *Burkholderia cenocepacia* 370

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Abstract—To study the role of Quorum Sensing (QS) regulation in the control of the cellular processes of *Burkholderia cenocepacia* 370, plasmid pME6863 was transferred into its cells. The plasmid contains a heterologous gene encoding AiiA *N*-acyl-homoserine lactonase, which degrades the signaling molecules of the QS system of *N*-acyl-homoserine lactones (AHL). An absence or reduction of AHL in the culture was revealed with the biosensors *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NT1/pZLR4, respectively. The presence of the *aiiA* gene, which was cloned from *Bacillus* sp. A24 in the cells of *B. cenocepacia* 370, resulted in a lack of hemolytic activity, reduced the extracellular proteolytic activity and decreased the cells' ability to swarming migration on the surface of the agar medium. The introduction of the *aiiA* gene did not affect lipase activity, fatty acids synthesis, HCN synthesis, or biofilm formation. Hydrogen peroxide was shown to stimulate biofilm formation by *B. cenocepacia* 370 in concentrations that inhibited or weakly suppressed bacterial growth. The introduction of the *aiiA* gene into the cells did not eliminate this effect but it did reduce it.

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INTRODUCTION

The bacteria of *Burkholderia cepacia* complex include *Burkholderia cenocepacia* strains inhabiting various ecological niches that are isolated from human and animals, soil, water, plant rhizosphere. Several *B. cepacia* strains, including *B. cenocepacia*, cause intrahospital infections, mainly in patients with cystic fibrosis (mucoviscidosis) and chronic granulomatosis or in individuals with decreased immunity [1, 2]. The bacteria from this complex are known to possess QS regulation systems. QS systems include low-molecular signaling molecules of various chemical natures and regulatory proteins that interact with the signal molecules. An increase in the density of bacterial population results in accumulation of signaling molecules up to the necessary borderline value, which may cause a rapid transcription activation (induction) of specific genes in the whole population. QS systems play a key role in the regulation of various bacterial metabolic processes and they are considered global factors of bacterial gene expression [3, 4]. QS systems control many important cellular processes, such as pathogenesis caused by pathogenic bacteria, biofilm formation, and the synthesis of toxins, antibiotics and other secondary metabolites, enzymes, etc.

B. cenocepacia contain at least three systems of QS regulation. Two of them—CepI/CepR, CciI/CciR—use *N*-acyl-homoserine lactones (AHL) as signal molecules. CepI AHL-synthase produces *N*-octanoyl-homoserine lactone (C8-HSL) in a major quantity and *N*-hexanoyl-homoserine lactone (C6-HSL) in a minor quantity. In contrast, CciI AHL-synthase catalyzes C6-HSL synthesis in a major quantity and C8-HSL in a minor quantity. AHL interacts with CepR and CciR regulatory proteins. These QS systems control various cellular functions in *B. cenocepacia*, including the synthesis of virulence factors, biofilm formation, cell migration on the media surface, etc. [5–11]. Moreover, another QS system functioning using *cis*-2-dodecenoic acid (BDSF) as a signal molecule was detected in these bacteria [6, 7, 10, 12]. *B. cenocepacia* also synthesizes 2-heptyl-4-chinolon (HHQ), a substance involved in cellular communication [13].

Accordingly, several QS systems function in *B. cenocepacia*, and they provide fine regulation of bacterial metabolism under various environmental conditions.

In the present study, the wide host spectrum plasmid pMB6863, which contains a heterologous *aiiA* gene cloned from *Bacillus* sp. A24, was inserted into

Table 1. Bacterial strains used in the present study

Bacterial strains	Strains characteristics	Source
Burkholderia cenocepacia 370	Clinical isolate	Collection from Gamaleya Research Institute for Epidemiology and Microbiology of RAMS
Chromobacterium violaceum (CV026)	Biosensor for AHL identification. Violacein production. Sm^r mini-Tn5 Hg^r *cviI::Tn5xylE* Km^r	[17]
Agrobacterium tumefaciens NT1/pZLR4	Biosensor for AHL identification AHL, Gm^r Cb^r	[18]
E. coli S17-1/pME6863	S17-1(λ-pir), bearing pME6863 plasmid containing cloned *aiiA* gene, Tc^r	L.S. Chernin, Israel
Burkholderia cenocepacia 370/pME6863	Strain bearing pME6863 plasmid containing cloned *aiiA* gene, Tc^r	The present study

