



National Academy of Sciences of Ukraine

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Zabolotny Institute of Microbiology and Virology

Department of Extremophilic Microorganisms Biology

Thermodynamic Prognosis to Search for Super Resistant to Cu(II) Microorganisms as the Basis for Environmental Biotechnology

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**Environmental
contamination by
copper**

Copper pollution of the environment destroys natural ecosystems. The particular sources of copper contamination are industrial wastewater, metal mines, and tailing sites

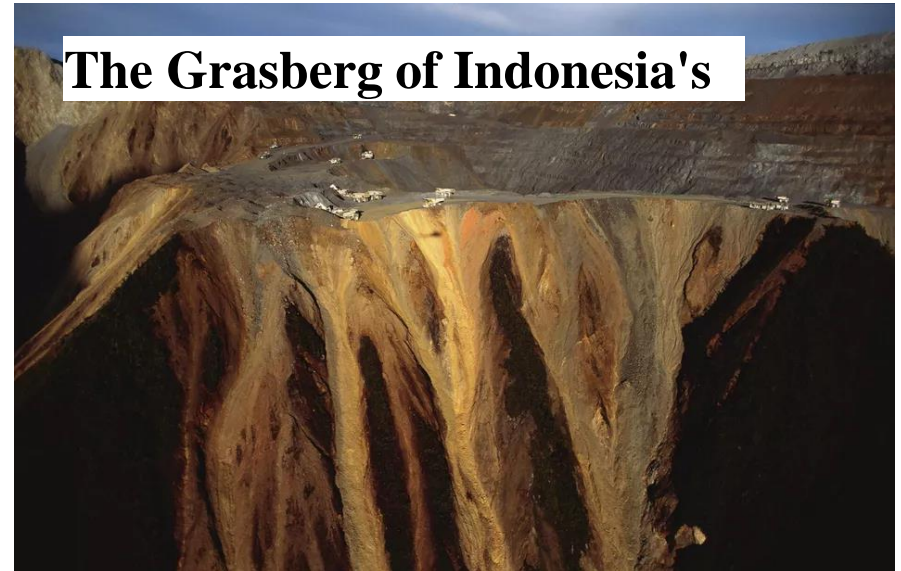
**The aim of the work was
to predict theoretically and confirm experimentally
the capability of microorganisms from natural
ecosystems (chernozem, Arctic and Antarctic soils):**

- to grow and interact with Cu(II) at high concentrations (up to 1.0 M);**
- to remove soluble toxic Cu(II) compounds from solutions.**

Environmental Pollution by **Copper**

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Copper mines as a sources of toxic copper compounds contamination



Environmental Pollution by **Copper**

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Excessive use of pesticides



Stikine River in Alaska



Zambia pollution by copper



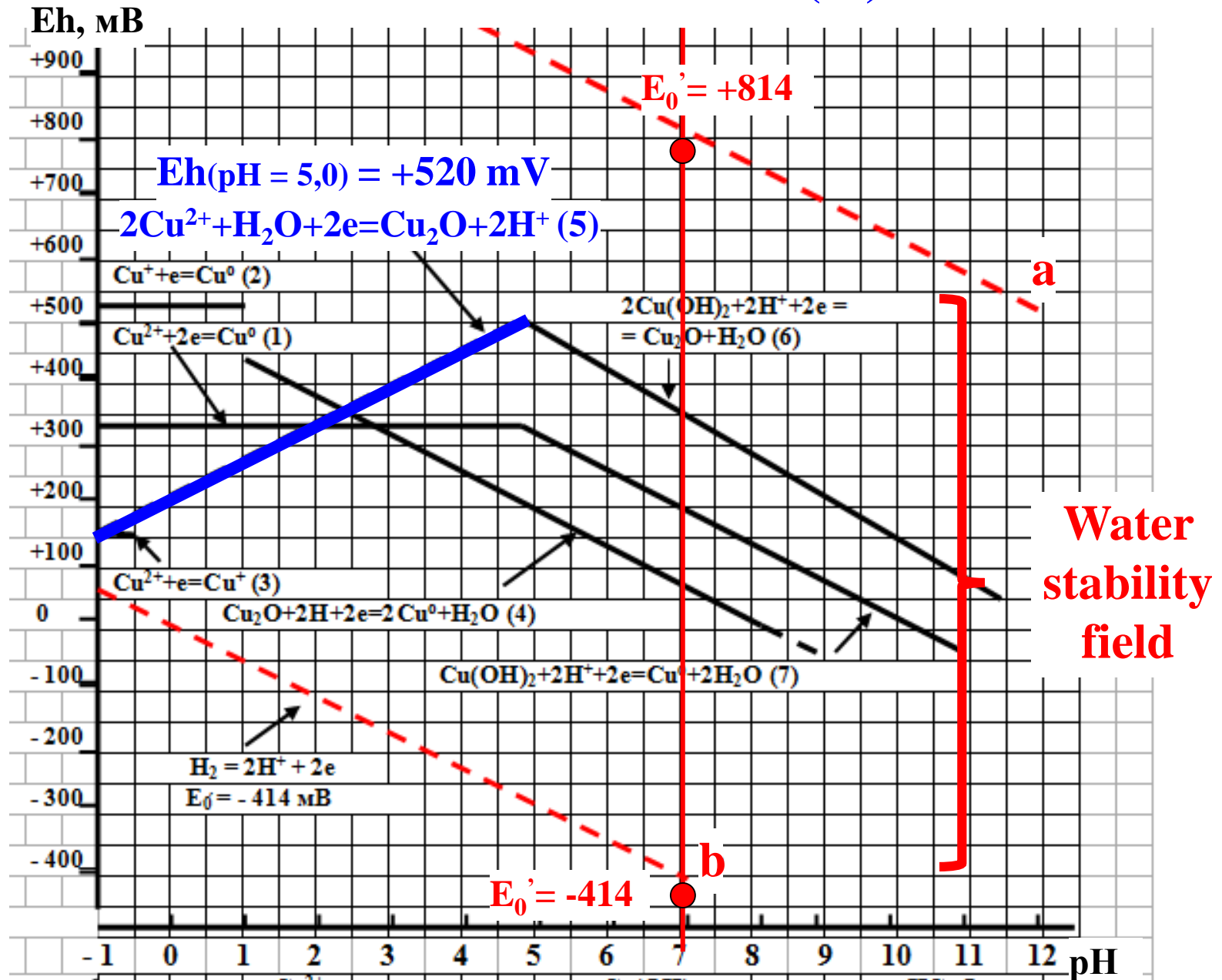
Outline of The Presentation

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- ✓ The characterization of theoretically positions of thermodynamic prognosis of the interaction of microorganisms with copper(II) compounds;
- ✓ The experimental confirmation of capability of microorganisms to grow at the presence of Cu(II) at high concentrations (up to 1.0 M);
- ✓ Genome sequencing and identification of super resistant to Cu(II) microorganisms;
- ✓ The experimental confirmation of mechanisms of microbial interaction with Cu(II);
- ✓ The specific copper resistant genes screening in genomes of isolated strains.

