UDC 579.222:579.841.1

A. Semenets, M. Finogenova, M. Galkin

BIOFILM FORMATION OF *PSEUDOMONAS AERUGINOSA* PA01 ΔWSPF1 STRAIN WITH HIGH C-DI-GMP LEVEL

Odessa National Mechnikov University,
Dvoryanska str, 2, Odesa, 65082, Ukraine, phone: +38 (0482) 63 57 61,
e-mail: assems@ukr.net

Bis-(3-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is in the spotlight of the scientists as the result of last achievement of microbial genomics and great interests in microbial communities [6, 8]. Depends on concentration of this regulator bacteria shifts its life-form from motile to sessile (biofilm formation) [7]. Aim was to determine swarming motility and biofilm formation abilities in *P. aeruginosa* PA01 ΔwspF1, with high c-di-GMP level.

K e y w o r d s: cyclic-di-GMP, *P. aeruginosa* PA01, *P. aeruginosa* PA01 ΔwspF1, adhesion, biofilm, swarming motility.

Cytoplasmic c-di-GMP is a bacterial secondary messenger, that regulate numerous of physiological processes: cell-to-cell communication, biofilm formation, motility, virulence, etc. [1-3]. It is found that c-di-GMP affects all stages of the biofilm formation process in *Pseudomonas aeruginosa* from the beginning of adhesion to biofilm decay. This compound regulate biosynthesis of matrix components, quorum sensing signal molecules, biosurfactants. [4, 7]. The aim of this work was studding a features of *P. aeruginosa* PA01 ΔwspF1 swarming motility and biofilm formation in fact that this strain characterize with high level of c-di-CMP in its cells.

Materials and methods

In this work two strains of *P. aeruginosa* were used. *P. aeruginosa* PA01 - the wild type strain was obtained from the collection of Odessa Mechnikov National

University microbiology, virology and biotechnology department. PA01 ΔwspF1 with high level of c-di-GMP was kindly provided by Dr. Olena Rzhepishevskaya from University of Umea, Sweden. The methods of research were described in another article [9].

Results and discussion

In this study we focused attention on comparison of hidrophobicity, z-potential, motility, and exopolysaccharides and biosurfactants secretion in two *P. aeruginosa* strains – PA01 (wild type), and PA01 ΔwspF1. According to literature data its known that in *P. aeruginosa* PA01 intracellular content of this messenger equals 3.5 fmol/mg proteins, and in *P. aeruginosa* PA01 ΔwspF1 there is an undetectable amount of this compound [5].

Determination of planktonic and attached cells were carried out after 30 and 60 minutes of incubation (fig. 1). Optical density of inoculums were 0,047 for *P. aeruginosa* PA01 and 0,051 for *P. aeruginosa* PA01 ΔwspF1.

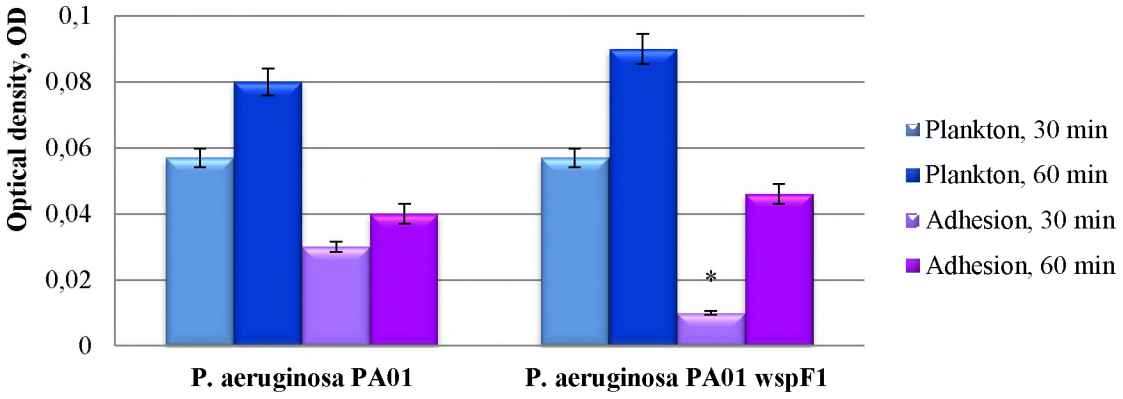

Fig. 1. Attached and free cells amount of *P. aeruginosa* test strains

Note: * - significant difference compared with *P. aeruginosa* PA01

Obtained results show that from the beginning of cultivation *P. aeruginosa* PA01 ΔwspF1 show lower than wild type strain ability of attachment to solid surface. After 30 min of incubation attached cells amount of *P. aeruginosa* PA01 were in 2,5 higher. In the next 30 min of incubation attached cells amount increased in both cases, but it was on 20% higher in case of *P. aeruginosa* PA01 ΔwspF1 compare the

P. aeruginosa PA01. After 60 min of incubation there is a tendency of higher increasing of planktonic cells amount in *P. aeruginosa* PA01.