the cells of clinical isolate of *B. cenocepacia* 370 (isolated and identified at the State Scientific Center of Antibiotics and the Gamaleya Research Institute for Epidemiology and Microbiology of the Russian Academy of Medical Sciences) in order to investigate the role of the QS system in the regulation of cellular processes in *B. cenocepacia*. This gene encodes the homoserine lactonase AiiA, which degrades AHL [14]. Insertion of the homoserine lactonase gene results in AHL activation and hence inhibits QS system functioning, thus disturbing the regulation of cell processes depending on QS. This approach seems to be highly perspective for the study of regulatory role of QS systems; however, it was used only for several bacteria. Many questions related to the effect of homoserine lactonase in cells remain unresolved.

The *B. cenocepacia* 370 strain demonstrated potential factors of pathogenicity, such as hemolytic activity, intercellular protease activity, lipase activity, and chitinolytic activity, but it possessed low virulence as reported in experiments in mice [15]. An insert of pMB6863 plasmid into strain cells resulted in a sharp decrease in AHL in the culture and affected several bacterial properties.

MATERIALS AND METHODS

Bacterial strains and inoculation conditions. The bacterial strains used in the study are shown in Table 1. Luria Broth (LB) media and agar-containing (1.5%) LB (LA) and M9 with the necessary additives were used for bacterial growth in the present study [16]. The bacteria were incubated under 30°C. Domestically produced antibiotics were used in the following concentrations (μg/mL): ampicillin—100–200; kanamycin—100; gentamicin—40. Tetracycline hydrochloride (Sigma) was used in a concentration of 20 μg/mL.

Identification of AHL production. Two biosensors were used to identify AHL production. The first, the *Chromobacterium violaceum* CV026 biosensor, was seeded in streaks on the surface of LA media and was crossed by streaks of tested cultures, followed by incubation for 24–48 h at a temperature 30°C. If the strain produced AHL, staining of indicatory strain (CV206) in a violet color was observed. The staining intensity was assessed visually [17].

The second, the *Agrobacterium tumefaciens* NT1/pZLR4 biosensor, was grown on LB media with ampicillin and gentamicin overnight under 30°C. Petri dishes with agar-containing M9 media with X-Gal (final concentration 80 μg/mL) were filled with 3 mL of agar (0.6%) M9 media including 0.5 mL of night culture of *A. tumefaciens* NT1/pZLR4. Strains tested for the ability to produce AHL were seeded as pricks on the surface of agar-containing media or liquid night culture was added into the wells in agar-containing media, followed by incubation at a temperature under 30°C for 24–48 h. The formation of a blue zone of X-Gal hydrolysis indicated for AHL synthesis by the tested strains [18].

The transfer of pME6863 plasmid into *B. cenocepacia* 370 cells was conducted according to [14] with slight modifications. The plasmid was transferred from *E. coli* S17-1 via conjugation with selection on LA media containing tetracycline hydrochloride and ampicillin. Cellular resistance to tetracycline hydrochloride was determined by a plasmid, and ampicillin was added to the media to delete the donor *E. coli* S17-1 strain containing the pME6863 plasmid. The presence of the *aiiA* gene in the *B. cenocepacia* 370 strain containing pME6863 plasmid was confirmed via PCR with specific primers (designed with the use of the *aiiA* gene sequence AF397400): AIIA-F 5'-TTCGTCCCAGCAGGAGGTCGTT-3' and AIIA-R 5'-GATGCCCTGGAGTATGGCCT-3'.

PCR was performed under the following conditions: 94°C for 3 min, 62°C for 2 min, 72°C for 2 min, followed by 30 cycles at 94°C for 10 s, 62°C for 10 s, and 72°C for 20 s, and final elongation at 72°C for

5 min. The PCR products were analyzed via electrophoresis in agarose gel.

Determination of Enzymatic Activities

Determination of intercellular proteolytic activity. The cells of the tested strains were seeded as pricks on agar-containing LA media with milk (0.5% fat milk equal to 1/3 of the total volume), followed by incubation for 48 h under 30°C. If the strains had intercellular proteolytic activity, zones of enzymatic hydrolysis of milk casein (lightning zones) were observed around the colonies. The value of enzymatic activity was determined on the radius of hydrolysis zones.

