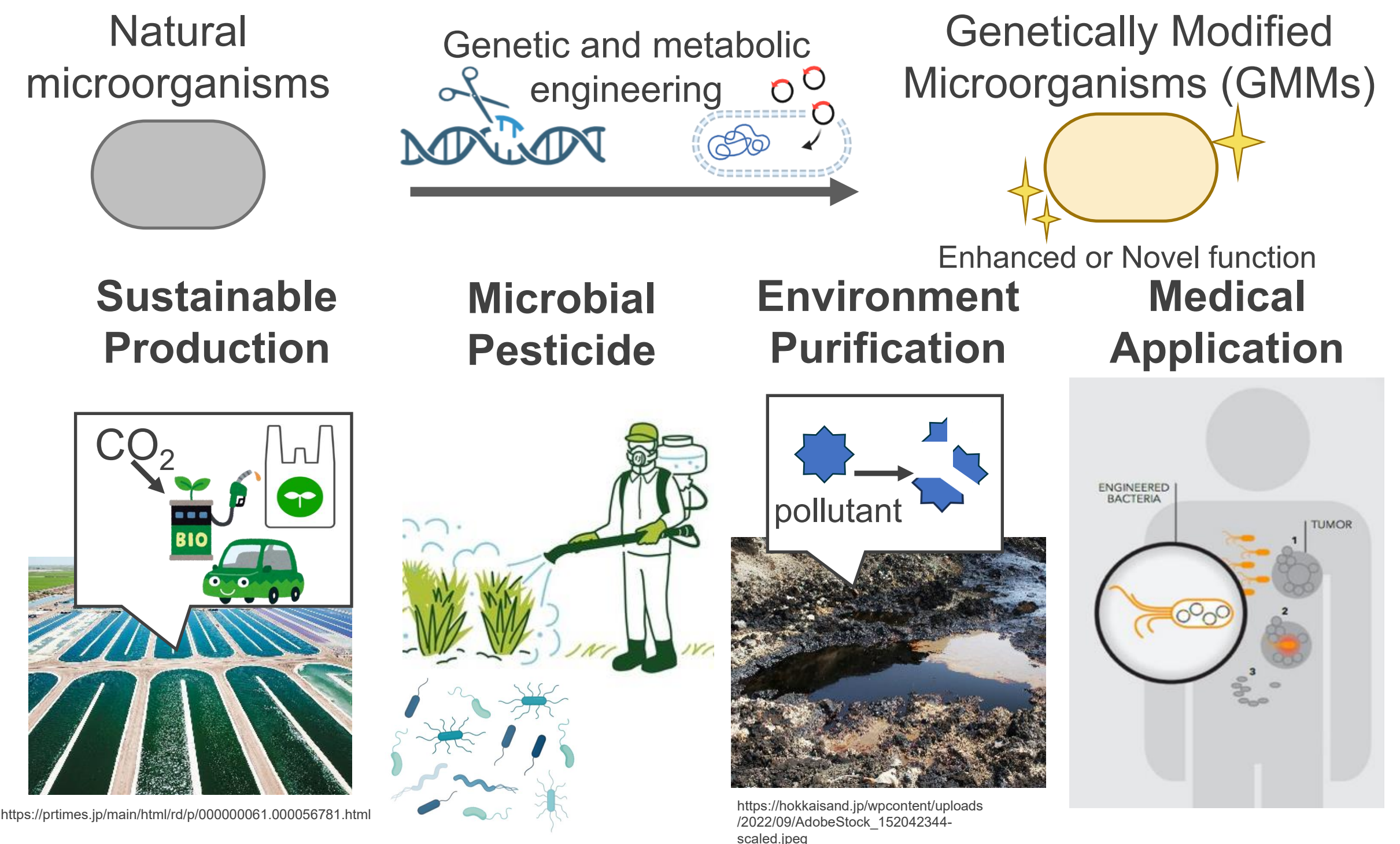


特殊なリン化合物に生育を依存させるバイオセーフティー技術の高度化

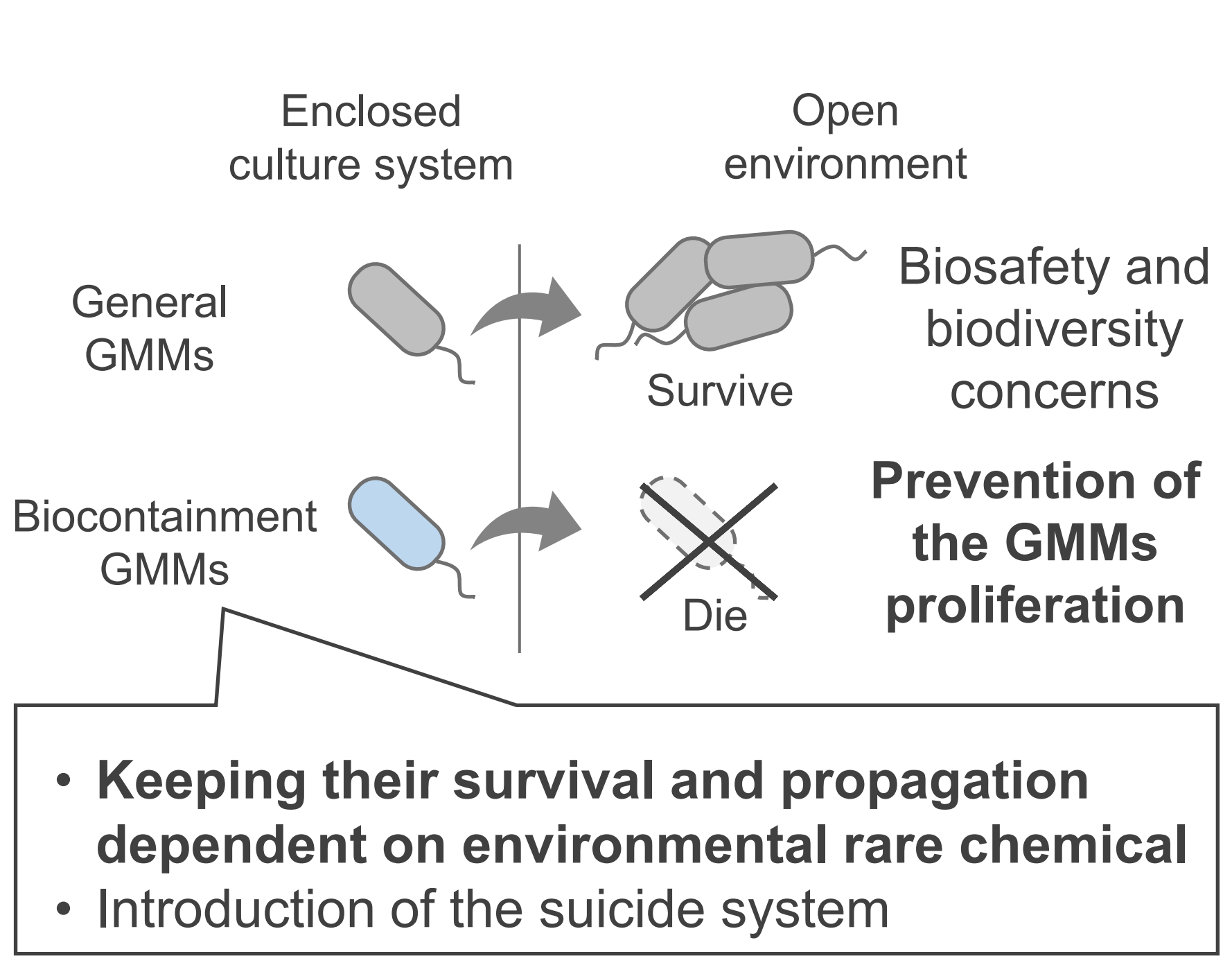
広島大学 統合生命科学研究科 三輪朱莉, 百川直輝, 黒田章夫, 廣田隆一

1. Introduction

