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[P03-377] Engineering substrate specificity of a phosphorus transporter to enhance biocontainment based on dependency on reduced phosphorus compounds

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Keywords：Hypophosphite、Substrate binding protein、ABC transporter、Substrate recognition、Biocontainment

Background

Genetically modified microorganisms have been applied in various fields such as energy production, agriculture, and environmental remediation. However, their potential release into natural environments poses biosafety and biodiversity concerns. Therefore, biocontainment strategies that prevent their proliferation outside controlled environments have recently attracted increasing attention. Previously, we established a biocontainment strategy based on dependency on phosphite (HPO_3^{2-}, Pt), a compound rarely found in natural environments, by modifying microbial phosphorus metabolism. This strategy was implemented by expressing the exogenous Pt/hypophosphite ($H_2PO_2^-$, HPt)-specific transporter HtxBCD (*Pseudomonas stutzeri* WM88), together with Pt dehydrogenase PtxD (*Ralstonia* sp. 4506), while disrupting all endogenous phosphorus transporters. Although this strategy provides highly stringent and cost-effective containment, recent studies suggest that anthropogenic Pt in the environment may potentially compromise its effectiveness.

Purpose, Method, Result

The purpose of this study was to develop a HPt-dependent biocontainment strategy that remains effective even in the presence of anthropogenic Pt. Site-directed mutations introduced into amino acid residues (W52 and D206), essential for substrate binding in the binding protein HtxB, abolished Pt transport while retaining HPt transport. *Escherichia coli* strain expressing the W52 or D206 mutant HtxBCD as the sole phosphorus transporter, and harboring an HPt oxidation pathway, exhibited strict HPt-dependent growth. In escape assays under non-permissive conditions, no escape mutants were detected for at least 21 days, with a detection limit of 1.5×10^{-11} per colony-forming unit. These results demonstrate that the HPt-dependent strategy provides containment performance comparable to that of the established Pt-dependent strategy and offers an additional safeguard for biocontainment strategies based on dependency on reduced phosphorus compounds.