The determination of lipase activity was performed as described in [19]. The cells from the tested strains were seeded as pricks on LA media containing Tween-20 (1%) and $CaCl_2$ (0.01%), followed by incubation for seven days under 30°C. If the strains demonstrated lipase activity, muddy zones were detected around the colonies (Tween-20 was cleaved with the formation of lauric acid, which resulted in the formation of insoluble salt of this acid in the calcium presence). The level of enzymatic activity was detected on the radius of nontransparent zones around the colonies and the level of media turbidity within a nontransparent zone.

Determination of hemolytic activity. The cells of the tested strains were seeded as pricks on bloody agar, followed by incubation for seven days under 28°C. Hemolytic activity was detected on transparent zones of hemolysis around the colonies.

Determination of biofilm formation. Fresh bacterial strains grown on Petri dishes with LA media were incubated in LB with respective antibiotics or without them under aeration for 24 h under 30°C. The cultures were dissolved 300 times in a fresh LB media without antibiotics. To assess the formation of biofilms, cell culture was grown in 96-well polystyrene plates (Medpolymer, Russia). 135 μL of cell culture and 15 μL of hydrogen peroxide solution were added to the wells, and in the control – 135 μL of cell culture and 15 μL of distilled water. The cells were grown for 24 h with weak mixing on a shaker (~105 rpm) under 30°C, followed by the determination of the optical density of unattached (plankton) cells under 595 nm. Biofilm formation was assessed after media removal, the washing of wells by water, and the staining of formed biofilms within 45 min under 24°C with crystal violet. After staining, the liquid was discarded, the wells were washed with water (three times), 96% ethanol was added for dye extraction from the biofilms, and the solution absorption was measured under $\lambda = 595$ nm 45 min after the start of the extraction. The staining intensity of the obtained solutions were the measure for the level of biofilm formation. Plankton cell and biofilm measurement was conducted on an iMark Microplate Reader (Bio-Rad, United States). A duration of cell growth equal to 24 h was optimal for biofilm formation; after that, the level of biofilm formation was unchanged or even decreased.

The biofilms were analyzed in 4–8 wells in each experiment, and the experiments were repeated three to four times. The biofilms were formed on well walls on the border of nutrient media and air but on well bottoms or the media surface in the wells.

Determination of bacterial cells' ability to migrate on the media surface (swarming migration). The determination of bacterial cells' ability to migrate on the media surface was performed according to [20]. Night cultures of the studied strains were dissolved 100 times in fresh LB media and grown for 2–3 h until the exponential growth phase. Three μL of night culture was placed on the surface of agar-containing media: LA 0.5% agar or M9 0.5% agar containing 0.4% glucose and 0.5% casamino acid and incubated for 48 h under 30°C. The presence and size of the zones of cell migration on the media surface (on the radius, mm) were detected visually.

HCN analysis. An analysis of hydrogen cyanide production was conducted with an Aquaquant-14417.0001 Testsystem (Merck). Cultures of the investigated strains were grown for 48–72 h under aeration and 28°C in LB media containing 2 g/L NaCl. Each strain was tested for HCN production twice.

Analysis of bacterial fatty acid content. The preparation of probes for the analysis was performed according to [21]. Chromatography-mass-spectrometry was carried out on a Mass-spectrometer Agilent-5850/5973 (Agilent Technologies, United States). The capillary column from fused silica (25 × 0.25 mm) was used for chromatographic division. A stationary HP-5ms phase of Hewlett Packard with a layer thickness of 0.2 μm was used. Chromatography was conducted in a mode of temperature programming of 135–320°C with a 7°C/min rate. The temperature of injector and interface was 280°C. Data analysis was conducted via built-in software. The substances in chromatographic peaks were identified by library software with the mass-spectra NIST database.