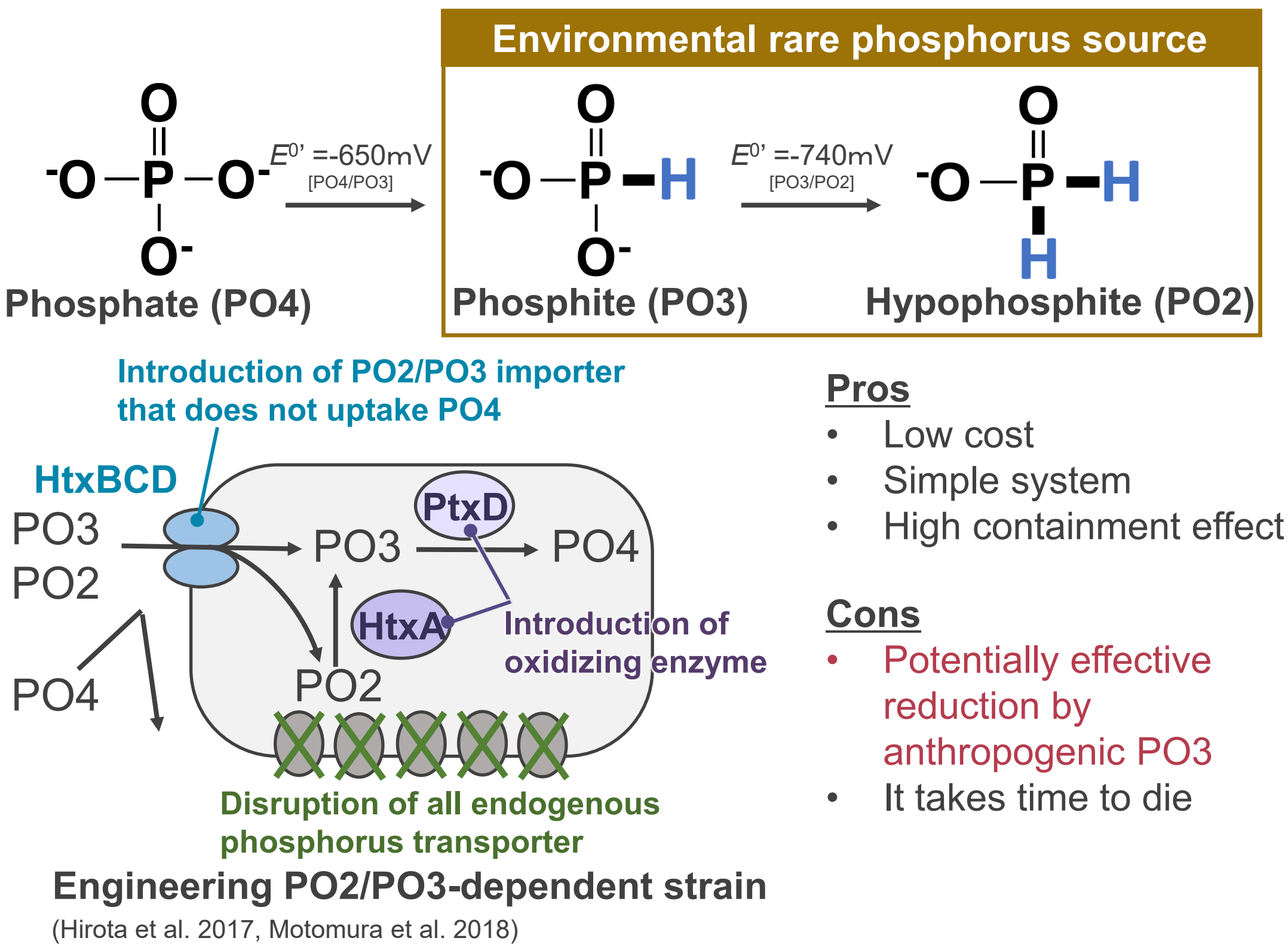
Outdoor use of genetically modified microorganisms (GMMs) is expected: from laboratory containment to environmental deployment