Examination of daily biofilms show that there were a significant differences in there general form and structure (fig. 2). *P. aeruginosa* PA01 biofilm consists of multicellular 3D-structures. At the same time, *P. aeruginosa* PA01 ΔwspF1 biofilm was flat and "monolayer" (fig. 2A). The difference from two strains also was noticeable on microcolony level (fig. 2B). *P. aeruginosa* PA01 microcolonies were good formed that consists of matrix enclosed cells. In addition, there are secondary microcolonies formation that enhance biofilm mass. In *P. aeruginosa* PA01 ΔwspF1 only the small structural units were detected.

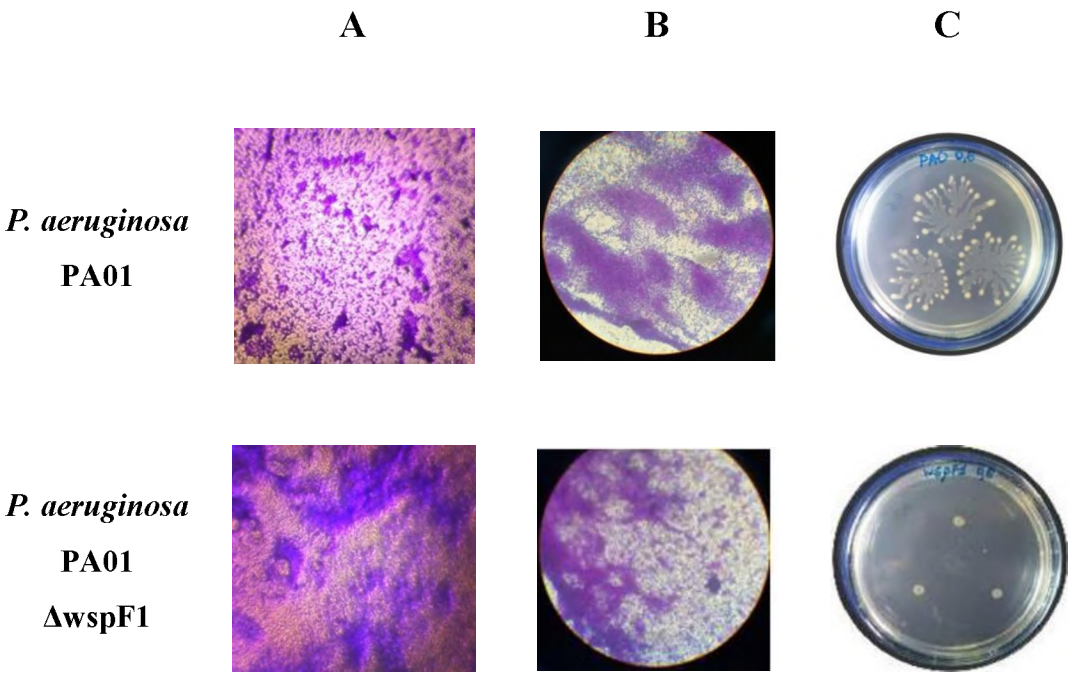

Fig. 2. Images of biofilms (A, B) and swarming motility (C) forming by wild and mutant strains of *P. aeruginosa*
(Magnification: A - × 200, B - × 400, crystal violet staining; C-16 mp)

Swarming experiments showed that *P. aeruginosa* PA01 ΔwspF1 swarming motility zones diameter were 5 ± 0 mm and it were in 0.11 times lower than at *P. aeruginosa* PA01 – 43 ± 3 mm. *P. aeruginosa* PA01 swarming zones have not clearly formed central "core". In the end of each "rays" there is a white thick colony. *P. aeruginosa* PA01 ΔwspF1 not able to motile by swarming (fig. 2C).

Quantity examination of the biofilm formation shows (fig. 3) that *P. aeruginosa* PA01 biofilm have in 1.3 times lower mass than *P. aeruginosa* PA01 ΔwspF1 ($p<0,001$). However, planktonic cells amount were higher in the case of *P. aeruginosa* PA01 ΔwspF1 by 10%. Pel and alginate exopolysacharides amount were similar in all strains biofilm matrix.