RESULTS

Introduction to the cells B. cenocepacia 370 the plasmid pME 6863 containing the gene aiiA by conjugation caused some difficulties related to the fact that it was necessary to carry out selection of transconjugants on a medium containing tetracycline hydrochloride but B. cenocepacia 370 strain turned out highly resistant to this antibiotic—to a concentration of 300 μg/mL. The presence of the pME6863 plasmid in cells of *E. coli* S17-1 led to resistance of the strain to 20–40 g/mL of tetracycline hydrochloride (weak cell growth was observed under 100 μg/mL) increased the resistance of *B. cenocepacia* 370. Finally, we succeeded in finding a concentration of tetracycline hydrochloride for transconjugant selection from 600 to 900 μg/mL, i.e., the plasmid insert resulted in a synergistic increase

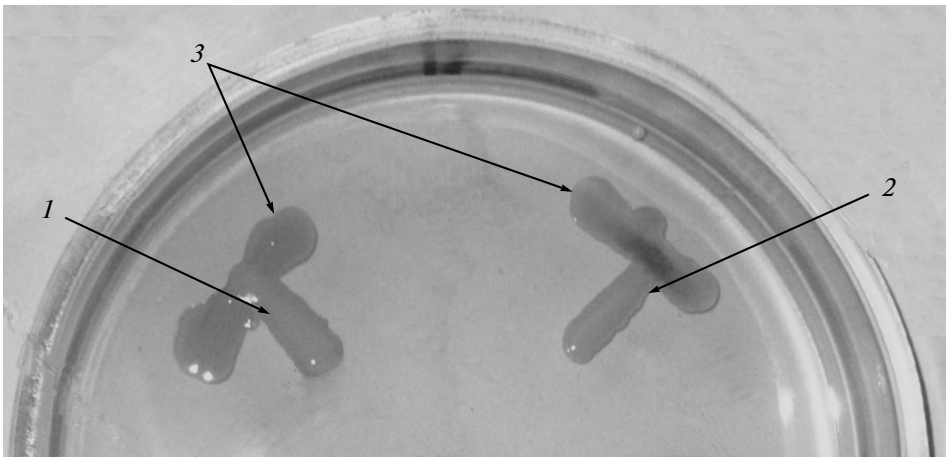

Fig. 1. Determination of AHL synthesis with the *C. violaceum* CVO26 biosensor. *1*—*B. cenocepacia* 370/pME6863; *2*—*B. cenocepacia* 370; *3*—*C. violaceum* CVO26.

of tetracycline resistance. The selection of *B. cenocepacia* cells containing the pME6863 insert also included the addition of 200 μg/mL ampicillin for counterselection of the *E. coli* S17-1/pME6863 donor strain. The presence of the *aiiA* gene in *B. cenocepacia* 370 cells was confirmed via PCR. The plasmid was stably maintained in *B. cenocepacia* 370 cells with strain growth on LB media without tetracycline hydrochloride under everyday strain reseeding for at least ten days; the strain bearing the plasmid maintained a high resistance to tetracycline after the reseedings.

The obtained strain bearing the pME6863 plasmid was tested for its ability to synthesize AHL as compared to the initial *B. cenocepacia* 370 strain. The *B. cenocepacia* 370 strain synthesized a small quantity of AHL (Fig. 1), which is characteristic for this species of bacteria.

As was previously established, *B. cenocepacia* 370 synthesized two main AHL types: C8-HSL and C6-HSL [15]. L. Eberl et al. (Switzerland) detected that this strain did not form AHL with long acyl chains (more than C10). According to the analysis conducted on dishes with the *C. violaceum* CV026 biosensor, the strain bearing the pME6863 plasmid demonstrated no AHL synthesis, unlike the initial strain (Fig. 1). The use of the *A. tumefaciens* NT1/pZLR4 sensor demonstrated decreased AHL production (data not shown) in the cells bearing the pME6863 plasmid. According to the calculation conducted with TotalLab software, the staining square (X-Gal hydrolysis) around the wells in agar-containing media with cultures of the studied strains was approximately twice as small as that in *B. cenocepacia* 370/ pME6863 strain as compared to the initial strain.

The CV026 biosensor is most sensitive to C6-HSL, and its sensitivity is lower by an order of magnitude than that for C8-HSL [17]; in contrast, the *A. tumefaciens* NT1/pZLR4 biosensor is less sensitive to C6-HSL but highly sensitive to C8-HSL (30–120 times) [18]. The data show that *aiiA* gene insertion in *B. cenocepacia* 370 cells probably results in an absence of C6-HSL and a decreased production of C8-HSL.