Biocontainment strategy for restricting the survival and propagation of GMMs outside controlled settings

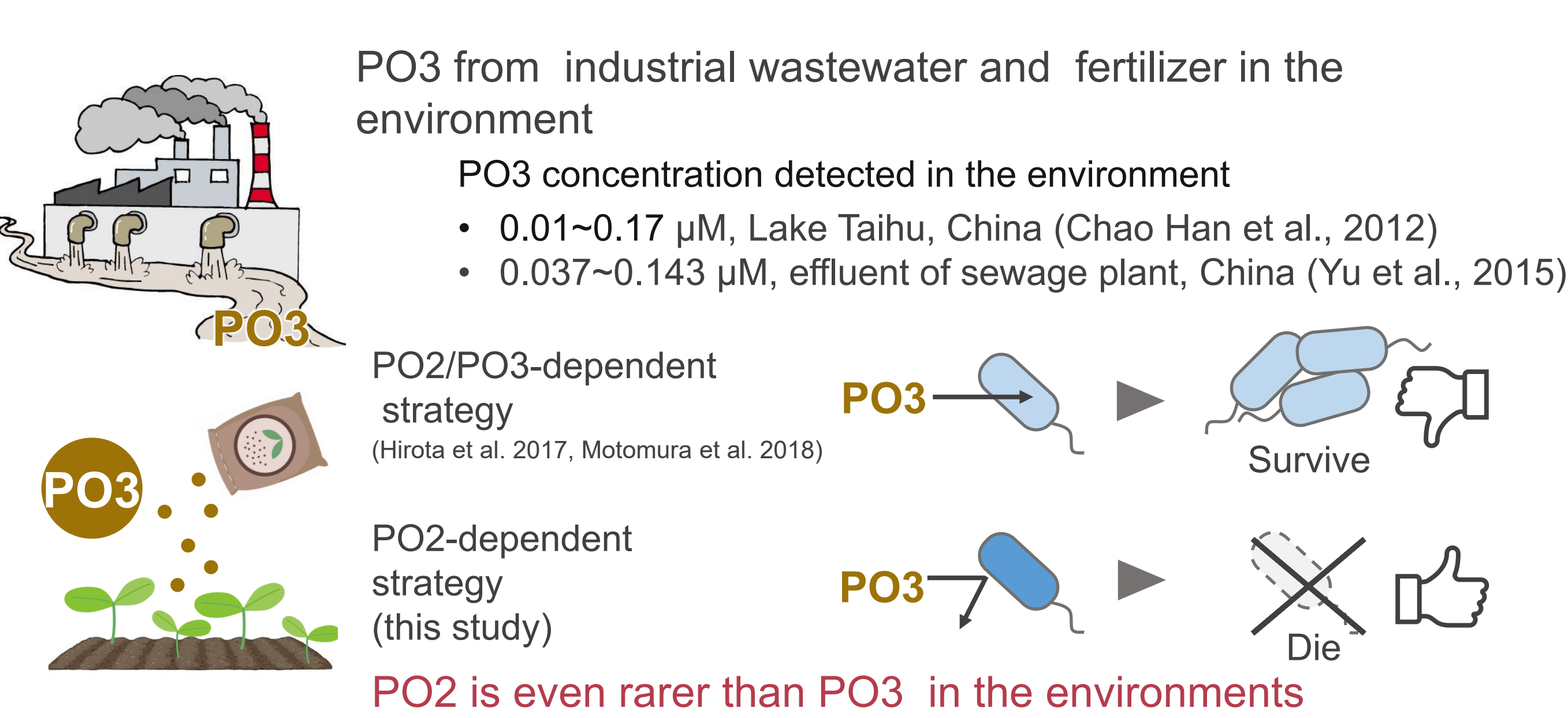


Synthetic phosphite-dependency: a practical and stringent biocontainment strategy



