THE ISOLATION OF COPPER RESISTANT RETRO MICROBIOME FROM ECO-FRIENDLY ECOSYSTEM OF "ATLANTIDA" KARST CAVE

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Copper (II) in low concentrations is a necessary trace element for microorganisms. However, Cu^{2+} at the concentration of 100 ppm and higher acquires the properties of a xenobiotic and becomes an extreme factor alien to natural ecosystems, including the microbiome. Therefore, the study of the regularities of microorganism's adaptation to copper as the alien extreme factor allows to investigate the patterns of microbial homeostasis to extreme factors and to identify the formation of adaptive mechanisms.

According to geological data, the "Atlantida" karst cave ecosystem was formed hundreds of thousands years ago. This cave is eco-friendly and completely isolated from any natural and man-made contaminants, including toxic Cu (II) compounds. Quantitative characteristics of retro microbiome growth at the presence of toxic Cu (II) is an indicator of the ability of microorganisms to adapt to the spontaneous influence of extreme factors. Thus, the aim of our work was to determine the quantitative regularities of resistance of cave "Atlantida" clay microbiome to toxic compounds of Cu (II).

Clay samples were collected from "Atlantida" karst cave in Khmelnitsky region of Ukraine. Microorganisms resistant to copper were determined by their ability to form colonies Microbial resistance was determined by the number of cell forming units (CFU) on the agar nutrient medium (NA) that contained the concentration gradient of Cu (II) (100-2500 ppm). Accumulation of Cu (II) in bacteria was confirmed by H_2S test. The reduction of Cu (II) was shown by the formation of insoluble brown Cu_2O in colonies.

The microorganisms resistant to toxic copper (II) were present in cave clay. They grew on the nutrient agar containing copper at high concentration, up to 200 ppm Cu^{2+} cation ($CuSO_4$ solution) and up to 2000 ppm Cu^{2+} (in complex with citrate). Chelation of copper (II) with citrate led to a drastic (by one order) increase of microbial resistance to this metal. The dependence between the number of live cells and copper concentration described by the hyperbolic curve, confirming the toxic effect of Cu (II) compounds on microorganisms. But the number of viable microorganisms was 7×10^3 CFU/g even at 2000 ppm of Cu (II).

Thus, the capability of the retro microbiome of "Atlantida" cave eco-friendly ecosystem to grow at the presence of toxic concentrations of Cu (II) was shown. The offered methodological approach is a novel universal method of isolation of copper-resistant microorganisms from natural extreme and retro ecosystems of the globe. The isolated copper-resistant microorganisms are prospective for industrial purification of copper-containing waste-waters and contaminated soils.