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INFLUENCE OF DISINFECTANT ON CRYSTALLOGENIC ACTIVITY OF PSEUDOMONAS AERUGINOSA IN VITRO

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*The **purpose of this work** was to clarify the crystallogenic properties of pseudomonads under the action of an antiseptic.*

***Material and methods.** The material for the study was 8 strains of P. aeruginosa isolated from patients of the burn Department. In accordance with the purpose and objectives of the study, the work was performed in 3 stages: assessment of the biological properties of isolated pseudomonad strains; determination of sensitivity to disinfectants by the square method; assessment of the crystallogenic (initiating) activity of pseudomonads in individual and joint form with the disinfectant. The tested antiseptic was "Desam" in the form of a standard 1% solution used for disinfection of surfaces and medical instruments.*

***Results.** It was found that all the studied Pseudomonas strains have the ability to activate the crystallogenesis of the basic substance (0.9% sodium chloride solution), which manifests itself both in qualitative and quantitative changes in the thesigraphic picture. It is shown that the addition of a common disinfectant ("Desam") to the system "Pseudomonas aeruginosa – 0.9% sodium chloride solution" significantly transforms its dehydration structuring. At the same time, strains of the microorganism resistant to disinfectants moderately reduce the crystal's gene activity (according to the main teziographic coefficient and belt coefficient). On the*

contrary, sensitive strains demonstrate a pronounced inhibition of the crystallogenesis of the basic substance. It allows to develop a new express method for determining the sensitivity of microorganisms to disinfectants.

Keywords: *pseudomonas aeruginosa; crystallogenic properties; disinfectants; monitoring*

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ВЛИЯНИЕ ДЕЗИНФЕКТАНТА НА КРИСТАЛЛОГЕННЫЕ СВОЙСТВА PSEUDOMONAS AERUGINOSA IN VITRO

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Целью работы *было выяснение кристаллогенных свойств псевдомонад под действием антисептика.*

Материал и методы. *Материалом для исследования послужили 8 штаммов P.aeruginosa, выделенных от пациентов ожогового отделения. В соответствии с целью и задачами исследования работа проводилась в 3 этапа: оценка биологических свойств выделенных штаммов псевдомонад; определение чувствительности к дезинфицирующим средствам методом квадрата; оценка кристаллогенной (инициирующей) активности псевдомонад в индивидуальной и совместной форме с дезинфицирующим средством. В качестве тестируемого антисептика использовали «Десам» в виде стандартного 1% раствора, используемого для дезинфекции поверхностей и медицинских инструментов.*

Результаты. *Установлено, что все изученные штаммы Pseudomonas обладают способностью активировать кристаллогенез основного вещества (0,9% раствора хлорида натрия), что проявляется как в качественных, так и в количественных изменениях в топографической картине. Показано, что добавление обычного дезинфицирующего средства («Десам») в систему « Pseudomonas aeruginosa – 0,9% раствор хлорида натрия» значительно трансформирует ее дегидратационную структуру. В то же время штаммы микроорганизма, устойчивые к дезинфицирующим средствам, умеренно снижают активность гена кристалла (по основному тезиграфическому коэффициенту*

и коэффициенту поясности). Напротив, чувствительные штаммы демонстрируют выраженное ингибирование кристаллогенеза основного вещества. Это позволяет разработать новый экспресс-метод определения чувствительности микроорганизмов к дезинфицирующим средствам.

Ключевые слова: *Pseudomonas aeruginosa; кристаллогенные свойства; дезинфектанты, мониторинг*

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Introduction

Currently, the phenomenon of biomineralization attracts the active attention of researchers. It is well-known that biomineralization is crystallization of various substances triggered by the presence of various microorganisms (primarily bacteria) [1, 7, 9]. Such an effect has found great use as a way of accumulating various toxic compounds, in particular, heavy metals. So, in the study of J. Liu et al. (2021) showed the ability of a number of pseudomonad strains to fix nickel from the medium [13], and this technology can be useful in creating a new generation of batteries and improved by including cadmium sulfide in their composition [14]. In another work of this research team, the possibility of using this bacterium to purify water from impurities of nickel, nitrates and phosphates is shown [12]. The works of other scientists confirm the effectiveness of the technology being created [22, 23]. The latter can also be accumulated from the environment by sideroform mycoorganisms [20]. Li W. et al. (2021) it was found that a number of ureolytic bacteria also have toxin-fixing properties, which can be used for the purification of industrial and household waste [11].

A separate area of application of biomineralization is the use of calcium precipitation induced by various bacteria for various technical tasks [3, 21, 24]. So, in the literature there is a series of works devoted to the production of urea using this principle [2]. In addition, C. Fang and V. Achal (2019) demonstrated the possibility of improving cement properties by biostimulating calcium precipitation processes [5], and in further studies (2021) developed a technology for obtaining bio-blocks from microbially synthesized urine [6]. At the same time, these materials can be supplemented with non-cement components (for example, metacaolin), also generated by biomineralization [10]. It is important that these processes are similar to the formation of nodules of various elements in nature [8].