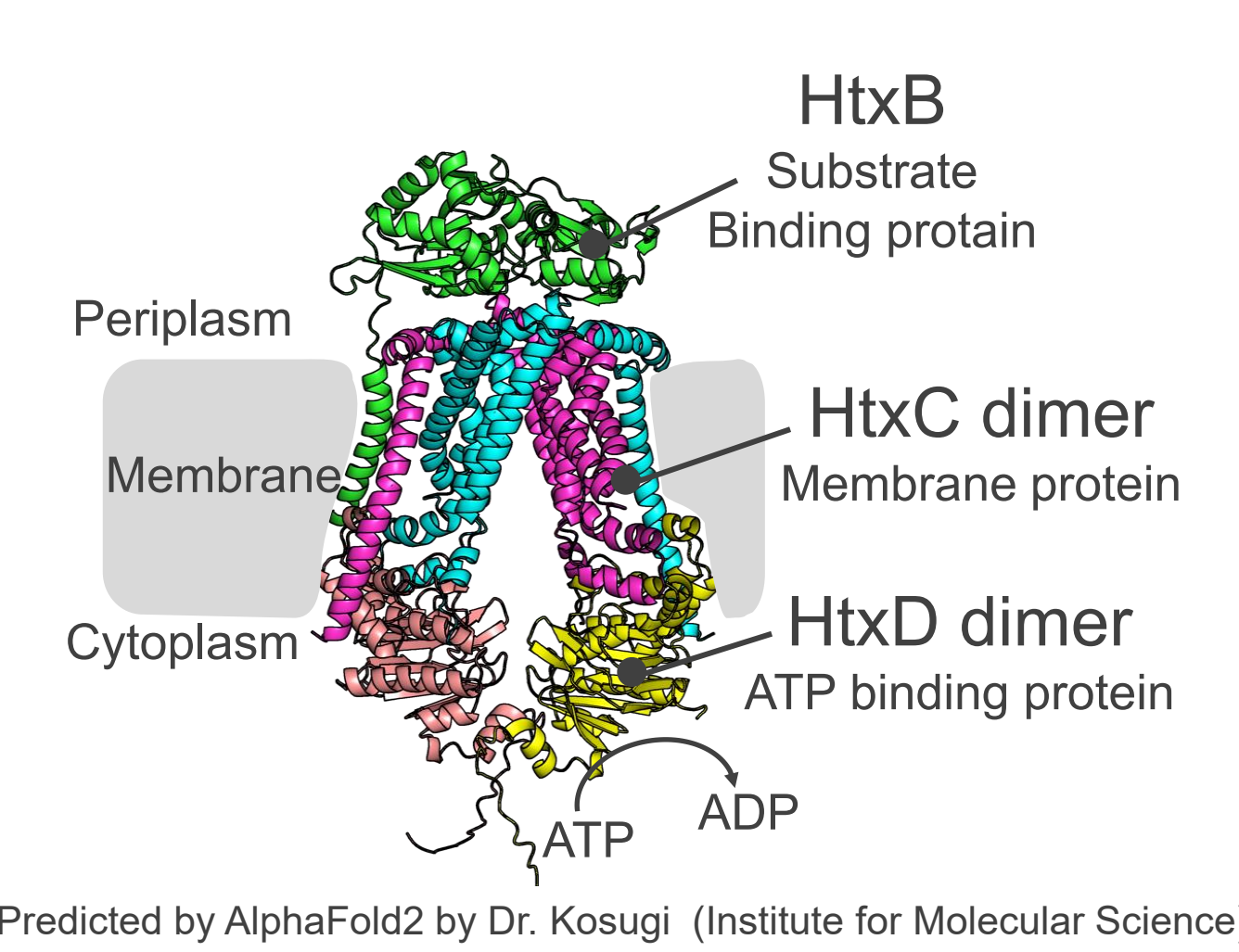
2. Purpose

From PO₃ to PO₂: developing a more stringent biocontainment strategy via PO₂-dependency



HtxBCD transporter

- Derived from *Pseudomonas stutzeri* WM88
- Substrate binding protein (SBP) dependent ABC importer
- Uptakes PO₂ and PO₃ but not PO₄