Table 2 includes data from the comparative determination of several enzymatic activities, swarming migration, and hydrogen cyanide synthesis by the culture of the initial *B. cenocepacia* 370 strain and a strain containing the pME6863 plasmid carrying the cloned homoserine lactonase AiiA gene. Experiments were performed on media lacking tetracycline in order to delete its effect on the cellular metabolism; these effects are possible, although the cells were resistant to tetracycline. It was demonstrated that cells containing

Table 2. Comparison of enzymatic activities, swarming-migration, and HCN synthesis in the cells of initial *B. cenocepacia* 370 strain and the strain bearing the pME6863 plasmid

Strain	Lipase activity*	Hemolytic activity**	Proteolytic activity***	Swarming, mm	CN^- synthesis, mg/L
B. cenocepacia 370	8.9 ± 0.5	2.0 ± 0	4.9 ± 0.6	4.1 ± 0.6	0.011 ± 0.001
B. cenocepacia 370/pME6863	9.3 ± 1.0	No zones	2.8 ± 0.5	2.7 ± 0.2	0.012 ± 0

* Radius of nontransparent zones (from the edge of bacterial growth), mm.

** Radius of hemolysis zone, mm.

*** Radius of casein hydrolysis zones, mm.

Table 3. Fatty acid content in *Burkholderia cenocepacia* 370 and *B. cenocepacia* 370/pME6863 strain

Acid	Content, %	
	B. cenocepacia 370	*B. cenocepacia* 370/pME6863
Tetradecanoic	5.33 ± 0.21	5.54 ± 0.11
Pentadecanoic	0.69 ± 0.08	0.70 ± 0.01
cis-9-hexadecenoic	1.33 ± 0.03	1.24 ± 0.07
cis-11-hexadecenoic	1.94 ± 0.16	1.68 ± 0.03
Hexadecanoic	26.20 ± 0.22	28.86 ± 1.00
3-hydroxytetradecanoic	2.43 ± 0.27	3.13 ± 1.16
11-methyl-hexadecenoic	1.21 ± 0.17	1.63 ± 0.58
Cyclopropaneheptadecanoic	22.00 ± 0.05	20.00 ± 3.25
Heptadecanoic	0.57 ± 0.07	0.67 ± 0.00
cis-11-octadecenoic	7.41 ± 1.82	4.97 ± 0.07
cis-13-octadecenoic	1.09 ± 0.08	1.26 ± 0.15
octadecenoic	2.58 ± 0.24	2.35 ± 0.05
3-hydroxy-hexadecanoic	0.11 ± 0.02	0.37 ± 0.018
2-hydroxy-hexadecanoic	1.02 ± 0.42	0.98 ± 0.46
11-methyl-octadecenoic	2.24 ± 0.31	3.05 ± 1.20
Octadecadienoic (conjugated)	2.65 ± 0.33	2.95 ± 0.93
Cyclopropanenonadecanoic	21.20 ± 0.89	20.62 ± 2.55
In total	100.00	100.00

the pME6863 plasmid were characterized by a lack of hemolysin synthesis, unlike in the initial strain. The lipase activity was almost similar in both strains. Intercellular proteolytic activity was decreased ~1.8 times in the strain with the *aiiA* gene.

The ability of cells containing the pME6863 plasmid to migrate on the media surface (swarming migration) was ~1.5 times lower than in cells from the initial strain. No effect was detected for the presence of the *aiiA* gene on hydrogen cyanide synthesis by *B. cenocepacia* 370 cells. The insertion of the pME6863 plasmid into bacterial cells caused no influence on cell growth.

We investigated the fatty acid content in the *B. cenocepacia* 370 strain and the strain containing the pME6863 plasmid via gas chromatography-mass-spectrometry (Table 3). The samples of the studied strains contained saturated, unsaturated, and hydroxyl acids. In total 17 fatty acids were identified in the spectra. The main fatty acids include hexadecanoic (26–28%), cyclopropaneheptadecanoic (20–22%), cyclopropanenonadecanoic (21%), *cis*-11-octadecenoic (5–7%), and tetradecanoic (5.3–5.5%). The fraction of remaining fatty acids varied from 0.11 to 3%. The fatty acid spectra in both strains were similar. No significant differences in the fatty acid content of the strains were observed, i.e. a decrease in AHL synthesis did not affect the fatty acid content. A slight decrease in *cis*-11-octadecenoic acid was observed in the strain containing the *aiiA* gene but, in contrast, an increase in 3-hydroxyhexadecanoic acid in this strain was detected.