On the other hand, such technical applications of the phenomenon of microorganism-associated crystallogenesis suggest its great importance for biological systems and potential pathogenetic role. Indirect confirmation of this, in particular, is the work of MM. Elmassry et al. (2021), which shows the influence of biomineralization processes in pseudomonads on their virulence [4]. Our previous studies also experimentally demonstrated the ability of staphylococci and Escherichia to modify the crystallization of aqueous salt solutions [17], and this effect turned out to be dose-dependent [15]. In addition, we have established the pathogenetic role of crystallogenic bacterial symbiosis in the mechanisms of development of complicated forms of gastric and duodenal ulcer [16], revealed the involvement of crystallization processes in the pathogenesis of liver alveococcosis [19] and new coronavirus infection [18]. At the same time, the effect of disinfectants on the crystallogenic properties of microorganisms remains undisclosed.

That is why the aim of the work was to clarify the crystallogenic properties of Pseudomonas aeruginosa under the action of disinfectant.

Material and research methods

Study design

The material for the study was 8 strains of *P. aeruginosa* isolated from patients of the burn department. In accordance with the purpose and objectives of the study, the work was carried out in 3 stages:

1. assessment of the biological properties of the isolated Pseudomonas strains;
2. determination of sensitivity to disinfectants by the square method;
3. evaluation of the crystallogenic (initiating) the activity of pseudomonads in individual and joint form with the disinfectant.

The tested antiseptic was “Desam” in the form of a standard 1% solution used for disinfection of surfaces and medical instruments.

The first stage of the work is carried out in accordance with the classical methods of bacteriology.

Laboratory study

In order to describe the crystallogenic activity of the studied bacteria for each strain, regardless of the degree of its resistance to disinfectants, a micro-preparation was prepared on a Petri dish according to a single algorithm. It is important to note that all complex samples intended for further drying contained equal amounts of components. The general view of the sample is shown in Figure 1.

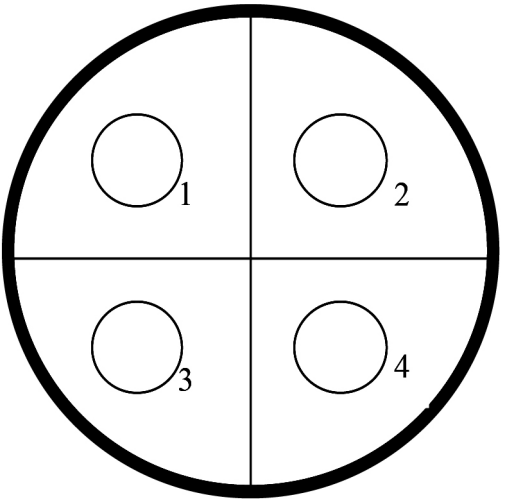


Fig. 1. Teziographic plate for estimation of pseudomonas sensitivity to disinfectants (1 – Control A (0.9% solution of sodium chloride), 2 – Control B (1% disinfectant solution), 3 – pseudomonas + 0.9% solution of sodium chloride, 4 – pseudomonas + 0.9% solution of sodium chloride + 1% disinfectant solution)

The analysis of the results of crystal formation was carried out using the algorithm developed for the assessment of tezigraphic facies for biological substrates [17, 19], which was adapted by us for the assessment of the crystallogenic properties of microorganisms [15, 16]. For this purpose, a universal system of criteria was used, which allowed us to describe the features of the initiator potential of the studied Pseudomonas strains from a qualitative and quantitative point of view.

Statistics

The results were processed using the Statistica 6.0 program. All the data were processed with standard algorithms of descriptive statistics and were present as Mean±SD. The Student's t-test was used for detection of statistical differences.

Results and Discussion

Based on the preliminary microbiological studies conducted on the sensitivity of the isolated strains, conducted using the " squares "method as the" gold standard", it was found that the ratio of sensitive and resistant strains was found to be 5 : 3. Among the sensitive strains – №904, 1268, 84, 88, 82, strains No.

133, 137, and 649 are classified as resistant. The proportion of strains that are sensitive and resistant to a 1% solution of Desam is clearly shown in Figure 2. It should be noted that most of the analyzed strains were sensitive to the applied working concentration of the tested disinfectant, which should be considered as a positive point.