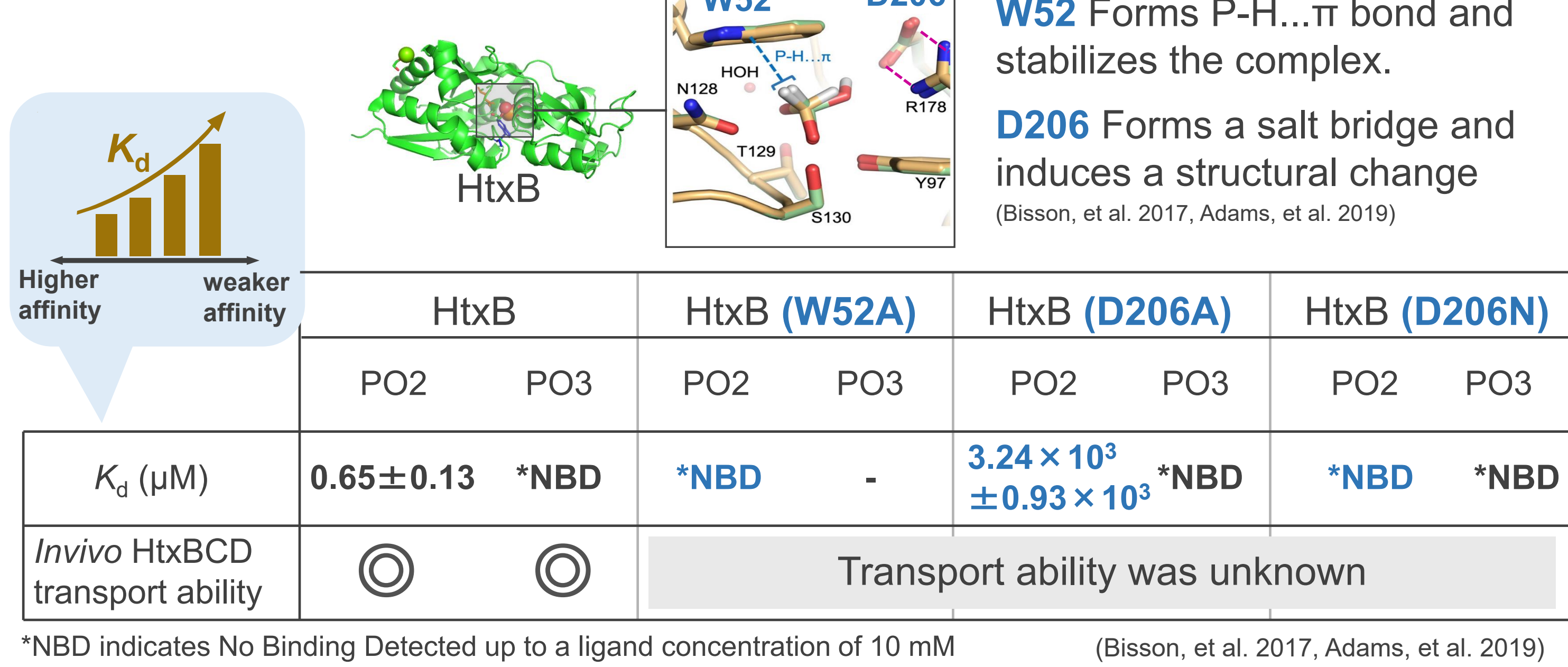
SBP usually determine specificity, but HtxBCDE is an exception

	HtxBCD <i>P. stutzeri</i>	PstSCAB <i>E. coli</i>	MalEFGK ₂ <i>E. coli</i>	ModAB ₂ C ₂ <i>E. coli</i>
Imported substrate	PO ₂ PO ₃	PO ₄	Maltose Maltodextrin	Molybdate
K _d (μM)	0.65 Bisson et al. 2017	*NBD Ledvina et al. 1998	0.8 Machtel et al. 2019	3.0 Hagen et al. 2011

