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Prevention of *Pseudomonas aeruginosa* biofilm formation by pyocins

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Background. *Pseudomonas aeruginosa* causes intrahospital infections, which are difficult to treat due to multiple antibiotic resistance of these strains, resulting also from their ability to form biofilm [1]. It has been shown that *P. aeruginosa* strains synthesize bacteriocins (pyocins) — antibiotic-like substances that are highly active against closely related cultures and can be considered an alternative to classic antibiotics [2]. **Aim.** To study the ability of pyocins to prevent biofilm formation of *P. aeruginosa*. **Methods.** Pyocins were obtained from *P. aeruginosa* UCM B-333 by [3] and brought in 96-well plate before the addition of *P. aeruginosa* UCM B-3 and UCM B-10 suspensions. The study of biofilm formation was carried out for 3 days. At different time intervals, the cell titres in planktonic and biofilm forms were determined, and the intensity of the formed biofilm was assessed using the light and fluorescence microscopy with acridine orange and ethidium bromide dyes. **Results.** Active substances of *P. aeruginosa* UCM B-333, represented by pyocins S1, S5, and S9, as well as microcin-II-like bacteriocins [4] affected the biofilm formation. Compared to the control indices, a single preventive use of pyocins with 51.2 thousand AU/mL activity led to a decrease in the titre of *P. aeruginosa* strains in planktonic form by 14500 times in 24 hours and by 4200 times in 48 hours. The effect of bacteriocins on highly antibiotic-resistant bacteria in biofilm form was delayed in time: the number of microorganisms decreased by one order during the first hours, and by 92 times during 24 h of growth. The influence of pyocins on the biofilm formation led to a decrease in the percentage of formed biofilm within the range of 39–50% during the first hours of contact and up to 53% at 24 hours. Even at 48 h, indices of biofilm coverage of the samples were 6% lower than the control values. The pyocins action was shown to be aimed at reducing the size of biofilm structures and the density of its matrix and connected with the nuclease activity of pyocins S1 and S9, which destroy eDNA in the biofilm matrix**.** When using pyocins with 20.5–51.2×10^3 AU/mL activity, single cells remained in biofilm composition, and with lower activity — their groups. But, in both cases, cells were not viable due to the pore-forming activity of pyocin S5. **Conclusions.** A single preventive application of pyocins with 20.5–51.2×10^3 AU/mL activity affects *P. aeruginosa* UCM B-3 and UCM B-10 biofilm formation. It prevents the reproduction of microorganisms in planktonic (up to 14500 times) and biofilm (up to 92 times) forms and also slows down the biofilm formation (up to 53%) within 24–48 hours.

Keywords: biofilm, pyocins, prevention of *Pseudomonas aeruginosa* biofilm formation.

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