



BIOREMEDIATION OF HEAVY METAL-POLLUTED WATERS BY ASSESSING THE EFFICIENCY OF ACUMULATION OF COPPER IN SELECTIVE STRAINS OF RECOMBINANT MICROORGANISMS

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Introduction

The purpose of this study was to use different genetically modified bacteria and yeasts to treat synthetic water with high concentrations of copper through the bioremediation process.

Copper Tolerance Assay of Yeast and Bacterial Strains

Different strains of *E. coli*, *S. cerevisiae*, and *P. pastoris* were grown on LB or YPD solid media containing different concentrations of copper sulfate: 0 (negative control), 0.5, 1.0, 2.5, 5.0, 10.0, 25.0, and 50.0 mM.

Escherichia coli

Saccharomyces cerevisiae

Pichia pastoris

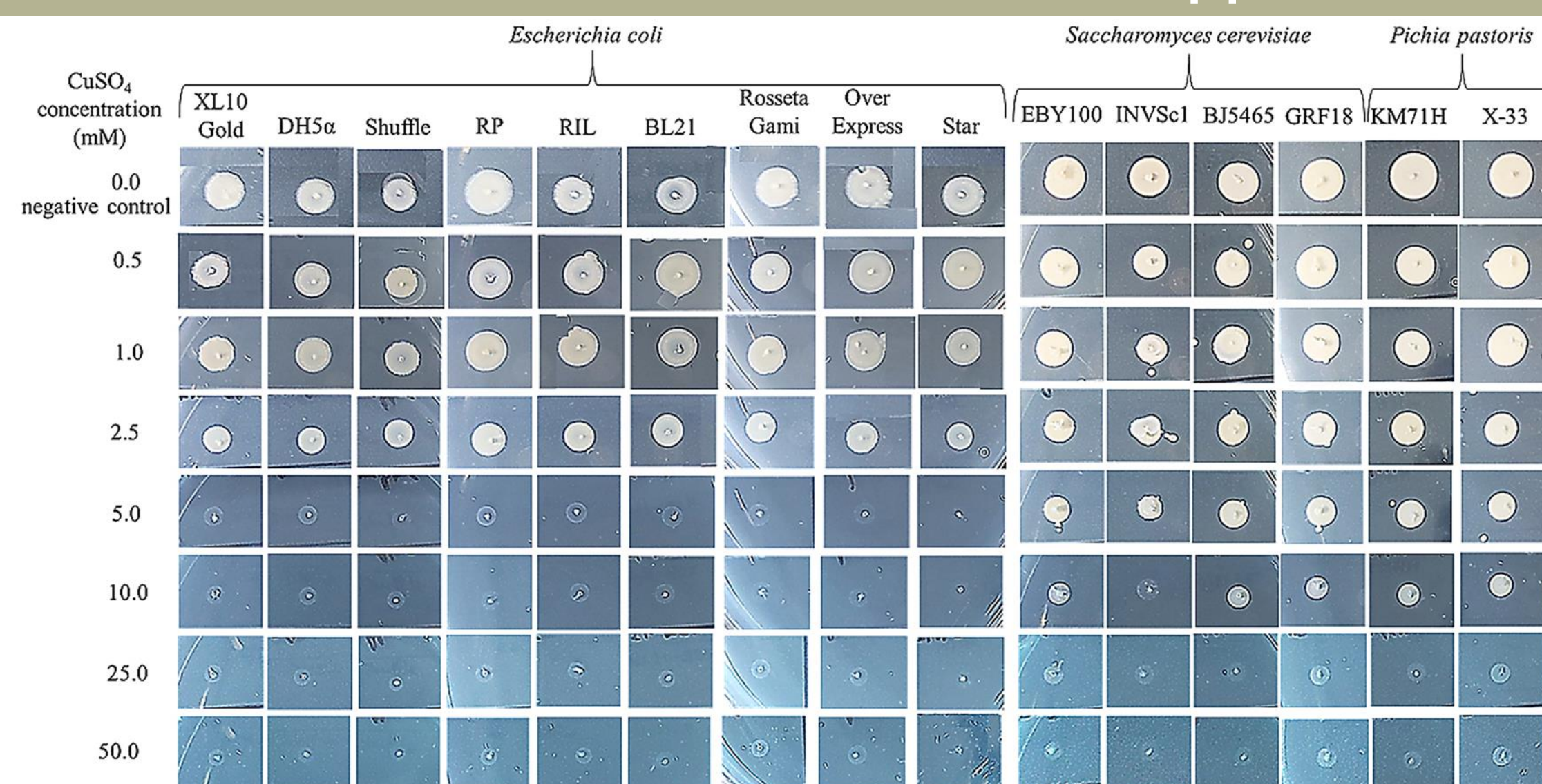
XL10 Gold
DH5α
BL21 (DE3)
BL21 (DE3) OverExpress
BL21 (DE3) RP
BL21 (DE3) RIL
BL21 (DE3) Shuffle
BL21 (DE3) Rosseta Gami
BL21 (DE3) Star