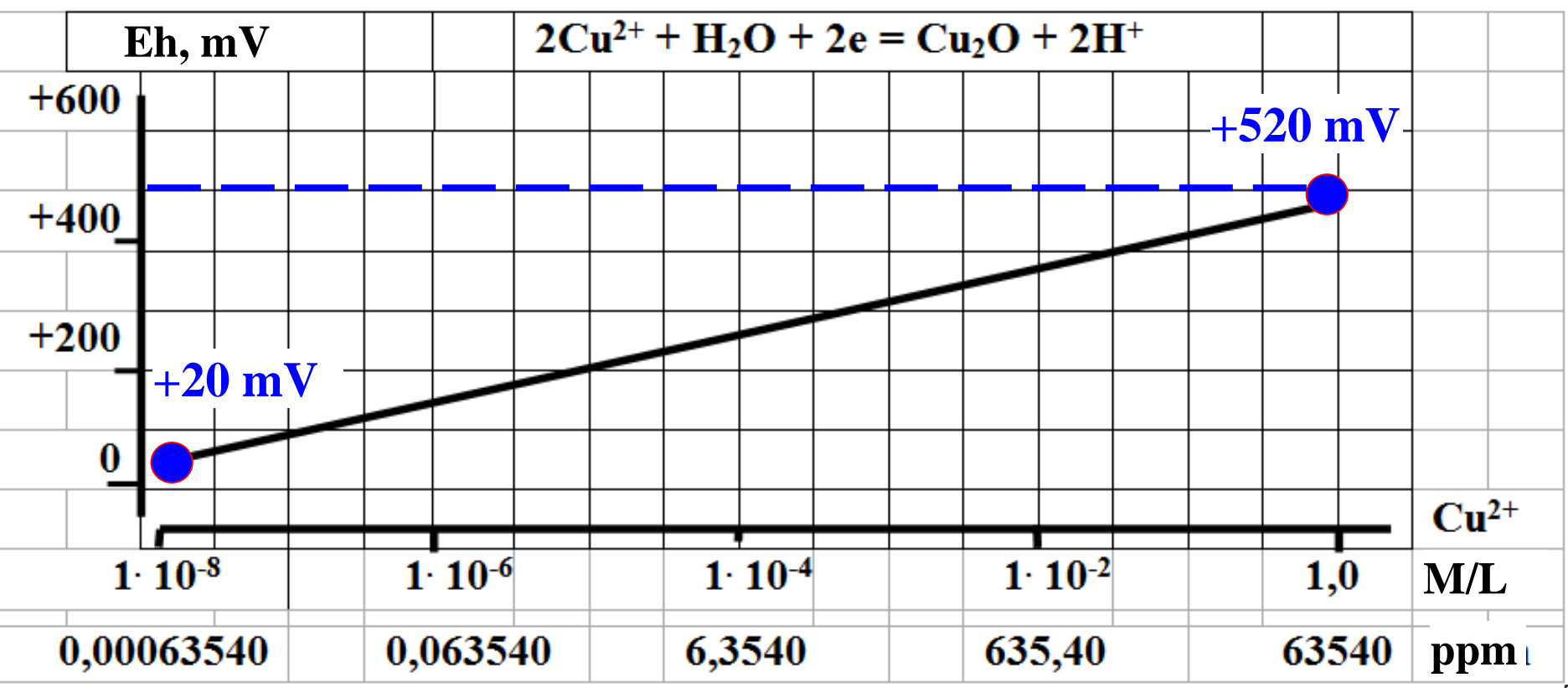
Thermodynamic Prognosis of Microbial Interaction with Cu(II)

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Changing the RedOx Potential in The Range of Concentration of **Cu(II)** from $1 \cdot 10^{-8}$ to 1.0 M/L

The value of the standard redox potential **Cu²⁺** (E_o') is located in the zone of thermodynamic stability of water (from - 414 to + 814 mV) in the range of concentrations from $1 \cdot 10^{-8}$ to $1,0$ M/L