The QS regulating systems functioning with AHL as signal molecules are known to be involved in the regulation of biofilm formation in several bacteria. For instance, it was reported for *Pseudomonas aeruginosa* and *Burkholderia cepacia* [5, 7, 11, 22]. However, the comparison of the level of biofilm formation in the *B. cenocepacia* 370 strain with the strain bearing the pME6863 plasmid revealed no significant differences. We recently reported that the application of subinhibitory hydrogen peroxide concentrations on *P. aeruginosa* cells resulted in the stimulation of biofilm formation. This effect depended on QS regulation—the insertion of the pME6863 plasmid, which contains the cloned *aiiA* gene, into the cells. This removed the stimulation effect of biofilm formation [23]. In our experiments, the application of hydrogen peroxide, which weakly inhibits *B. cenocepacia* 370 growth, also caused a significant, 10-fold stimulation of biofilm formation with an increase of H_2O_2 concentration to 20 μg/mL. The insertion of the pME6863 plasmid into the cells was characterized by the same regulation mechanism; however, the effect value slightly decreased (Figs. 2a and 2b).

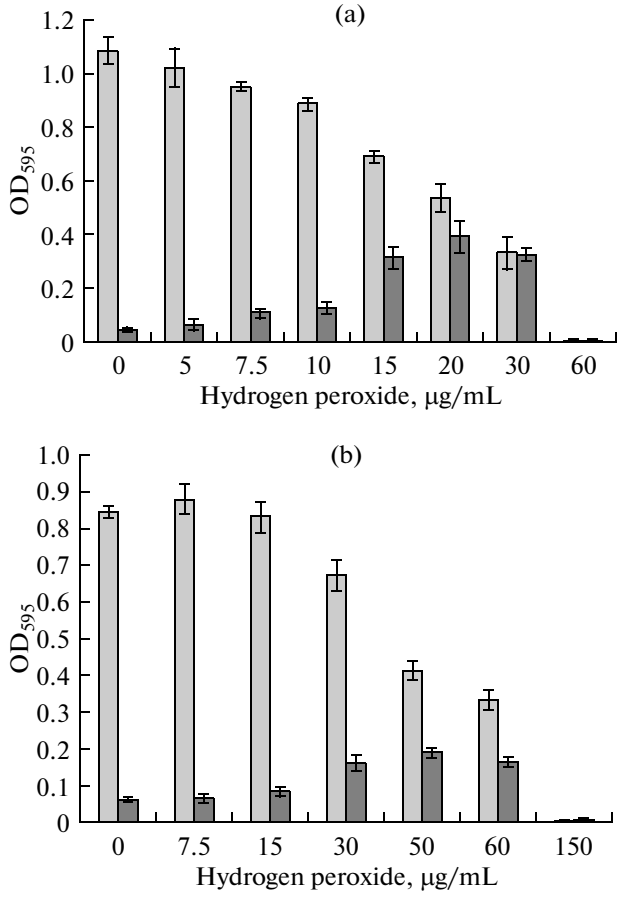

Fig. 2. Influence of hydrogen peroxide on biofilm formation and plankton growth of *B. cenocepacia* 370 (a) and *B. cenocepacia* 370/pME6863 (b). Biofilms are marked by dark columns, plankton growth are marked by white columns.

DISCUSSION

The insertion of the heterologous AiiA gene, which encodes for homoserine lactonase, was used in several studies as a convenient approach for the study of the interaction of QS regulating systems using AHL for their functioning with bacterial gene expression. This approach seems to be especially rational in cases when the bacteria synthesize several AHLs and contain more than one QS regulating system, since various AHLs might partially replace each other while interacting with receptor proteins.