EBY100
INVS c1
BJ54 65
GRF1 8

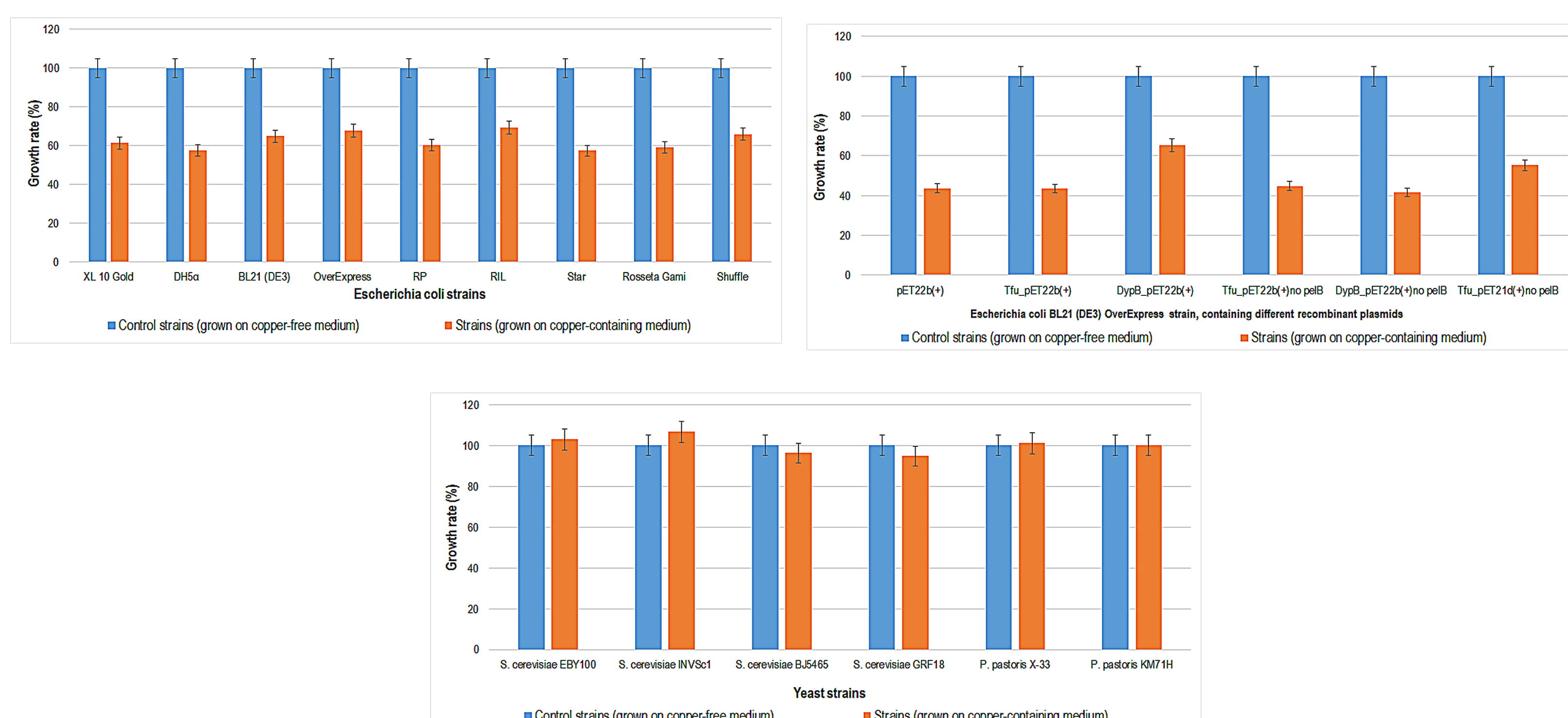
KM71 H
X-33

Each plate was inoculated with 5 µL of each preculture. Plates were incubated for two days at 35 °C or 30 °C. Copper tolerance was given by experiments related to the viability of bacteria and yeasts on plates. This are the results obtained.