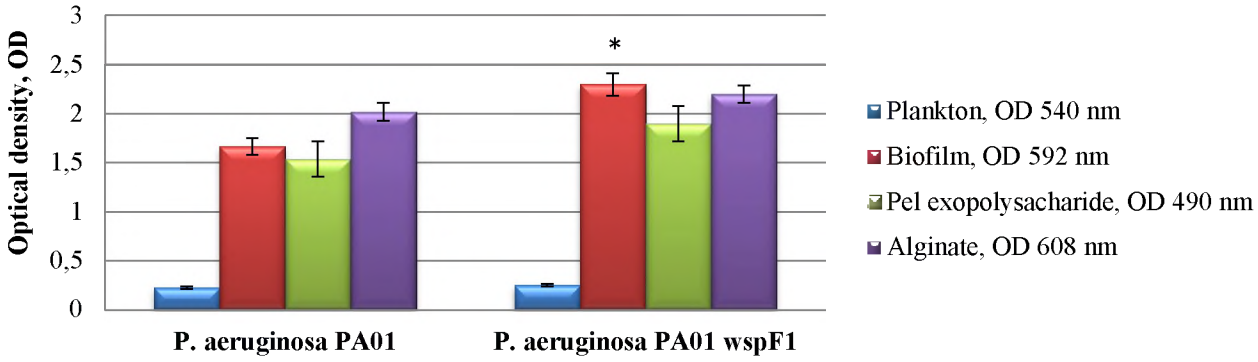

Fig. 3. Biofilm mass and exopolysaccharides ammount.

Note: * - significant difference compared with *P. aeruginosa* PA01

Cell size and physical-chemical properties of the cells surface study show that examined strains have a difference in hydrophobicity. More over, strains hydrophobicity changed during the cultivation (table).

Table

Cell size and physical-chemical properties of the *P. aeruginosa* strains cells surface

Strain	Cell hydrophobicity, %		d, nm	z-potential, - mV
	3 h	24 h		
P. aeruginosa PA01	42,8 ± 2,3	14,2 ± 1,7	666,4 ± 46,4	23,4 ± 1,2
P. aeruginosa PA01 ΔwspF1	41,6 ± 1,9	23,5 ± 1,4*	677,3 ± 46,1	24,7 ± 1,5

Note: * - significant difference compared with *P. aeruginosa* PA01

In logarithmic phase of growth, after 3 h of incubation cell hydrophobicity of *P. aeruginosa* PA01 were higher in 1.03 times then in *P. aeruginosa* PA01 ΔwspF1. Transition to stationary phase accompanied with opposite changes of hydrophobicity

especially for wild strain – 3 fold decreasing and only 1.8 fold decreasing in mutant type strain. Cell diameter in *P. aeruginosa* PA01 overnight culture was 10% less than in *P. aeruginosa* PA01 ΔwspF1. Z-potential of the cells were the same.

More over, decreasing in biofilm formation ability on the background of c-di-GMP decreasing make this system an attractive target for novel antimicrobial drugs.

REFERENCE

1. Borlee B. R., Goldman A. D., Murakami K., Samudrala R., Wozniak D. J., Parsek M. R. *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix // Mol. Microbiol. – 2010. – V. 75. – P. 827–842.
2. Byrd M.S., Sadovskaya I., Vinogradov E., Lu H., Sprinkle A.B., Richardson S.H. Genetic and biochemical analyses of the *Pseudomonas aeruginosa* Psl exopolysaccharide reveal overlapping roles for polysaccharide synthesis enzymes in Psl and LPS production // Mol. Microbiol. – 2009. – V. 73. – P. 622–638.
3. Caiazza, N. C., Merritt J. H., Brothers K. M., O'Toole G. A. Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14 // J. Bacteriol. – 2007. – V. 189. – P. 3603–3612.
4. Hickman J. W., Tifrea D. F., Harwood C. S. A chemosensory system that regulates biofilm formation through modulation of cyclic diguanylate levels // PNAS. – 2005. – V. 102. – P. 14422–14427.
5. Jones J.C., Newsom D., Kelly B. et al. ChIP-Seq and RNA-Seq Reveal an AmrZ-Mediated Mechanism for Cyclic di-GMP Synthesis and Biofilm Development by *Pseudomonas aeruginosa* // PLoS Pathog. – 2014. – V. 10(3): e1003984. doi:10.1371/journal.ppat.1003984
6. Lee V.T., Matewish J.M., Kessler J.L., Hyodo M., Hayakawa Y. A cyclic-di-GMP receptor required for bacterial exopolysaccharide production // Mol. Microbiol. – 2007. – V. 65. – P. 1474–1484.

7. Parsek M. R., Greenberg E.P. Sociomicrobiology: the connections between quorum sensing and biofilms // Trends in Microbiol. – 2005. – V. 13. – P. 27-33.
8. Römling U., Galperin M.Y., Gomelsky M. Cyclic di-GMP: the First 25 Years of a Universal Bacterial Second Messenger // Microbiology and Molecular Biology Reviews. – 2013. – V. 77, № 1. – P. 1–52.
9. Semenets A.S., Galkin M.B., Filipova T.O. Biofilm formation by Pseudomonas aeruginosa strains with different level of cyclic di-guanosine monophosphate biosynthesis // Microbiology and biotechnology. – 2016. – V. 33. – P. 19-29.