湖南農業大學
Hunan Agricultural University

Mechanism of seed endophytic bacteria R5 enhancing cadmium tolerance in rice

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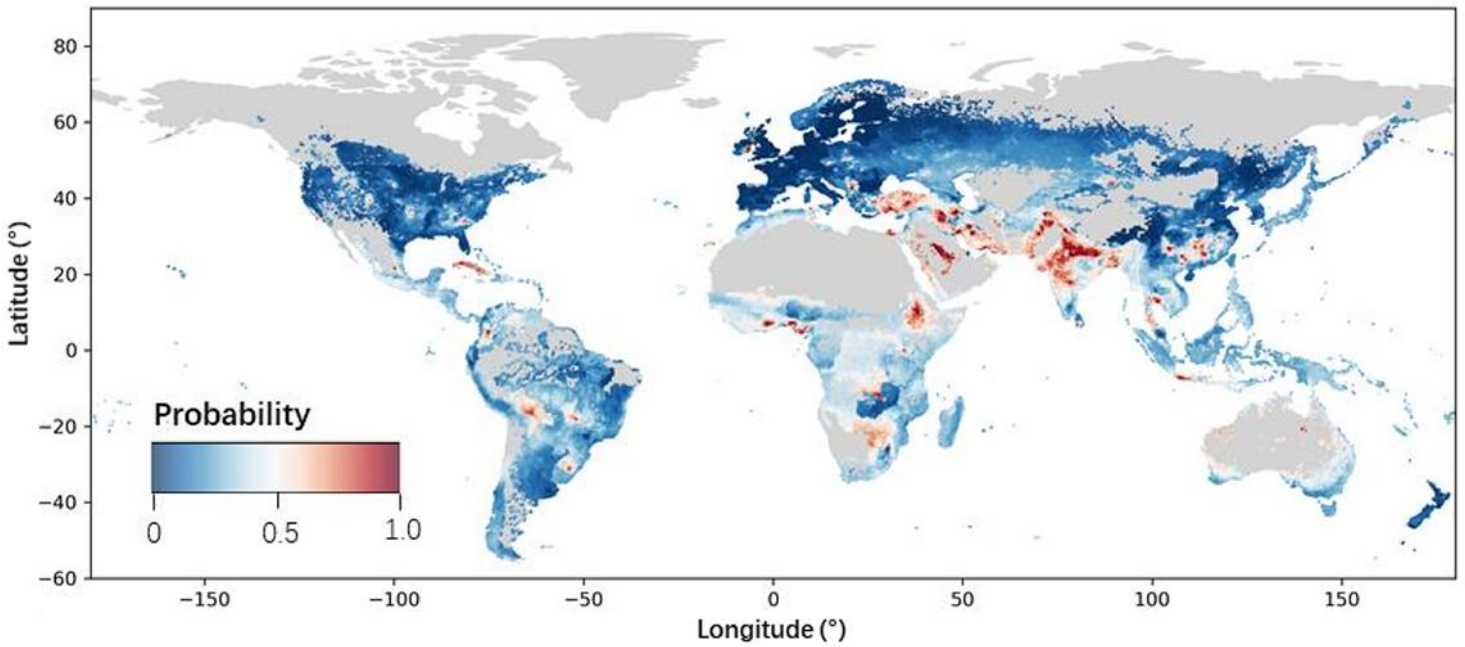
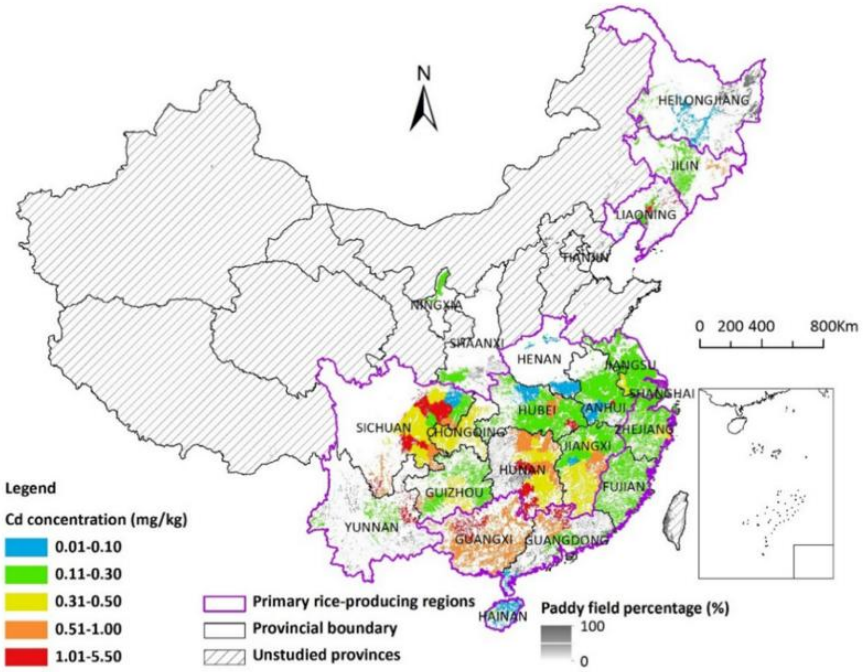
Cadmium (Cd) Pollution Threatens Rice Safety



