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# EXPERIMENTAL STUDY OF THE PHENOMENON OF MICROORGANISM-ASSOCIATED CRYSTALLOGENESIS

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**ABSTRACT** — THE AIM OF THE STUDY was to clarify the character of the initiatory activity of pathogenic and non-pathogenic microorganisms in regard to salt solutions. We selected colonies of two microorganisms: *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*St. aureus*) [ $10^6$ ,  $10^8$ ,  $10^{10}$ ,  $10^{12}$ ,  $10^{14}$ ,  $10^{16}$ ,  $10^{18}$  and  $10^{20}$  CFU/ml of solution]. A 10% solution of sodium chloride was used as the initiated substance, which made it possible to perform a tezigraphic test. On the basis of model experiments with colonies of some pathogenic and non-pathogenic microorganisms, a new phenomenon was confirmed — microbial initiation of crystal formation (the phenomenon of microorganism-associated crystallogenesis). This phenomenon is universal for microagents, but it also has specific features imposed by the species of the microorganism and its current functional state.

**KEYWORDS** — biocrystallogics, microorganism-associated crystallogenesis, *Staphylococcus*, *Escherichia*.

## INTRODUCTION

The metabolic activity of various microorganisms is widely discussed in the literature. At the same time, various properties of bacteria are studied and applied in some industries [1, 2, 6, 7]. In these works, numerous facts of the connection of the processes of crystal formation with the activity of microbes are presented. The most studied are representatives of the genus *Pseudomonas*, which are able to initiate the crystallogenesis of various organic and mineral compounds from solutions [1, 2, 5]. Similar properties were found in other microorganisms (*Listeria innocua*, *Escherichia coli*, *Helicobacter pylori*, *Desulfovibrio desulfuricans*, *Agrobacterium tumefaciens* [1–3, 5] etc.). A significant practical interest is the ability of microorganisms to accumulate many metals, including noble metals

(gold, silver, platinum) in the form of microcrystals in the perimembrane space or intracellularly [1, 2, 6, 7].

Thus, *the aim of the study* was to clarify the character of the initiatory activity of pathogenic and non-pathogenic microorganisms in regard to salt solutions.

## MATERIAL AND METHODS

We selected colonies of two microorganisms: *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*St. aureus*). The strains were obtained at the Kirov Research Institute of Hematology and Blood Transfusion. In order to clarify the role of the concentration of the bacterium, the same test amounts of bacteria were used for both microorganisms:  $10^6$ ,  $10^8$ ,  $10^{10}$ ,  $10^{12}$ ,  $10^{14}$ ,  $10^{16}$ ,  $10^{18}$  and  $10^{20}$  CFU/ml of solution. A 10% solution of sodium chloride was used as an initiated substance, which made it possible to perform a tezigraphic test [4]. This allowed to quantify the *initiatory potential* of the bacteria — the ability to influence the crystallization pattern of the salt solution. Statistical processing of the results was performed using variation statistics algorithms, Microsoft Excel 2007 and Statistica 6.1 for Windows.

## RESULTS

The study of crystallogenic properties of 10% sodium chloride solution has allowed to establish that there is a dynamics accelerating the expressiveness of shifts in crystal formation of hypertonic saline with an increase in the content of microagent. It is important that this trend applies to both micro-organisms. A more detailed study of the distribution of tezigrams along the line of the increasing initiating dose of the microagent, taking into account all controlled concentrations, revealed a two-stage nature of the reaction of sodium chloride on the metabolic activity of the microorganism. It includes the initial absence of significant variations compared to the reference sample of the reference substance. As the number of microbial bodies increases, a concentration for each microorganism is clearly recorded, which initiates the dynamics of crystallogenesis transformation in the marginal zone. It is expressed in the formation of islands of single crystals surrounding relatively large pyramidal structures formed by sodium chloride. It is essential that the

concentration, at which such changes are detected in *Escherichia* and *Staphylococcus*, varies, and it is significantly less in the pathogenic microagent. Thus, for *E. coli*, this threshold concentration is  $10^{12}$  CFU/ml, while for *St. Aureus*, even with the minimum (of the studied) number of bacteria ( $10^6$  CFU/ml), significant transformations of the structure of the marginal zone are registered.

The third of the variants of initiation of sodium chloride crystallization by bacteria associated with the number of micro-agents identified by us is the increasing neocrystallogenesis in the central and intermediate zones of the dried samples. Based on this, it is possible to distinguish the second threshold concentration, and its values species-specific ( $10^{14}$  CFU/ml for *E. coli* and  $10^{10}$  CFU/ml for *S. aureus*).

In accordance with the chosen method of describing dried samples, we performed not only a qualitative, but also a quantitative assessment by applying a system of criteria. The analysis showed that the qualitative transformations associated with the metabolic activity of bacteria are fully confirmed when evaluating the quantitative criteria of the diagnostic test. In particular, the monotonous increase in the level of the main tezigraphic coefficient is the result of first marginal, and then total (extending to all zones of the *facia*) neocrystallogenesis, reflecting the *initiator potential* of the microorganism. At the same time, the increase in the number of crystal structures is not accompanied by an increase in structure index. Thus, in terms of the main tezigraphic coefficient, and, consequently, in terms of the initiatory capacity (when using a 10% sodium chloride solution as a base substance), *St. aureus* significantly surpasses *E. coli* at all the studied concentrations ( $p < 0.05$ ), and if the *initiator potential* of *E. coli* is insignificant at small values, then it is initially high in staphylococci (increased crystal formation of sodium chloride by 3.5 times). At high concentrations, the clear differentiation between bacteria is somewhat smoothed out due to a pronounced increase in the initiator potential of *E. coli*.

Similar trends were found when assessing the *correctness* of the crystallogenesis of the sodium chloride solution modulated by microorganisms (according to *facia* destruction degree), but no significant differences were at low concentrations of bacterial agents ( $p < 0.05$ ). In addition, a pathogenic bacterium (in our case, *Staphylococcus aureus*) at medium and high concentrations has a fairly clear destructive effect on the crystallization of sodium chloride solutions than an *Escherichia coli*. These changes are verified by correlation analysis ( $r = -0.103 \pm 0.062$ ).

## CONCLUSION

On the basis of model experiments with colonies of some pathogenic and non-pathogenic microorganisms, a new phenomenon was confirmed — microbial initiation of crystal formation (the phenomenon of microorganism-associated crystallogenesis). This phenomenon is universal for microagents, but it also has specific features imposed by the species of the microorganism and its current functional state.

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