Tolerance of Yeast and Bacterial Strains on Solid Copper-Containing Media



Tolerance of Yeast and Bacterial Strains on Liquid Copper-Containing Media



Growth conditions of microorganism

E. coli, *S. cerevisiae*, and *P. pastoris* strains were cultured in liquid media. The growth of microorganisms was started at an optical density of 600 nm (OD 600 nm) of around 0.1, then the cultures were incubated for 24 h at 35 °C or 30 °C. Second, 200 µL of five recombinant *E. coli* strains precultures containing plasmids with peroxidase genes were first inoculated in 10 mL LB containing 100 µg/mL ampicillin and then incubated for 2 h at 35 °C at 300 rpm. In the exponential phase (OD 600 nm around 1.0), the expression of recombinant peroxidases was induced by adding to the cultures IPTG 1 mM. These were further incubated at 35 °C, 300 rpm for 7 h. To each 3 mL from each culture, 3 mL of specific medium, LB, YPD, and LB-ampicillin- IPTG containing 2 mM CuSO₄ were added.

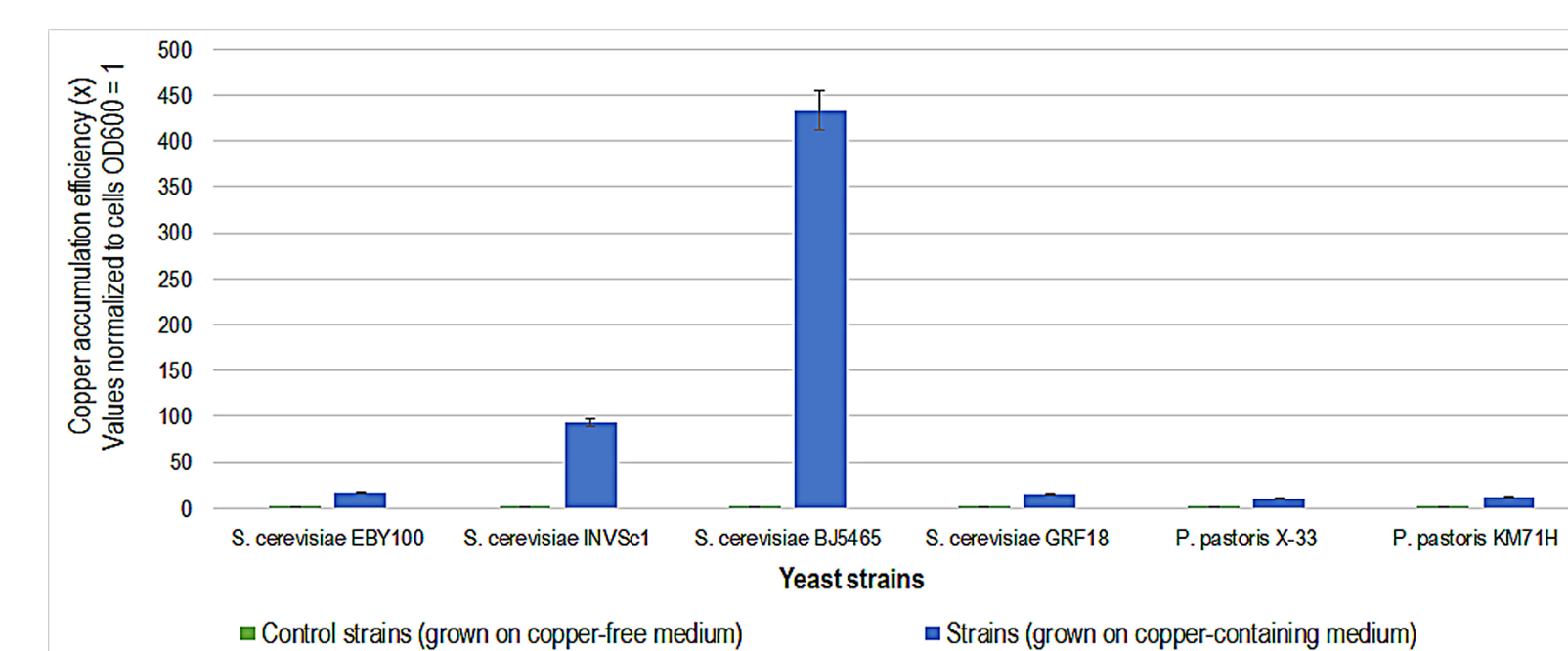
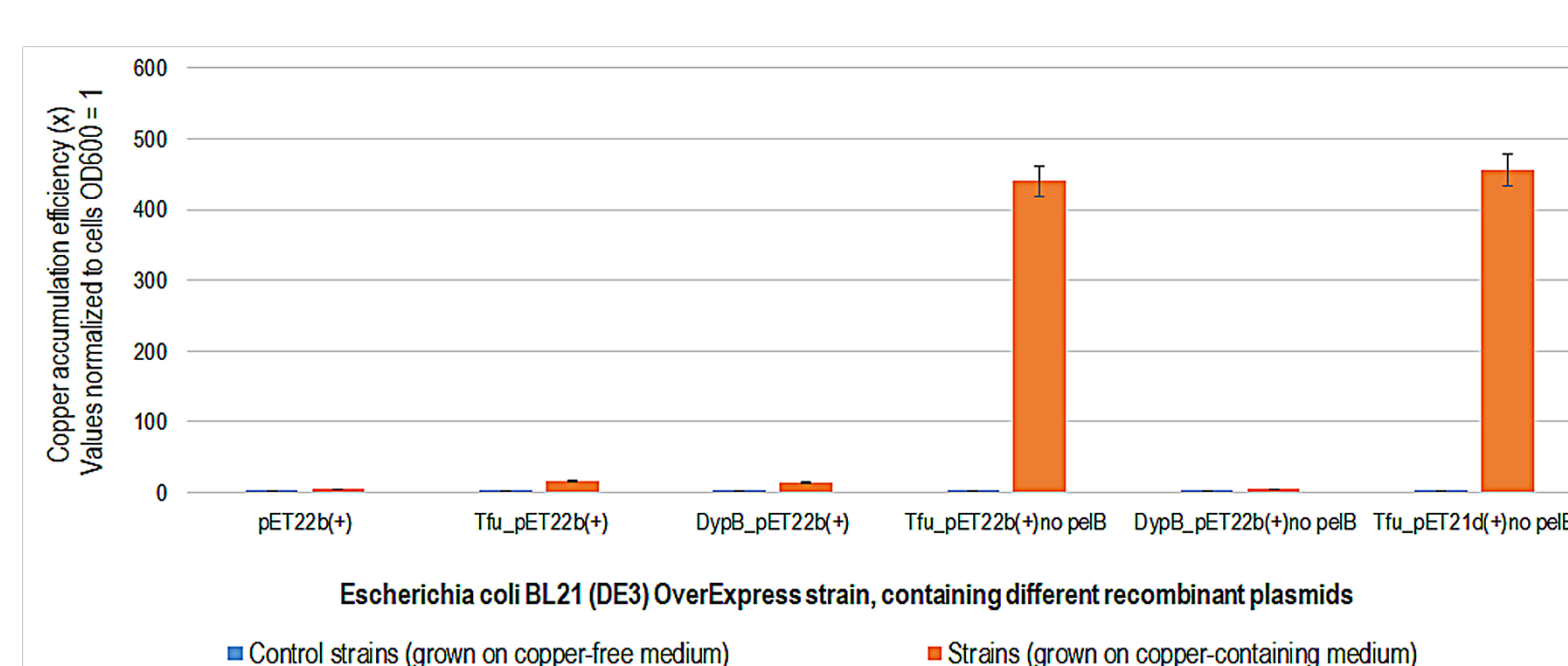
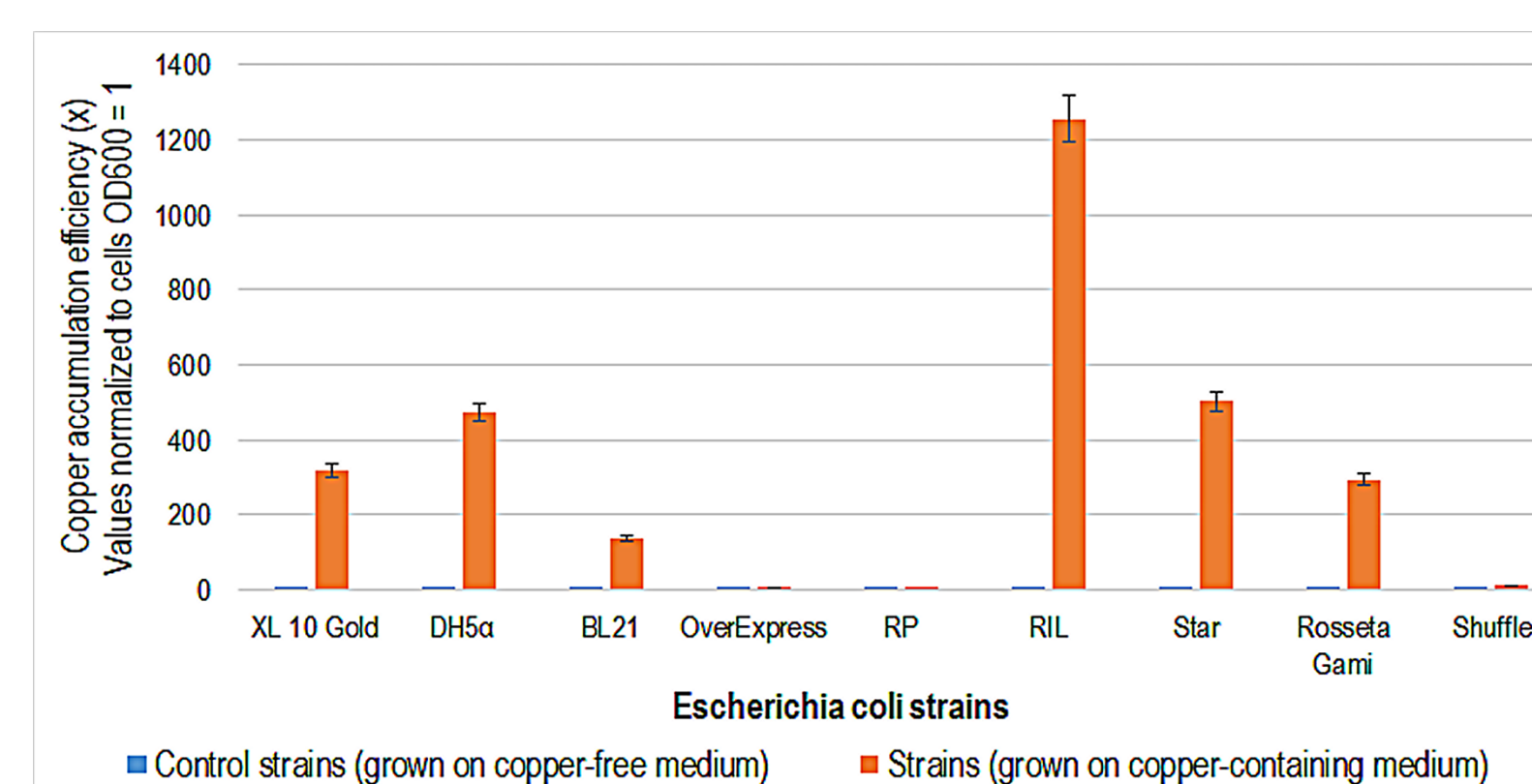
Cell Sample Preparation