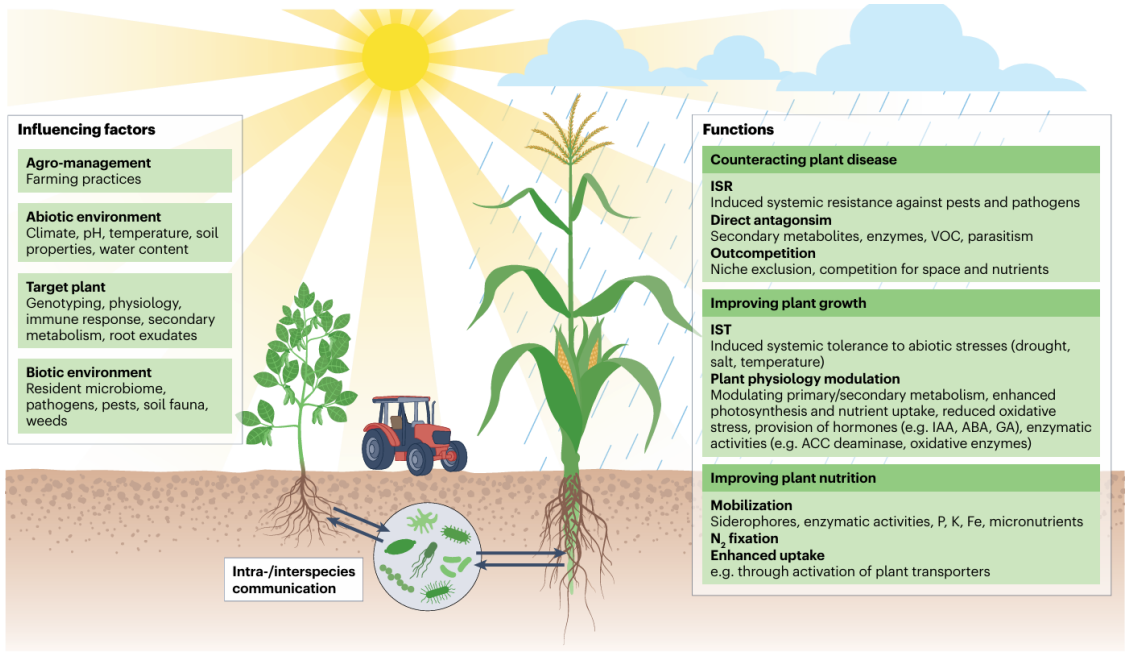
(Hou *et al.*, *Science*, 2025)