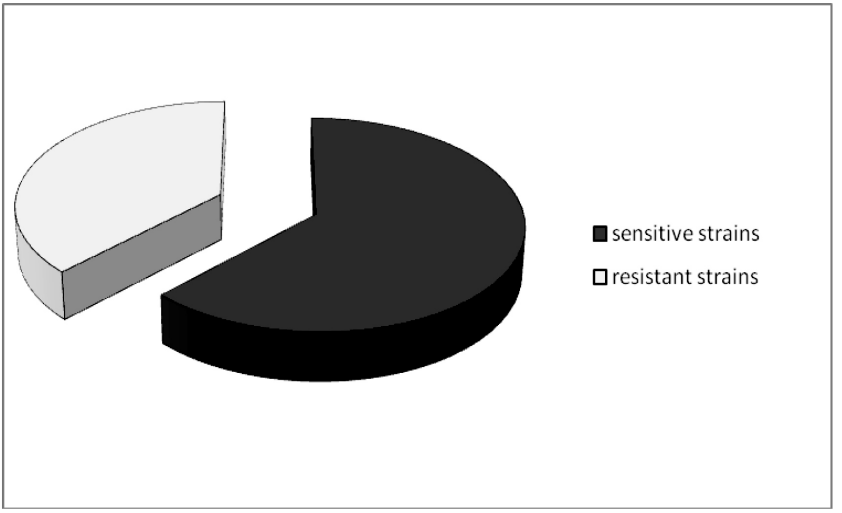


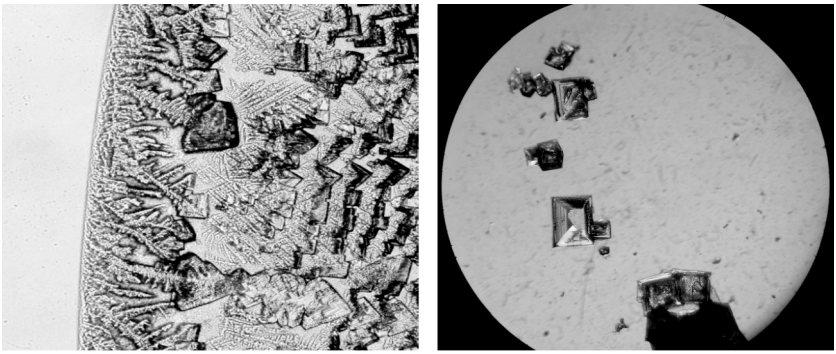
Fig. 2. Ratio of sensitive and resistant (to 1% disinfectant solution) strains of P. aeruginosa

The results of the microbiological determination of the sensitivity of pseudomonads formed the basis for the next stage of the work, at which the crystallogenic (initiating) the activity of pseudomonads in individual and joint form with the disinfectant.

Our analysis of the modulating properties of the studied Pseudomonas strains allowed us to establish that the latter are quite active initiators of the crystallogenesis of a solution of sodium chloride of physiological concentration (Fig. 3). This is reflected in the fact that the density of the crystallization centers in the experimental sample (a mixture of "microorganisms – saline solution") significantly exceeds that in the control facies of the individual base substance (2.37 times; $p<0.05$), directly determining the value of the main tezigraphic coefficient Q.

It is interesting to note that the crystalloscopic picture of the sample, which includes the biosystem "bacteria-basic substance", does not differ in the variants of the morphotypes formed from the control micro-product. This makes

it possible to assume that the presence of pseudomonads does not ensure the appearance of neocrystallogenesis, in particular, due to the metabolites of the microorganism itself, whereas in this case there is only a mechanical activation of the crystallogenesis of the base compound (a solution of sodium chloride of physiological concentration) by bacteria. The mechanism of this variant of the implementation of the microorganism-associated crystallization is most likely to be the extracellular variant of the latter [16, 17, 19], and one of the main factors regulating the modulation of crystallization is, from our point of view, the tinctorial properties of the cell wall of *Pseudomonas aeruginosa* [4].



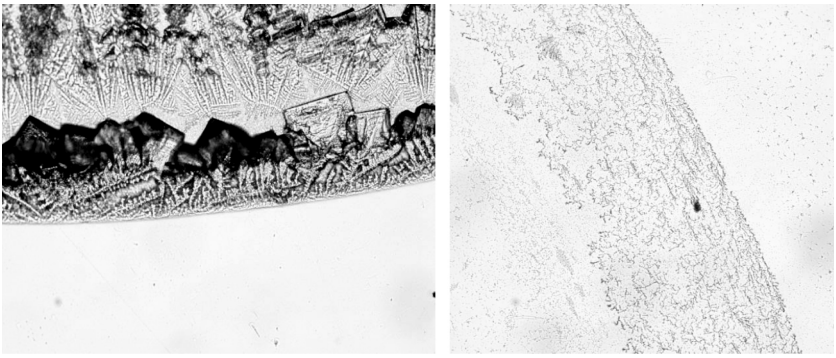
P. aeruginosa + 0.9% solution of sodium chloride

0.9% solution of sodium chloride

Fig. 3. Tezigram of system "P. aeruginosa + 0.9% solution of sodium chloride" and control of 0.9% solution of sodium chloride (magn. x56)

The last stage of the work, which is of direct practical importance, was to establish the possibilities of screening the sensitivity of pseudomonads to the considered disinfectant based on the visualization of their crystallogenic activity.