Stereochemical analogy of **Toxic Metals** and **Macroelements**

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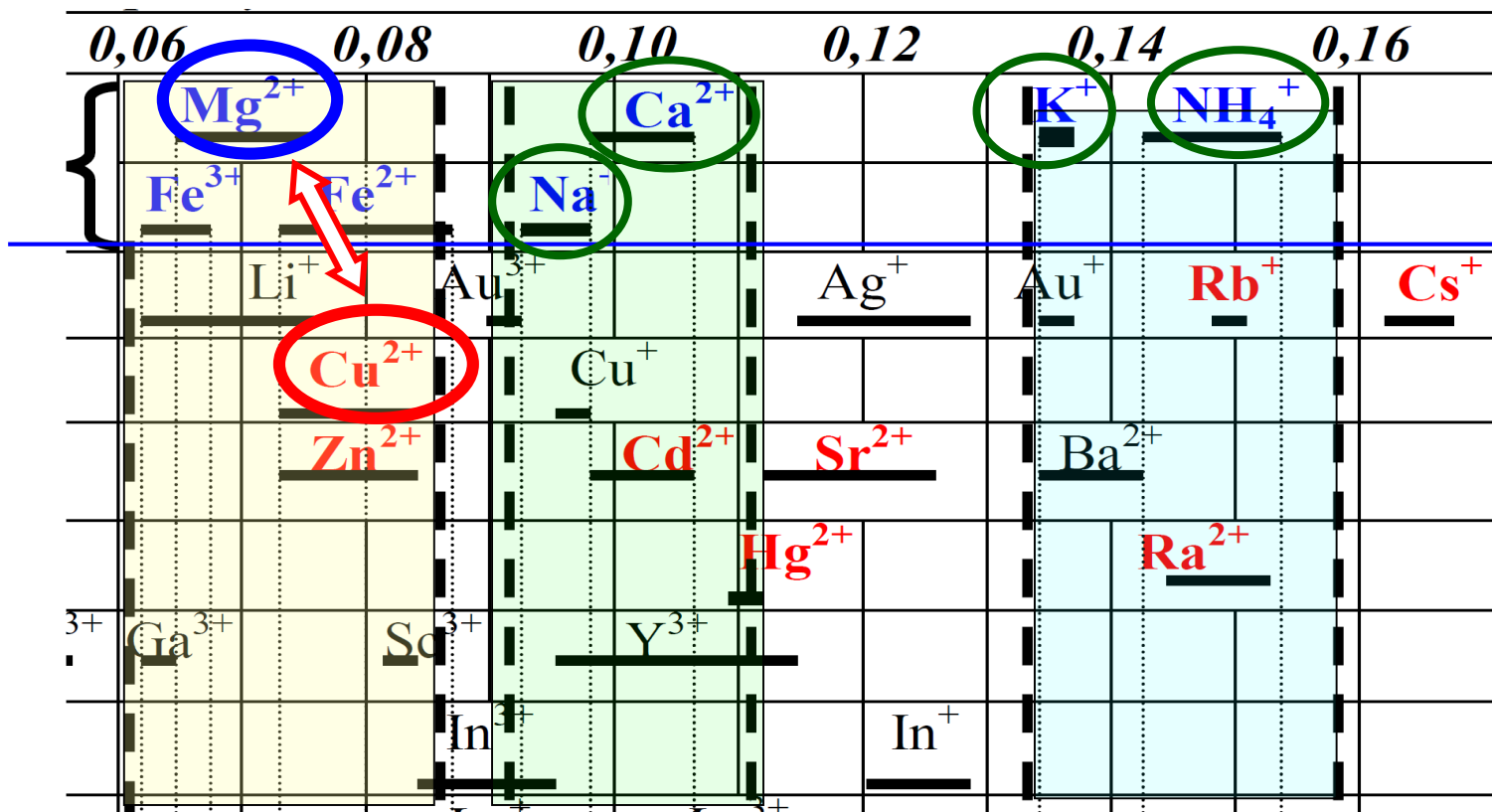
Stereochemical analogy - proximity or equality of ion radii

Ion radii of **Mg²⁺** and **Cu²⁺** is about 0,075 nm

Microbial transport systems are mistaken and "capture" **toxic metals** together with **macroelements**