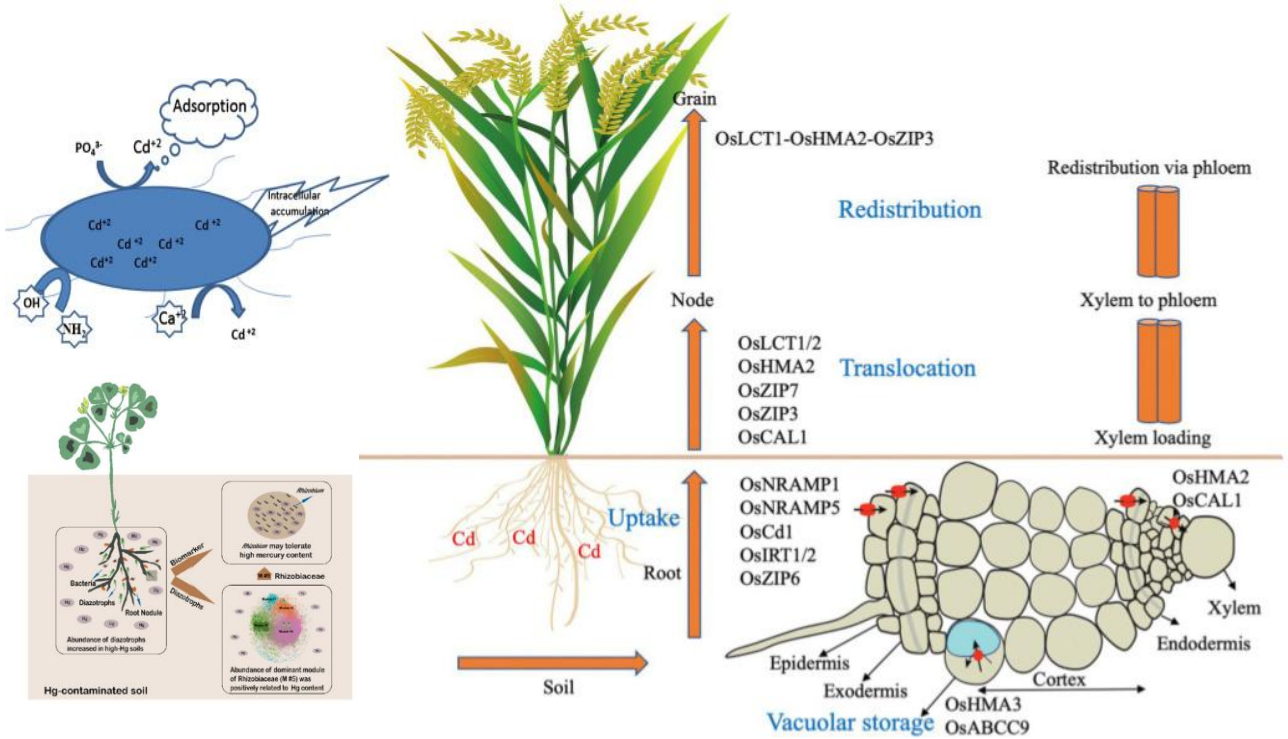
(Liu *et al.*, *Environmental Science & Pollution Research International*, 2016)

- Cadmium (Cd) is a toxic heavy metal in agricultural soils.
- Rice accumulates Cd readily, posing food-safety risks.
- Biological strategies are needed to reduce grain Cd.

How Microbes Can Mitigate Cadmium Stress



(Stéphane *et al.*, *Nature reviews Microbiology*, 2024)



