THERMODYNAMIC PROGNOSIS TO SEARCH FOR SUPER RESISTANT TO COPPER (II) MICROORGANISMS AS THE BASIS FOR ENVIRONMENTAL BIOTECHNOLOGIES

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Copper pollution of the environment destroys natural ecosystems. The particular sources of copper contamination are industrial wastewaters, metal mines, and tailing sites. The chemical copper detoxification methods are expensive and hazardous. The environmentally friendly alternative is the development of biotechnological methods for copper removal, which require microorganisms resistant to high concentrations of copper.

The aim of our work was to predict theoretically and confirm experimentally the capability of microorganisms from diverse ecological niches to grow and interact with Cu (II) at high concentrations up to 1.0 M and to remove soluble toxic Cu (II) compounds from contaminated solutions. The isolation of copper-resistant microorganisms was enabled by the thermodynamic prognosis of microbial interaction with metals. The microorganisms were isolated by cultivation in the liquid medium (NB) with a concentration gradient of Cu (II). Genomic DNA was extracted by standard phenol/chloroform method. The DNA was sequenced on the Illumina MiSeq platform using 2×300 paired-end chemistry (Novogene). A 16S rRNA sequence-based phylogenetic analysis was used for identification of all four bacterial isolates. Morphological and physiological characterization was used for identification of yeast isolate. Several positions of thermodynamic prognosis were experimentally confirmed. First, the one yeast and four bacterial copper resistant strains were isolated from diverse ecological niches. The isolates were resistant to Cu (II) at super high concentration up to 1.0 M. Morphological and physiological characterization let to identify the yeast isolate from Deception Island of Antarctic region as *Rhodotorula mucilaginosa* UKR5. A 16S rRNA sequence-based phylogenetic analysis showed that all four bacterial isolates belong to the genus of *Pseudomonas*. BlastN against the GenBank 16S ribosomal RNA database identified strain UKR1 from Kyiv region as *P. lactis*, UKR2 from Kyiv region as *P. panacis*, and the two isolates (UKR3 and UKR4) from Svalbard Island (Arctic Ocean) and Galindez Island (Antarctic) as P. *veronii*. Initial screening for genes encoding copper resistance mechanisms showed that all bacterial strains encode copper resistance proteins CopA, CopB, CopD, CopZ and two-component regulatory system CusRS, known to determine metal resistance in Pseudomonas genus. Second, all of isolates accumulated Cu (II) in colonies and reduced it to the insoluble Cu (I) form ($Cu_2O\downarrow$).

Moreover, preliminary studies revealed a high efficiency of toxic soluble Cu (II) removal from solution due to its precipitation in forms of $Cu(OH)_2\downarrow$ or $CuCO_3\downarrow$ by microbial communities. The results of this study are promising for development of industrial biotechnologies for copper-containing wastewaters purification and bioremediation of copper-contaminated ecosystems.