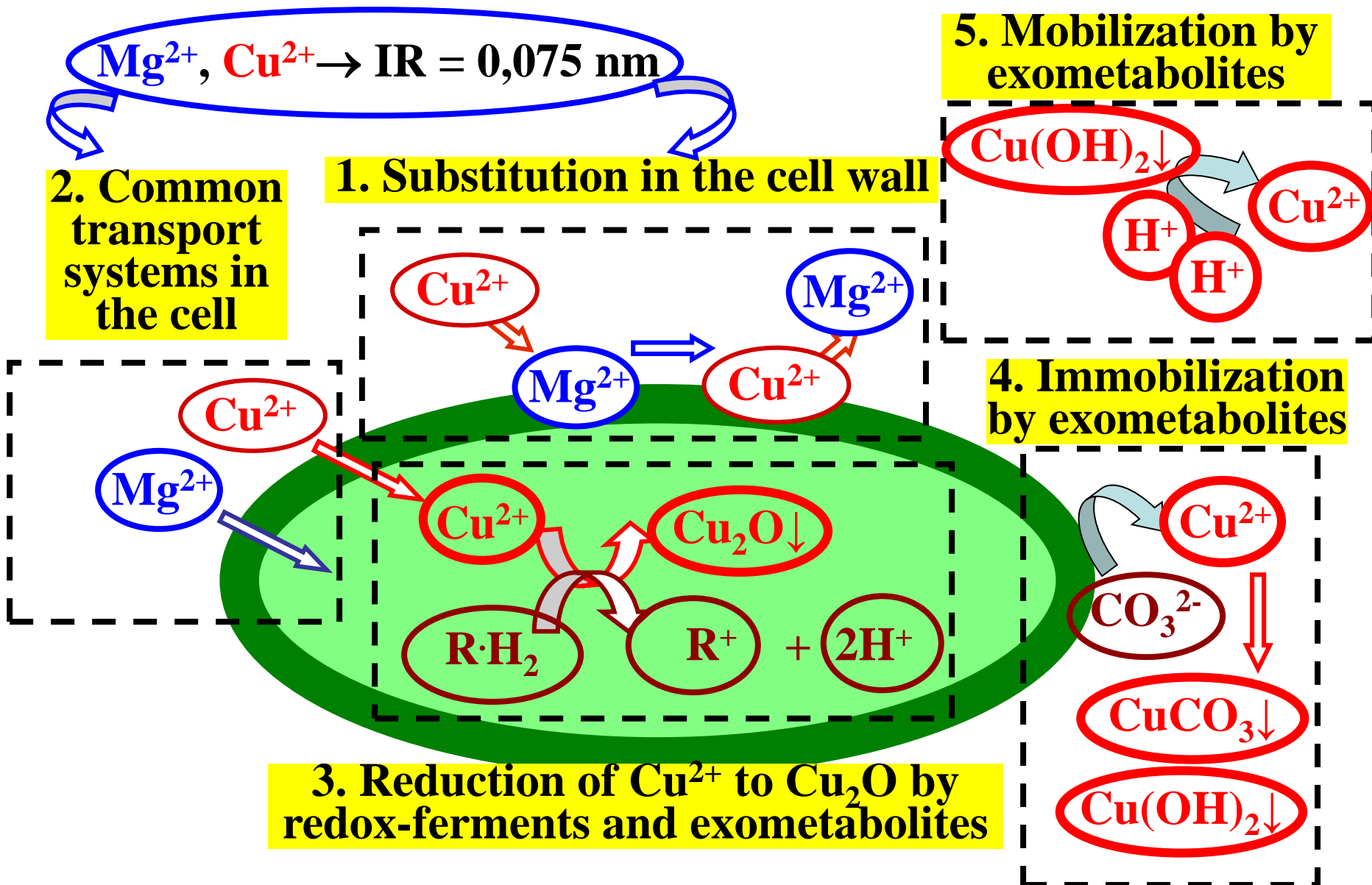
Macroelements

Ion Radii, *nm*



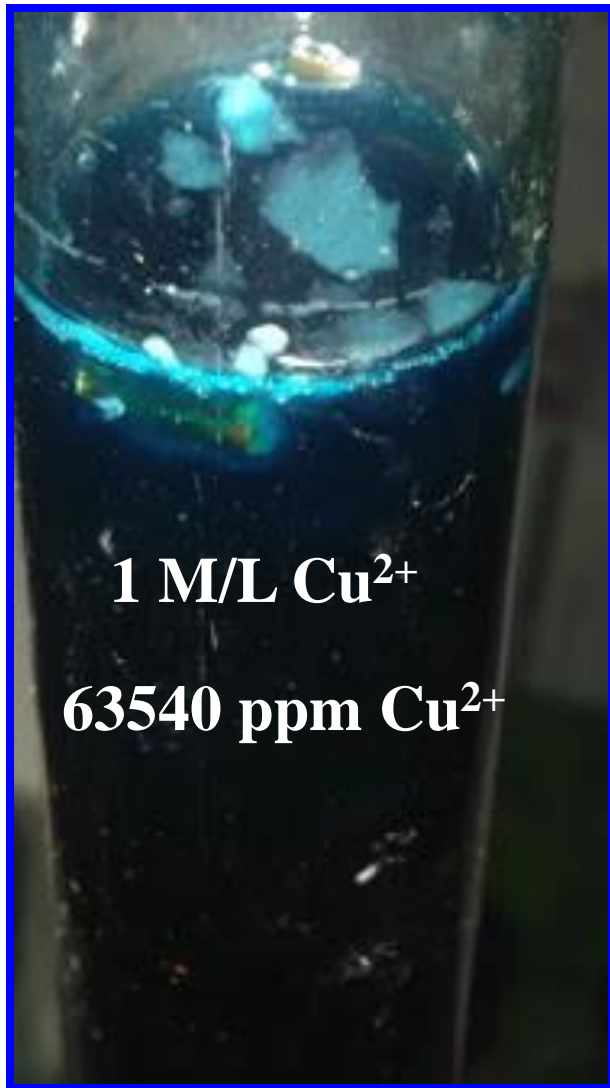
Microorganisms Interaction with Cu^{2+}

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Grows and Interaction of *Pseudomonas panacis* with Cu^{2+} at concentration of 1M\L

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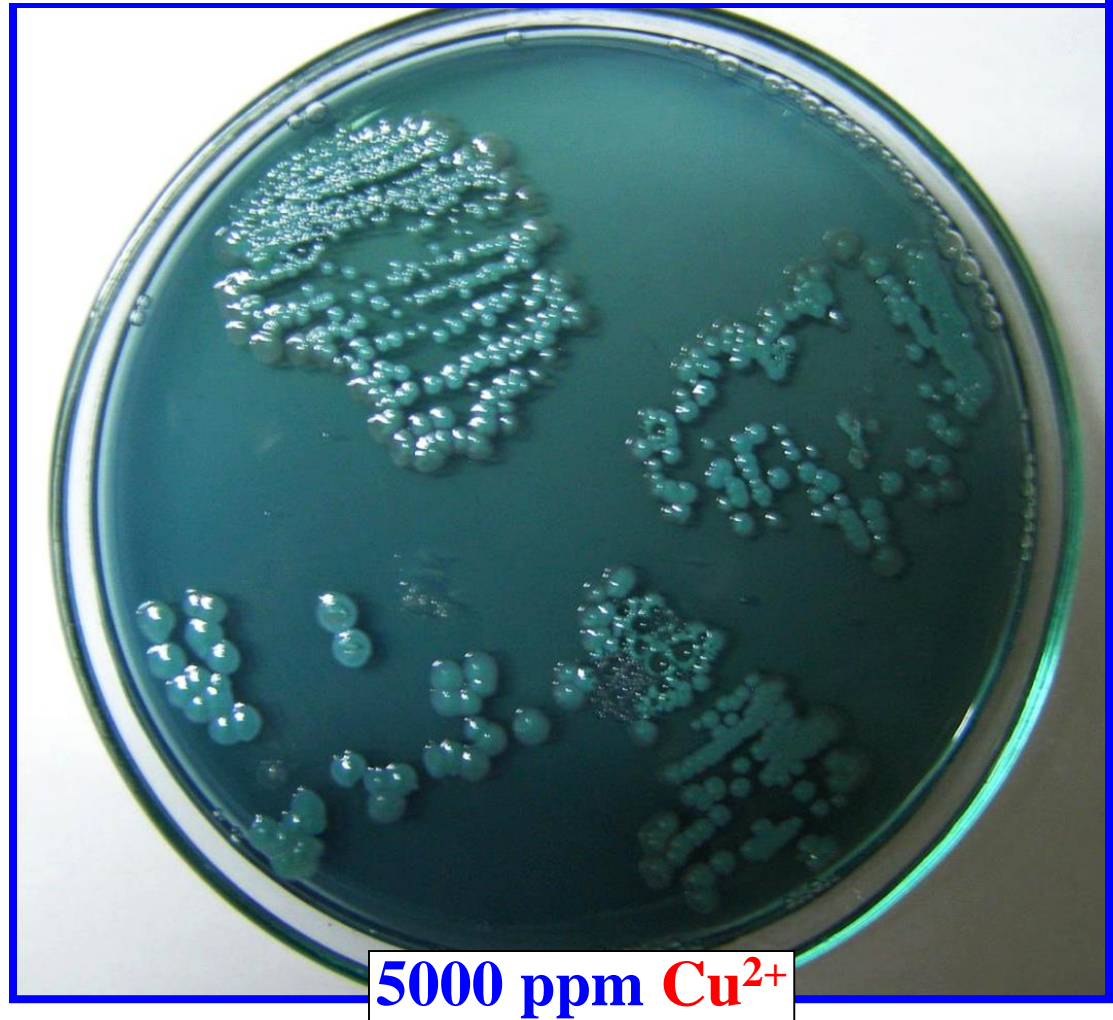