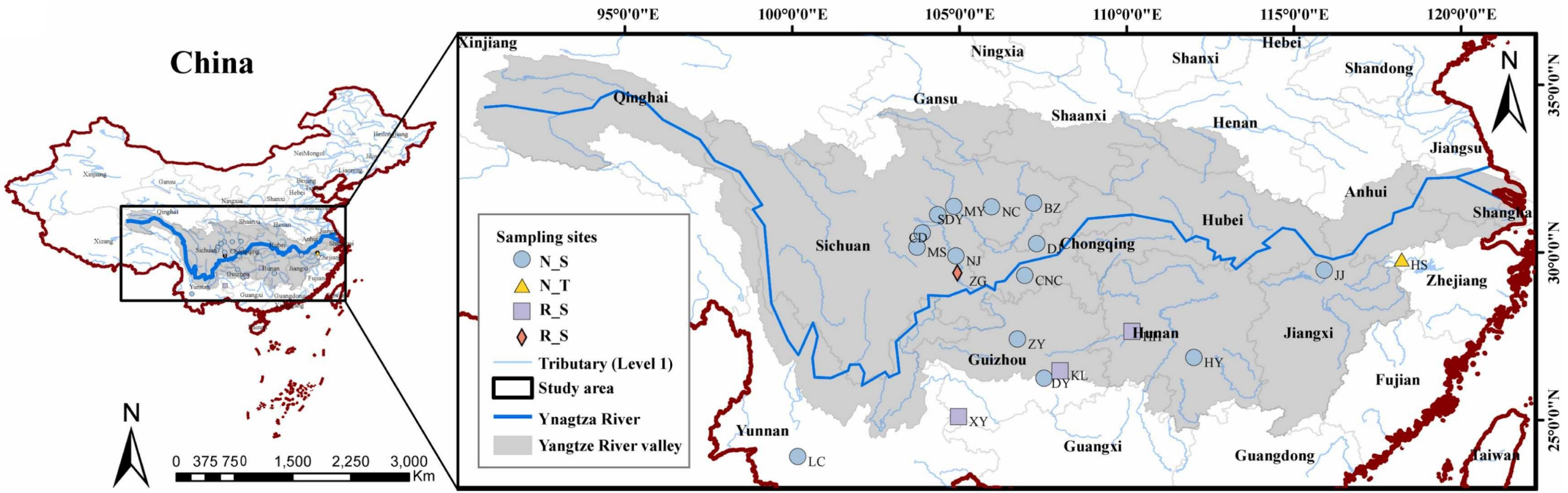
(Hao *et al.*, *Frontiers in Plant Science*, 2022)

- Microbial mechanisms:

  1. Cd immobilization in soil. **2.** Adsorption on microbial surfaces.

  3. Intracellular sequestration / precipitation. **4.** Inhibition or downregulation of plant Cd uptake.

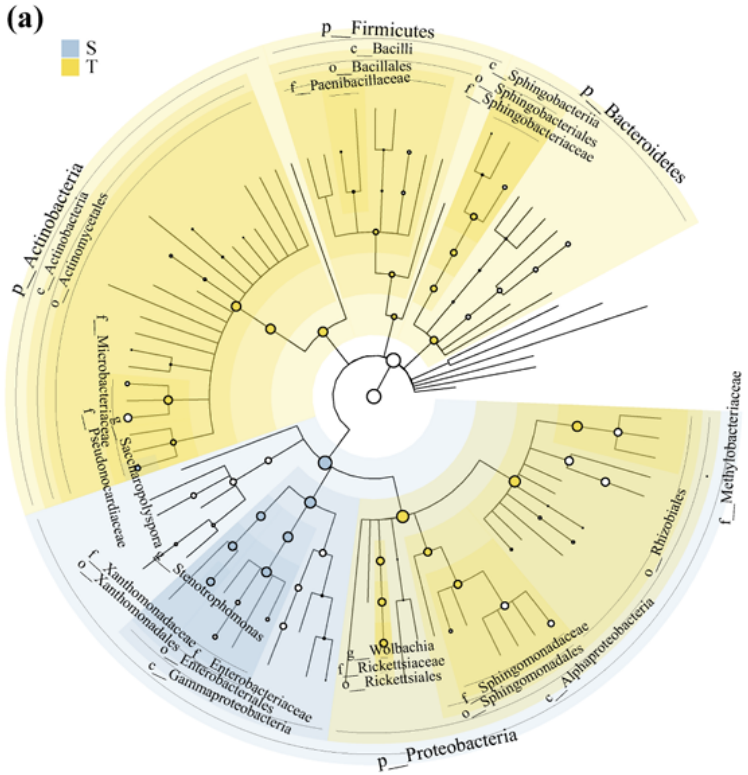
Isolation of Rice Seed Endophytic Bacteria