After 48 h of growth, 3 mL of each culture was centrifuged for 2 min at 11,000 g. After removal of the supernatant, cells were resuspended in 500 µL of 2% HNO₃ solution and left at room temperature for 72 h. The cell debris was additionally subjected to homogenization for 30 s at 20,000 rpm using a Micra D-1 homogenizer from Germany. Samples were centrifuged for 10 min at 11,000 g. The supernatant from each cell sample was subjected to analysis by inductively coupled plasma optical emission spectrometry (ICP-OES).

ICP-OES Analysis

The plasma was stabilized and optically profiled according to the manufacturer's recommendations (ISO 11885:2007–Water Quality). The accumulated copper in the cells was determined at a wavelength of 324.754 nm. Operating conditions were as follows: forward power of 1450W, coolant flow of 13.0 L/min, nebulization flow of 0.75 L/min, an auxiliary flow of 1.0 L/min, and a sample aspiration rate of 2 mL/min. All chemicals used were of high purity. Certified reference materials (VHG Labs, QWPTM-15) were used for quality control of chemical analyses.

Copper Accumulation Efficiency



Conclusion

The growth of the bacterial strains used in these experiments was inhibited upon exposure to 1 mM copper, while yeast cultures were unaffected at this concentration. ICP-OES analysis showed that the *E. coli* RIL strain had the best copper accumulation efficiency (1250 times more efficient than the control strain), while the DH5α and STAR strains were about 450 times more efficient. *E. coli* BL21 (DE3) OverExpress cells that internally expressed peroxidase had the best copper accumulation efficiency. The *S. cerevisiae* BJ5465 yeast strain is the most efficient in copper accumulation of the six yeast strains used, accumulating over 400 times more copper than the negative control strain. As a result, the method presented in this study effectively demonstrates both the efficiency of copper ion accumulation and the capacity of the strains' cell walls to bind copper. The bioengineered yeasts and bacteria, as well as various strains expressing metal-dependent enzymes used for the first time, are successful candidates for copper accumulation to remove above-limit copper concentrations from water. A promising objective for the management of heavy metal contaminated water remediation is developing genetically engineered strains that not only accumulate metals but also express proteins capable of binding multiple metal ions.

Acknowledgements

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