1 M/L Cu^{2+}

63540 ppm Cu^{2+}

Liquid Media

Accumulation of Cu^{2+} inside colonies



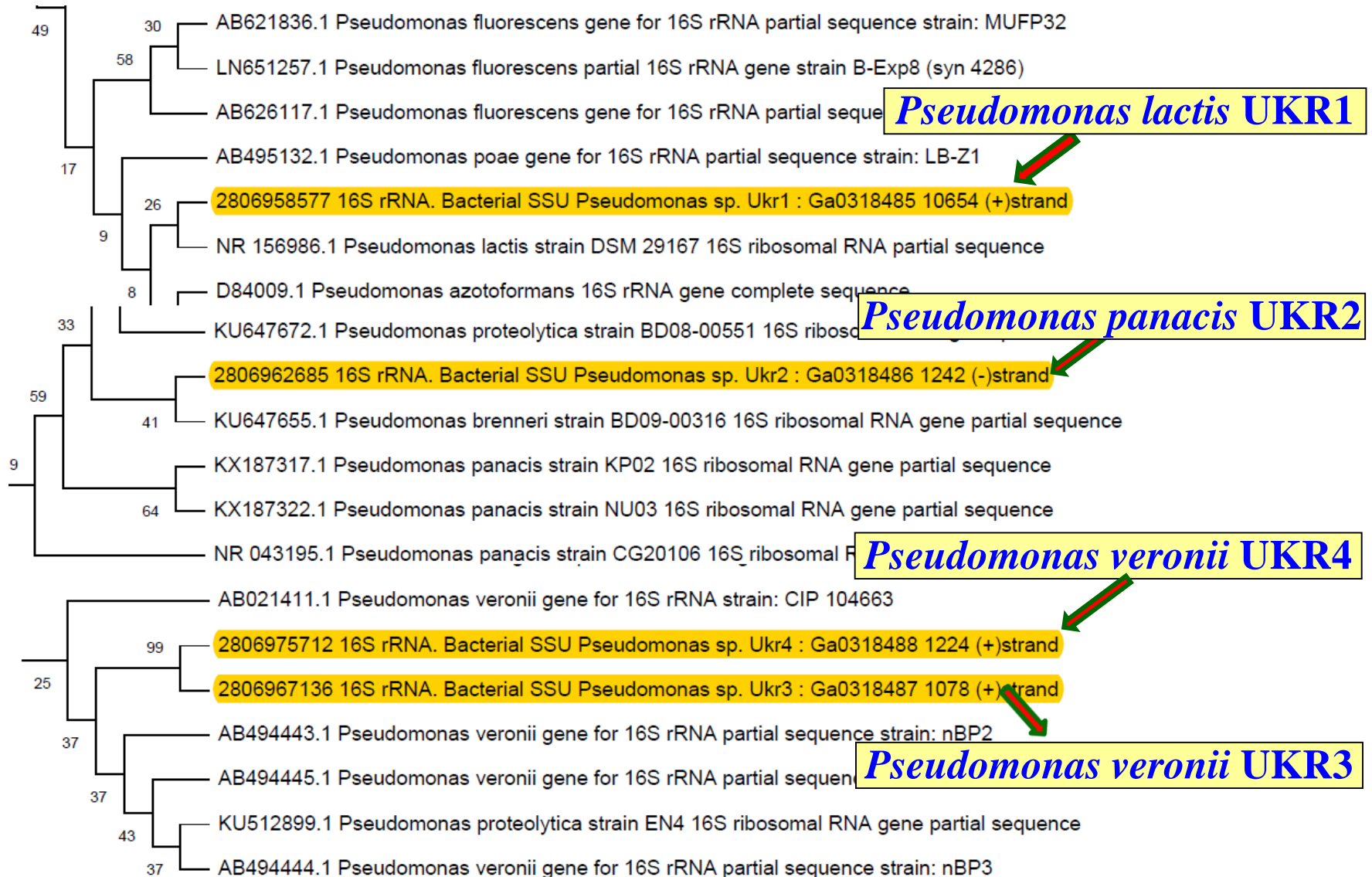
5000 ppm Cu^{2+}

Isolation of Cu^{2+} resistant Strains

Identification of Super Resistant to Copper(II) Microorganisms

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MPC [Cu^{2+}] = 63 546 ppm = 1 M/L



Identification of Super Resistant to **Copper(II)** Antarctic Yeast Strain

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MPC [Cu²⁺] = 63 546 ppm = 1 M/L