Analysis of the nature of individual crystallization of pseudomonads and antiseptics allows us to formulate the following working hypothesis: in the case of detection of the mixture "microorganism – 0.9% solution of sodium chloride – 1% solution of disinfectant" activation of crystallogenesis in comparison with the basic substance, it is assumed that the number of bacteria sufficient for the manifestation of the phenomenon of microorganism-associated crystallogenesis [15], and, consequently, the inadequate effectiveness of the antiseptic used. On the contrary, when detecting the inhibition of crystallization in the experimental sample, the sensitivity of the studied strain of psalmonad to the considered disinfectant can be indicated.



Resistant strain Sensitive strain

Fig. 4. Teziograms of system "pseudomonas – 0.9% solution of sodium chloride – 1% disinfectant solution" in connection with resistance to disinfectant (magn. x56)

This hypothesis was fully confirmed by the study of the data of the tezigraphic analysis of strains isolated from patients with a combustiological profile. Even a simple visual comparison of the tezigraphic pictures allows us to verify the differences between the micro-preparations (Fig. 4).

In order to quantify the tezigraphy data, a visuometric study of the obtained samples was performed using the above criteria.

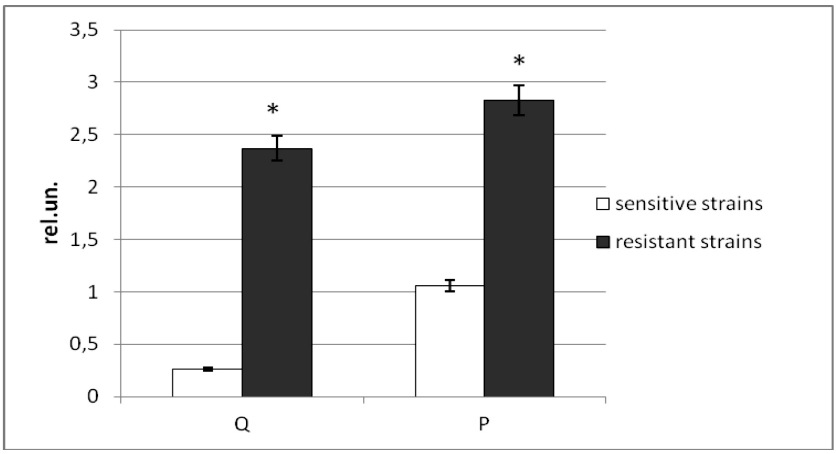


Fig. 5. Results of criterial analysis of tezigrams for sensitive and resistant to disinfectant strains of Pseudomonas (Q – main teziographic coefficient, P – belt coefficient; «*» – statistical value of differences between sensitive and resistant strains is $p<0,05$

The study of the main parameters of the description of the initiated crystallogenesis of mixtures allowed us to establish that the main tezigraphic coefficient Q, indicating the degree of modulation of the base substance under the influence of biomaterial, for sensitive strains was found to be at a significantly lower level (0.26±0.14 standard units) than for resistant ones (2.37±0.28; the statistical significance of the differences is $p<0.05$). This dynamic confirms the proposed working hypothesis, since when studying the newly created biosystem with a sensitive strain, an almost complete absence of the effect that initiates salt crystallization is found.

The belt coefficient P, which demonstrates the degree of heterogeneity of the studied biosubstrate in terms of the spread of molecular weights of the components, was also at a higher level in resistant strains (2.83±0.31 versus 1.06±0.19 in sensitive ones), which is an indirect sign of the metabolic activity of the Pseudomonas.

Conclusion

Based on the conducted crystalloscopic studies, it was found that all the studied Pseudomonas strains have the ability to activate the crystallogenesis of the basic substance (0.9% sodium chloride solution), which manifests itself both in qualitative and quantitative changes in the thesigraphic picture. It is shown that the addition of a common disinfectant ("Desam") to the system "Pseudomonas aeruginosa – 0.9% sodium chloride solution" significantly transforms its dehydration structuring. At the same time, strains of the microorganism resistant to disinfectants moderately reduce the crystal's gene activity (according to the main thesigraphic coefficient and the coefficient of zoning). On the contrary, sensitive strains demonstrate a pronounced inhibition of the crystallogenesis of the basic substance. It allows to develop a new express method for determining the sensitivity of microorganisms to disinfectants.

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