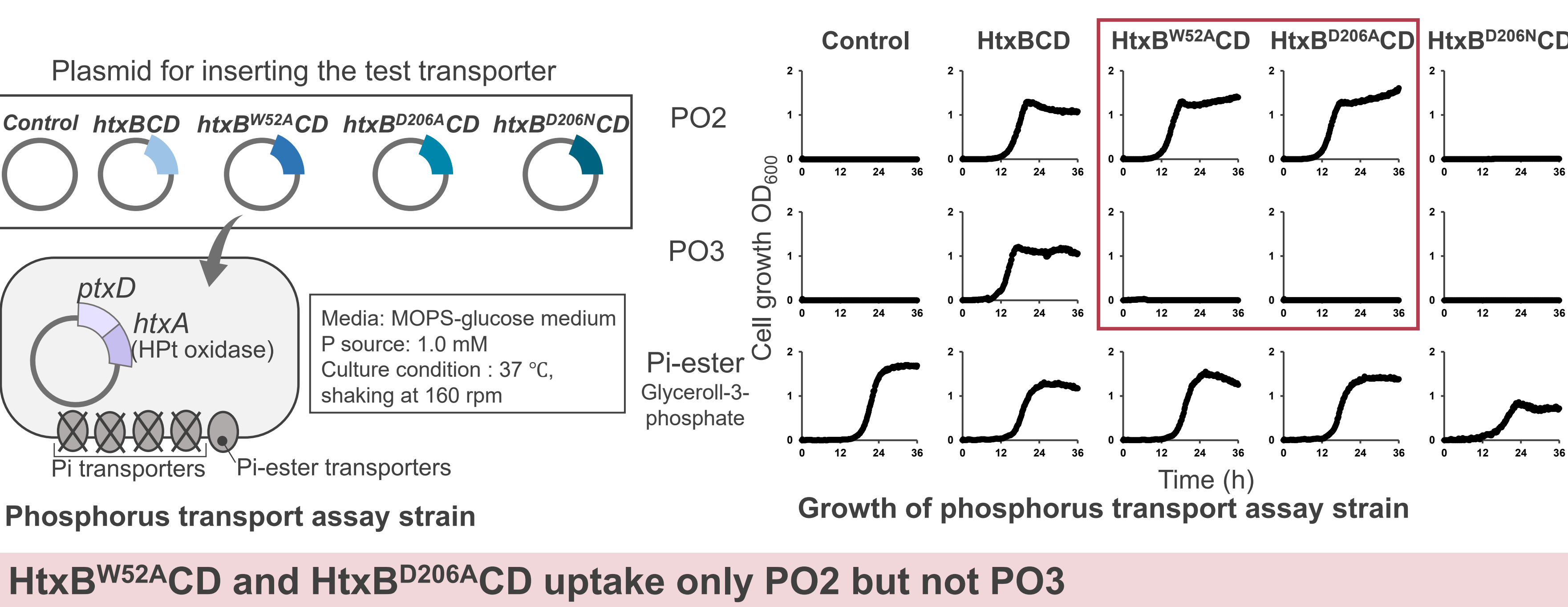
*NBD indicates No Binding Detected up to a ligand concentration of 100 mM

3. Result

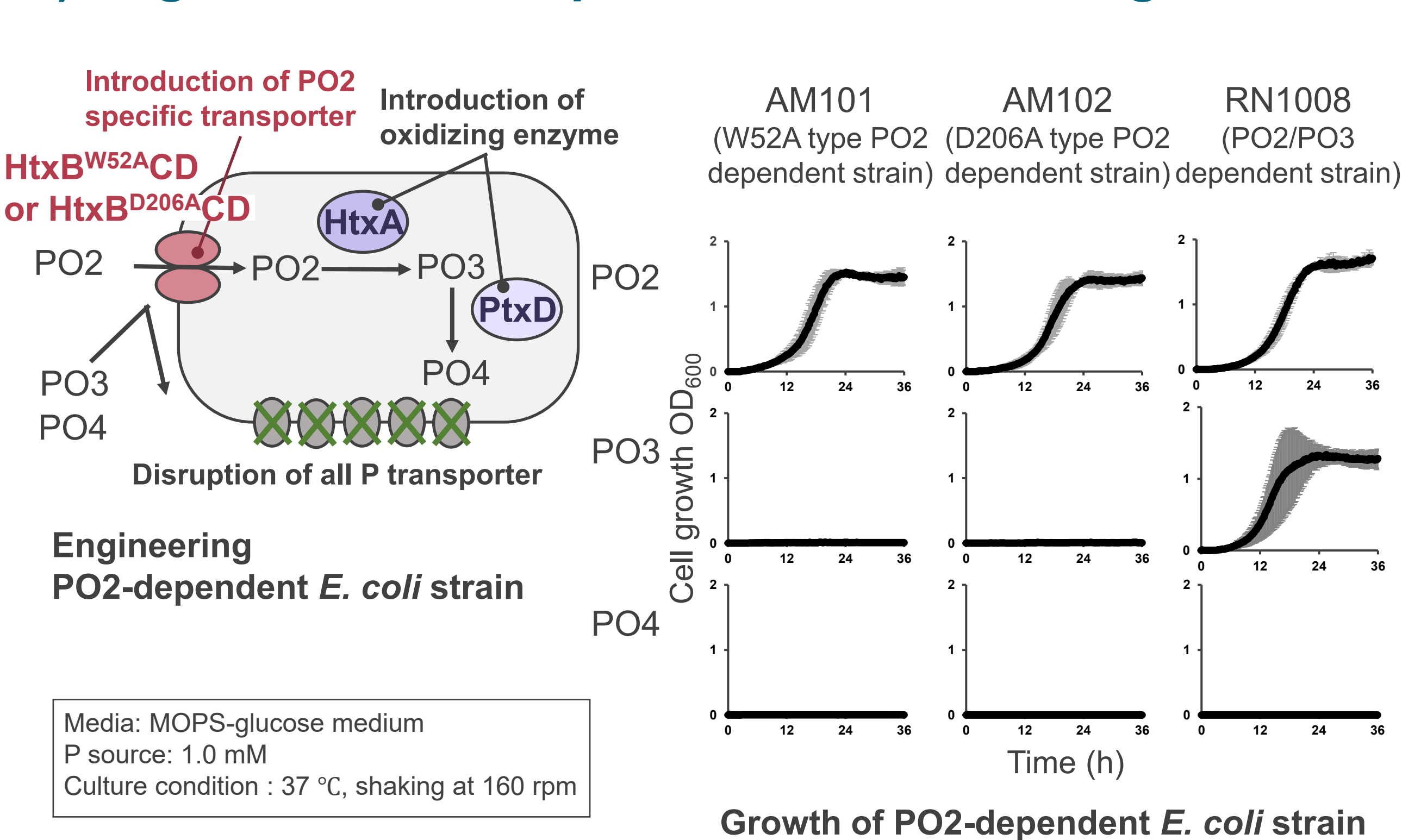
i) D206 or W52 mutation decrease in HtxB binding affinity to PO₂