Metabolic characteristic for identification

***Rhodotorula mucilaginosa* UKR5**

prepared with the help of Ph.D. Olga Yaneva

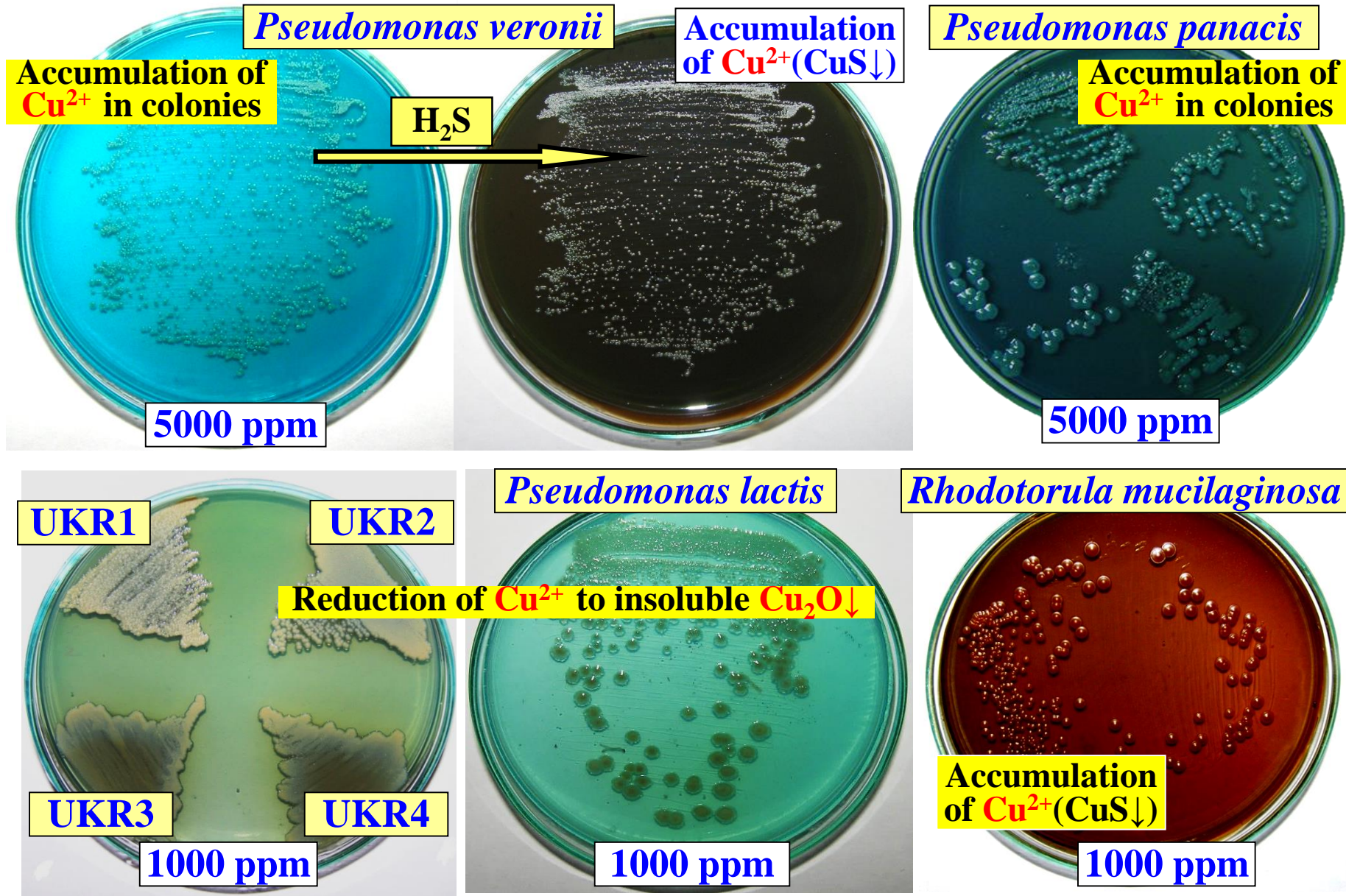
Carbon source						
Saccharose	Glucose	Ramnos	Maltose	Lactose	Galactose	Raffinose
+	-	-	+	-	+	+
Carbon source						
Ribit	Glycerol	Ethanol	Inulin	Sodium gluconate	Me-glycoside	N-acetyl- D-glucosamine
-	+	+	-	+	-	-
Carbon source						
Citric acid	Xylitol	D-Glucosamine	Lactic acid	Hexadecane	Gluconolactone	Glucuronic acid
+	+	-	+	-	+	-

+ presence of growth

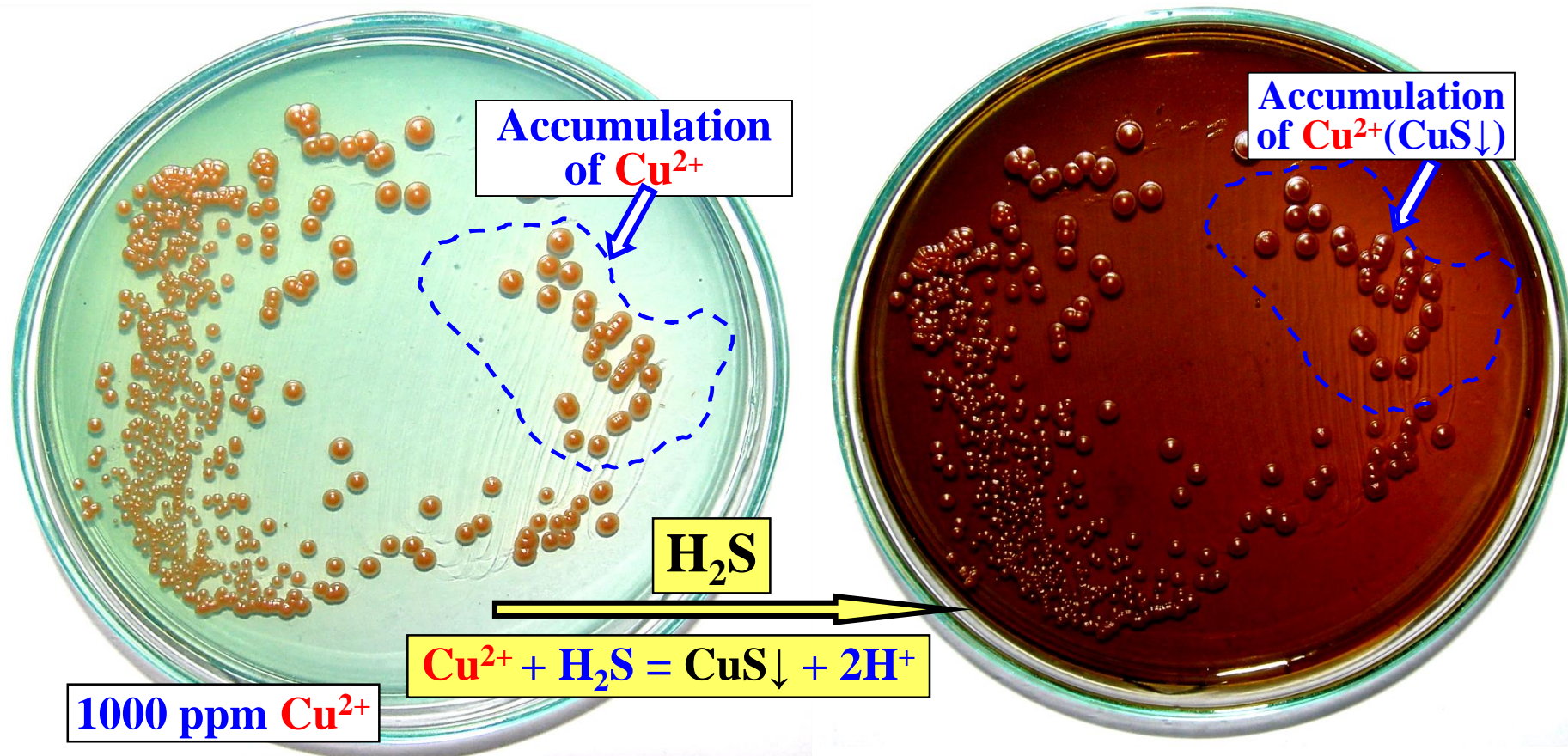
- absence of growth

The growth and interaction of isolated super resistant strains with toxic Cu(II)