The present study included an investigation of the dependence of several cellular processes in *B. cenocepacia* 370 on QS regulation via a comparison of these processes in the initial strain and in a strain with a pME6863 plasmid containing a cloned heterologous *aiiA* gene. It was shown that a decrease in AHL quantity in the culture due to their degradation by homoserine lactonase resulted in the suppression of hemolytic activity, since zones of hemolysis around the strain with the pME6863 plasmid were absent. Moreover, diminished intercellular proteolytic activity was also revealed, while the presence of the *aiiA* gene in cells did not affect lipase activity. A similar effect of the presence of this homoserine lactonase in cells from five *B. cenocepacia* strains was detected recently; no intercellular proteolytic activity was observed in the other three investigated strains [24]. No effect of *aiiA* gene in the cells of *Burkholderia thailandensis* on hemolytic activity was detected [25].

According to our data, the strain bearing the pME6863 plasmid possessed a decreased ability to migrate on the surface of agar-containing media (swarming migration). Bacterial migration on the media surface represents an important factor for biofilm formation; it is necessary for the primary cells' attachment to the surface and biofilm development in *P. aerugonosa* [26, 27]. Swarming migration in the investigated bacteria is known to depend on QS [27]. Our data concerning the influence of *aiiA* gene insertion on swarming are consistent with previous findings reported for several *Burkholderia* [24, 25] and *P. aeruginosa* PAO1 species [14]. Regulation of the expression of genes involved in cellular migration of swarming type probably depends on the QS in these two bacterial genera.

No changes in the dependence of HCN synthesis on QS was observed after the introduction of the pME6863 plasmid into *B. cenocepacia* 370 cells. With respect to other *Burkholderia* species and strains, no data concerning the effect of homoserine lactonase gene on its production were found. An introduction of this gene in *P. aeruginosa* PAO1 cells caused a rapid decrease in hydrogen cyanide synthesis [14]; it was shown that both QS systems using AHL—LasIR and RhlIR [28] were necessary for its synthesis. However, the QS probably did not regulate cyanogenesis in *P. aeruginosa* 2P24. A *P fluorescens* CHAO strain producing a high quantity of HCN possessed no AHL-directed QS system [29]. Accordingly, the dependence of cyanogenesis on this QS type appears is not general.

In the present study, the presence of homoserine lactonase, which causes a rapid decrease in AHL production in *B. cenocepacia* 370 cells, was shown to possess no influence on biofilm formation (although it reduced swarming migration). According to the data [24], the presence of *aiiA* gene differently affected biofilms formation in various *B. cenocepacia* strains: three strains were characterized by an approximately two-fold lower level of biofilm formation, while an increase in biofilm biomass was detected in another two strains.

Doses of antibacterial agents that are subinhibitory or weakly inhibiting bacterial growth are known may stimulate biofilm formation in bacteria. For instance, this was demonstrated for several antibiotics, including aminoglycoside, tetracycline, etc. [30, 31]. We observed this effect in *P. aeruginosa* PAO1 and *B. cenocepacia* under the action of nitrofurans, NO donors, plant phenols, and several phytohormones [32–34]. The present study demonstrated the stimulating

action of low concentrations of hydrogen peroxide on biofilm formation by *B. cenocepacia* 370. Introduction of the *aiiA* gene into cells of this strain resulted in the maintenance of stimulation of biofilm formation but with a lower value.

The stimulation of biofilm formation under low H_2O_2 doses in the presence of the *aiiA* gene in *B. cenocepacia* 370 cells might be explained in the following way: (1) according to the analysis with the *A. tumefaciens* NT1/pZLR biosensor, *B. cenocepacia* 370 cells synthesize some quantity of C8-HSL; it is probably enough to regulate the stimulation of biofilm formation; (2) another QS system of *B. cenocepacia* regulation that uses AHL as signal molecule [11] may be involved in the control of biofilm formation in *B. cenocepacia* 370 under the aforementioned conditions.

In the present study, we also investigated the dependence of QS on the fatty acid content in *B. cenocepacia* 370 cells; this aspect of metabolism in *B. cepacia* complex bacteria was not studied previously. The fatty acid content is known to represent a stable bacterial characteristic that is maintained in generations with a high level of significance and is used for their identification [35]. It was demonstrated that the presence of the *aiiA* gene in *B. cenocepacia* 370 cells hardly affected the fatty acid content, while several minor differences were observed in comparison with the fatty acid content in the initial strain.

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