ii) Transport ability of HtxB^{W52A}CDE, HtxB^{D206A}CDE and HtxB^{D206N}CDE

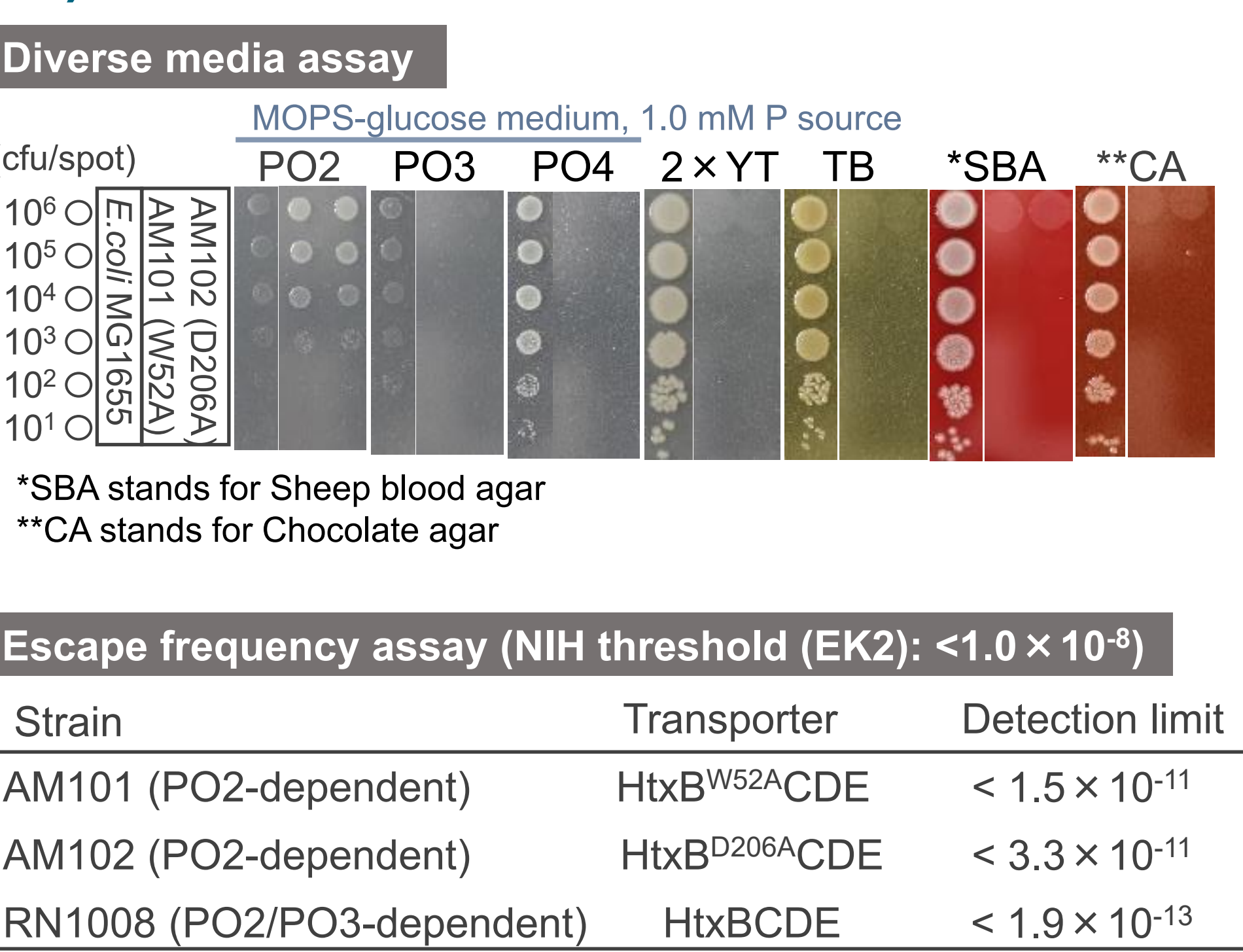


iii) Engineered PO₂-dependent strain and its growth



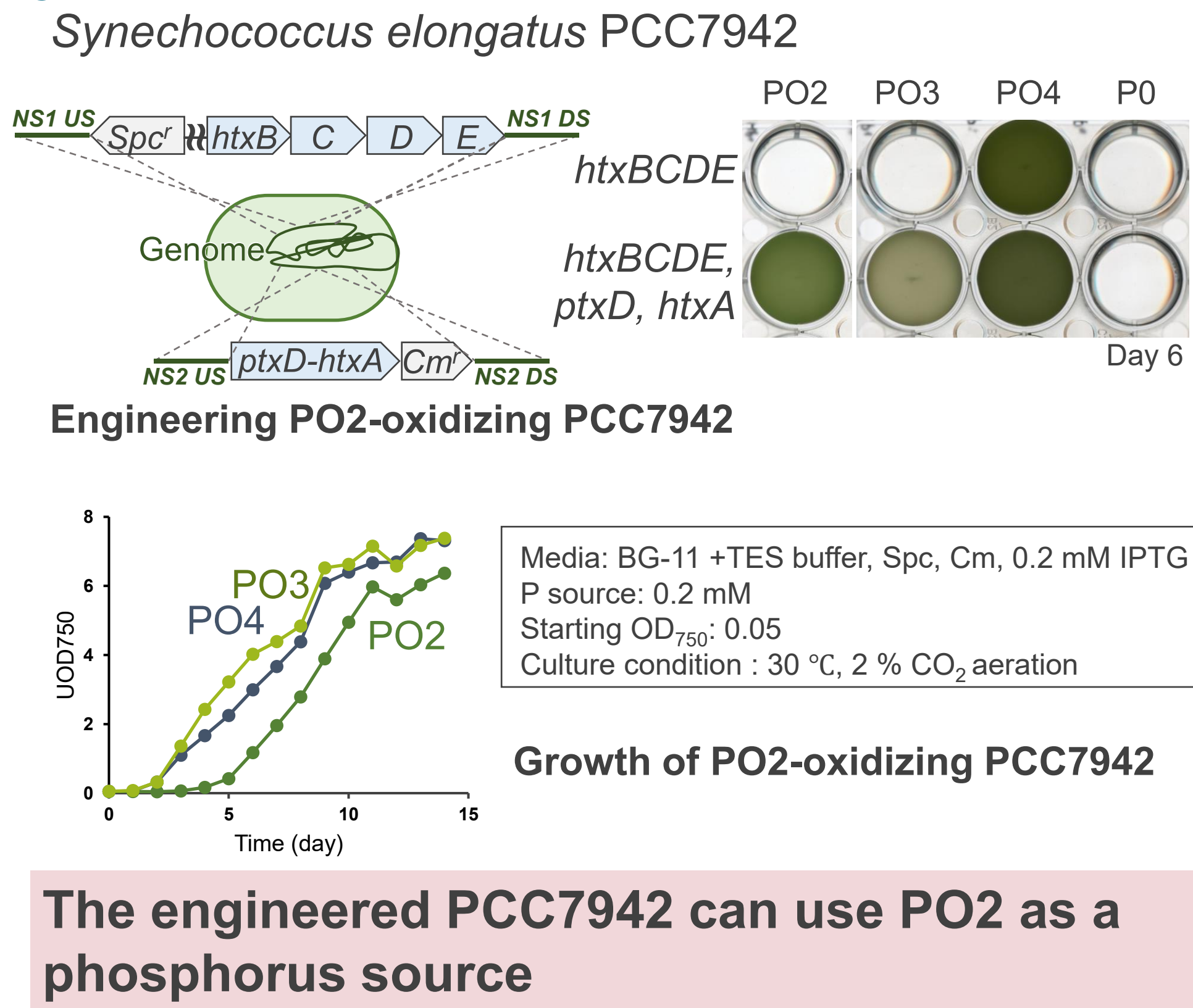
The engineered *E. coli* exhibited strict PO₂-dependency

iv) Biocontainment effect



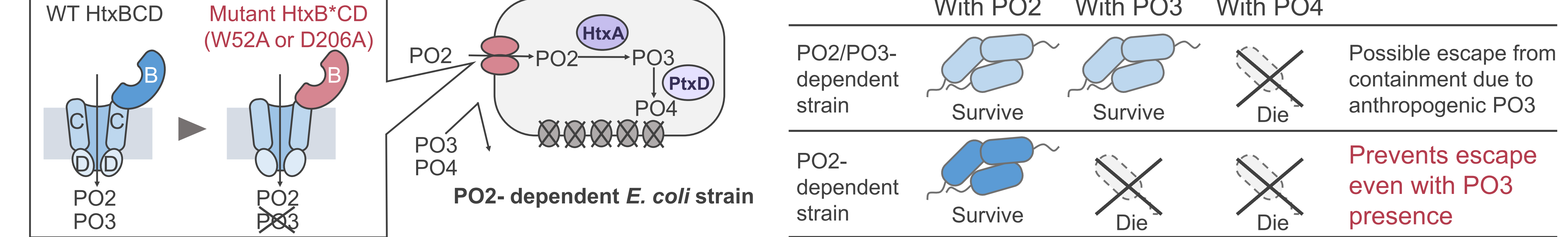
Strict PO₂-dependency and no detectable escape mutants in engineered *E. coli*

v) Engineered PO₂-oxidizing cyanobacterial strain



The engineered PCC7942 can use PO₂ as a phosphorus source

4. Conclusion



Perspective

- Host range expansion (marine cyanobacteria and eukaryotic algae, etc.)
- Investigation of PO₂ and PO₃ present in the environment
- Safety test in a microcosm simulating the environment