

No. 0610

Heavy Metal Remediation of Sea Water, Brine, and Bittern Using the Moderate Halophile *Halomonas elongata*

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Summary

Metal pollution is a growing threat to the environment and human health. High salinity and metal pollution are often observed together because inorganic metals and mineral salts are concentrated simultaneously in nature. Therefore, the key to a successful metal-bioremediation is to develop a remediation technology that can be used in high-salinity environments. Halophilic bacteria are typical inhabitants of high-salinity sites such as marine environments and salt fields. *Halomonas elongata* strain OUT30018, a moderate halophile isolated from high-salinity soil from Khon Kaen, Thailand, can survive heavy metal-stress under high salinity and alkaline conditions. Using *H. elongata* OUT30018, we aim to develop superior biotechnology for metal-remediation. We have developed a cell-surface engineering system for *H. elongata* OUT30018 using its own outer-membrane lipoprotein as an anchor. Expression of the synthetic phytochelatin (PC), EC8, composed of (Glu-Cys)₈Gly peptide fused to the lipoprotein anchor on the surface of *H. elongata* OUT30018 resulted in improved bioaccumulation of Cu, Cd and Zn in medium containing each metal separately, while in medium containing mixed metals (Cu, Cd and Zn), Cu is the main metal accumulated. Furthermore, to test the effect of charged amino acid residues next to Cys on metal-binding selectivity of PC-like peptides, we expressed alternative PC-like peptides, XC6, composed of (Xaa-Cys)₆ peptides (Xaa = Glu [E], Asp [D], Gly [G], or His [H]) fused to the lipoprotein anchor on the surface of *H. elongata* OUT30018. In the *H. elongata* OUT30018 cells displaying PC-like peptides with acidic amino acid residues (EC6 and DC6), bioaccumulation of Cu was greatly improved. Therefore, cell-surface engineered *H. elongata* OUT30018 is a promising tool for decontamination of heavy metals in salty water such as seawater and brine.