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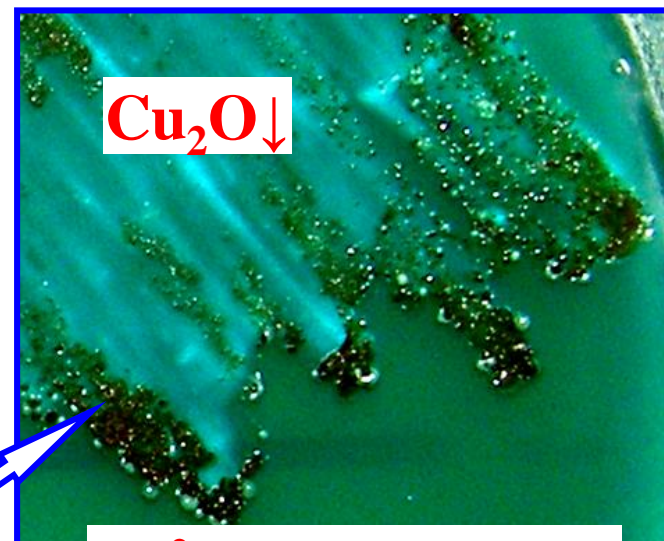
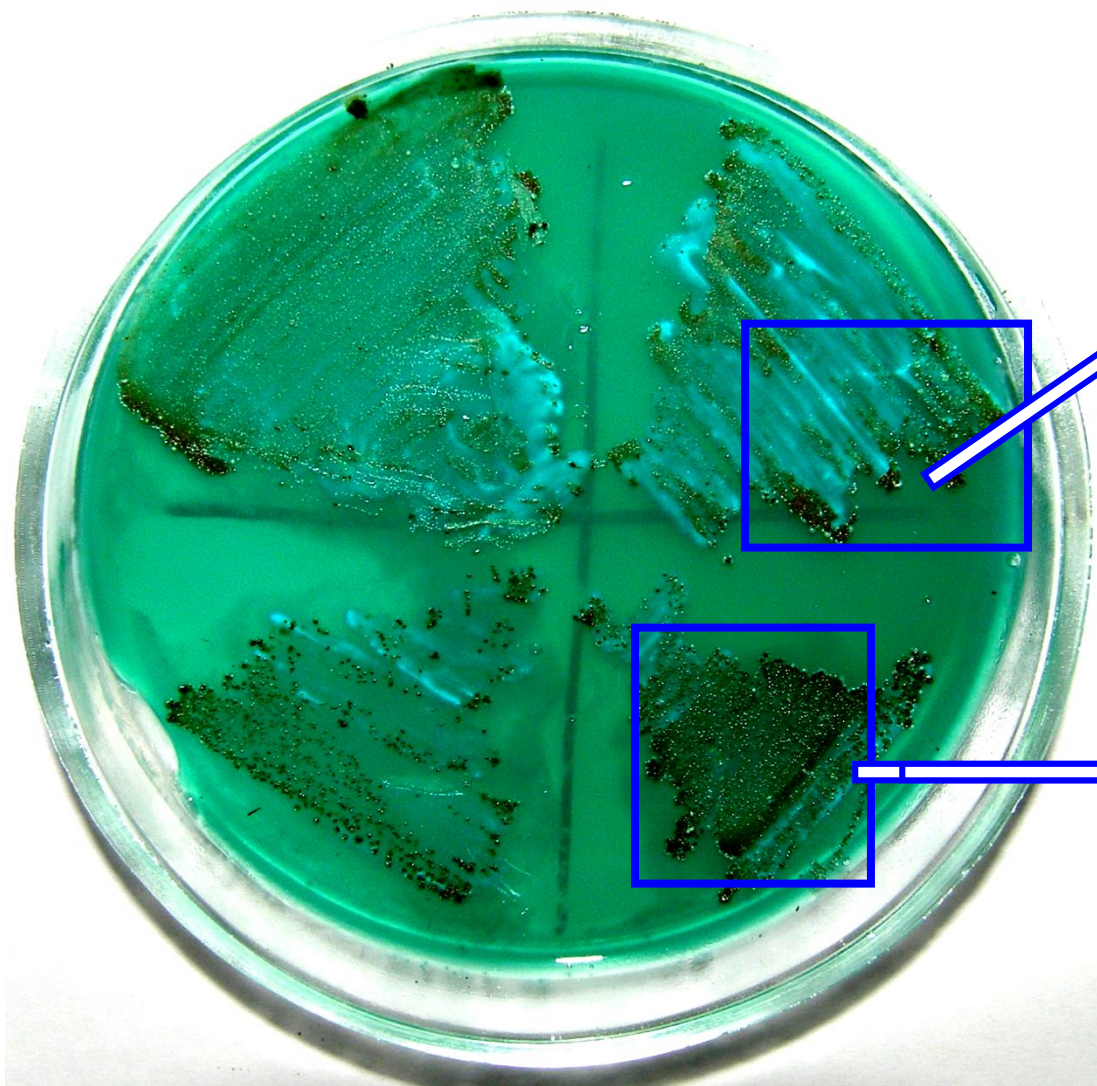
Accumulation of **Copper** (1000 ppm Cu^{2+})
Due to Stereochemical Analogy with Mg^{2+} by
Rhodotorula mucilaginosa UKR5



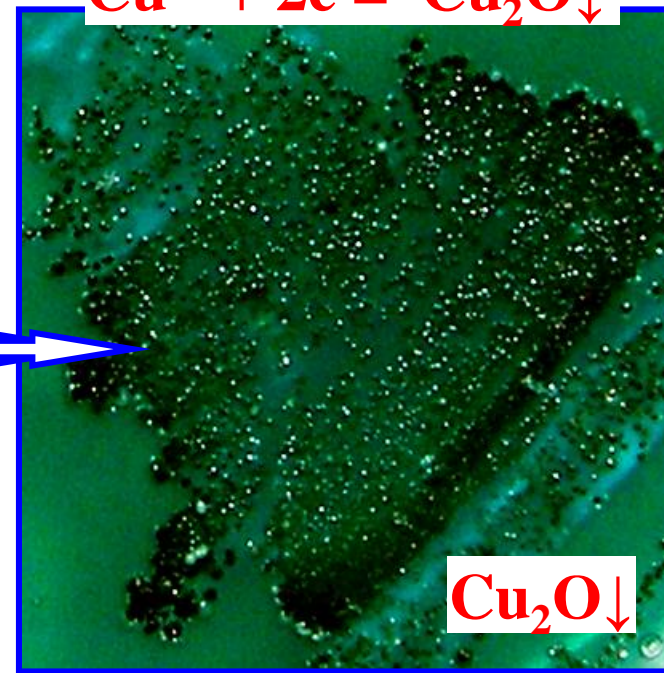
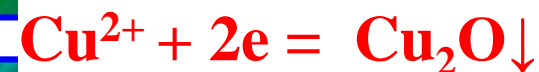
Reduction of Cu^{2+} to insoluble Copper(I) Oxide by *Pseudomonas veronii* UKR4

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2500 ppm Cu^{2+}



$\text{Cu}_2\text{O} \downarrow$



$\text{Cu}_2\text{O} \downarrow$

The Screening of Specific Copper Resistant Proteins in Genomes of Isolated *Pseudomonas* Strains

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CopA

Copper binding protein (periplasmic multicopper oxidase) uses ATP to pump Cu^+ and Cu^{2+} across cell membranes.

CopB

Outer membrane protein or P-type ATPase is exhibit high copper transport activity. CopB exports Cu^+ and Cu^{2+} from the cytoplasm.

CopD

Internal membrane protein executes copper uptake to the periplasm.

CopZ

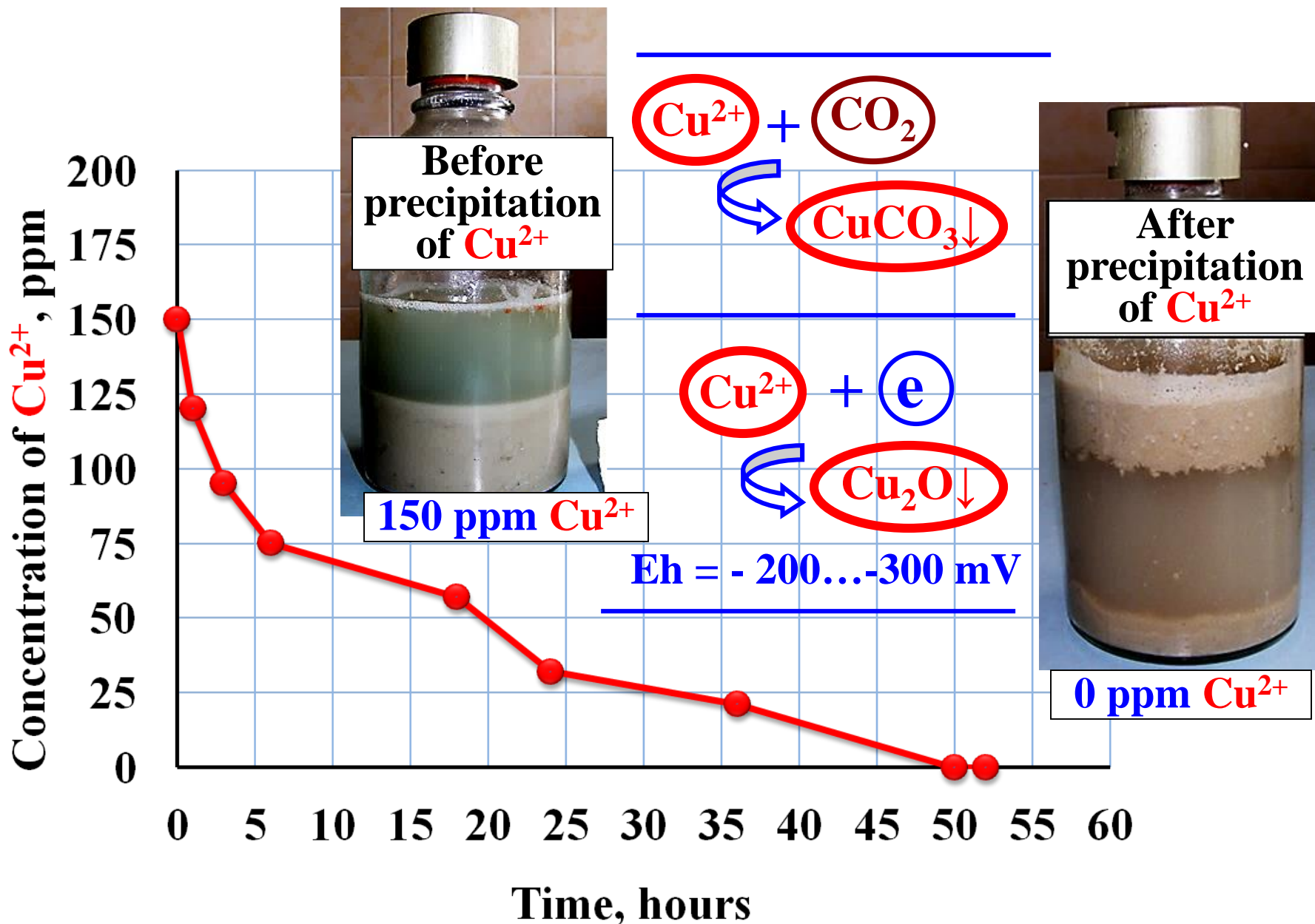
Chaperone that serves for the intracellular sequestration and transport of Cu^{2+} . Delivers Cu^{2+} to the copper-transporting ATPase CopA.

CusRS

Chromosomal two-component genes system that encode proteins homologous to known metal ion antiporters and maintain intracellular copper levels within a safe range

The Removing of Soluble Cu^{2+} During Hydrogen Fermentation of Multicomponent Food Waste

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Conclusions

1. The key points of the thermodynamic prognosis of the interaction of microorganisms with copper compounds were experimentally confirmed.
2. The isolated strains cultivated at the presence of Cu(II) at its super high concentrations (5 000 ppm ... 63 546 ppm Cu^{2+}), interacted with Cu(II) compounds – accumulated in cells and reduced to insoluble Cu_2O by microorganisms as well as precipitated to insoluble compounds.
3. Thermodynamic justified pathways of microbial interaction with Cu(II) were confirmed by initial screening for genes encoding copper resistance mechanisms. Genome of all isolated *Pseudomonas* strains contains copper resistance proteins - CopA, CopB, CopD, CopZ, as well as two component regulatory system CusRS.
4. The confirmed mechanisms of microbial interaction with Cu(II) are promising for development biotechnologies of super concentrated copper-containing wastewater purification and bioremediation of copper-contaminated ecosystems.

**Thank You for
attention!**

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J A Steadman