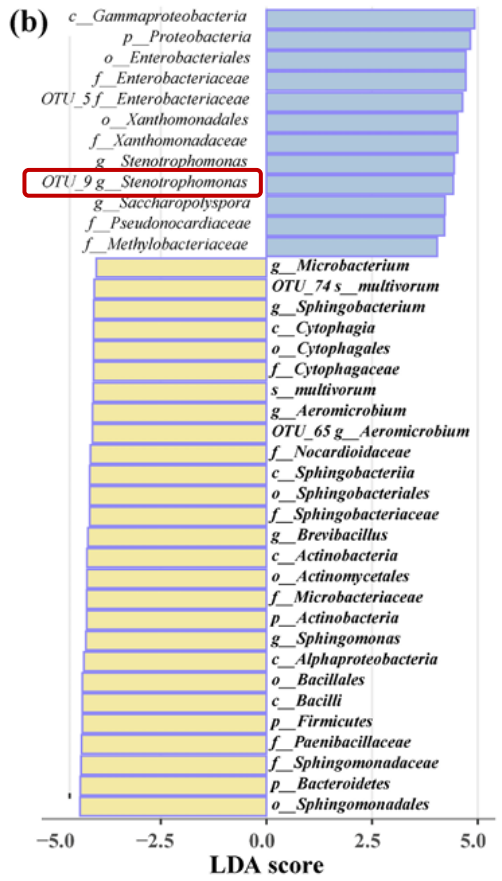


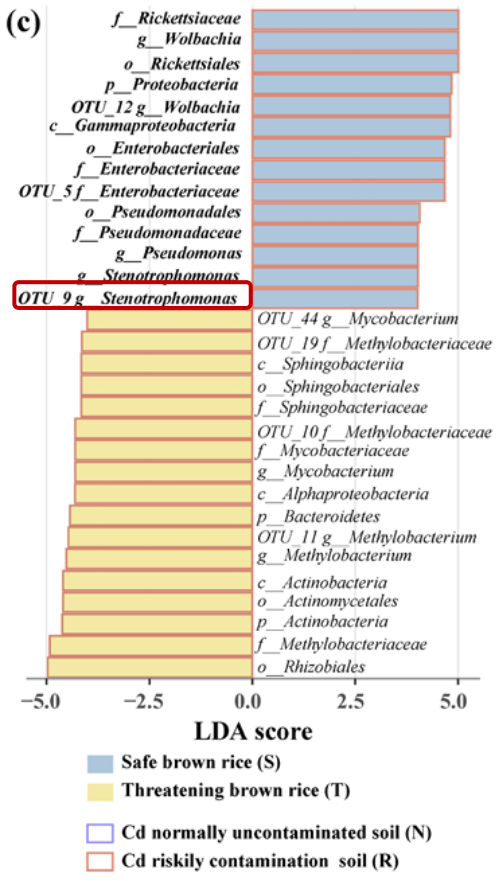
- Rice seeds collected from 19 locations in Yangtze River Basin.
- Endophytic bacteria isolated and cultured.
- Screening for Cd-related functional strains.

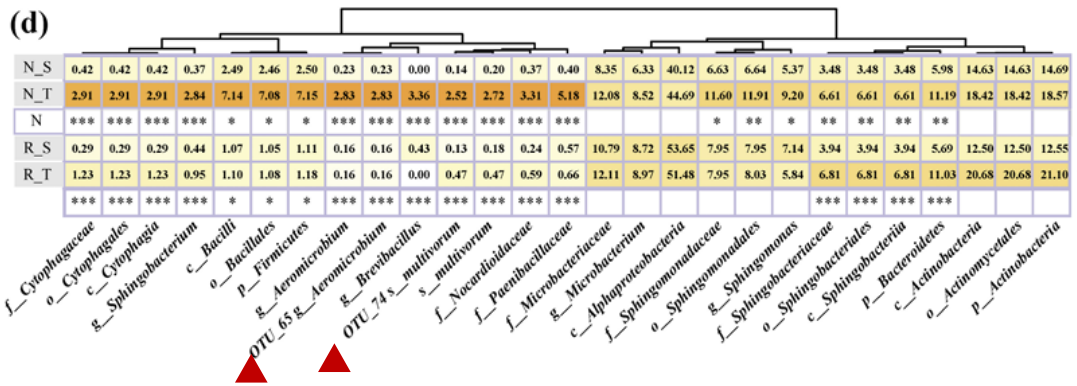
(Wang *et al.* , Ecotoxicology and Environmental Safety, 2023)

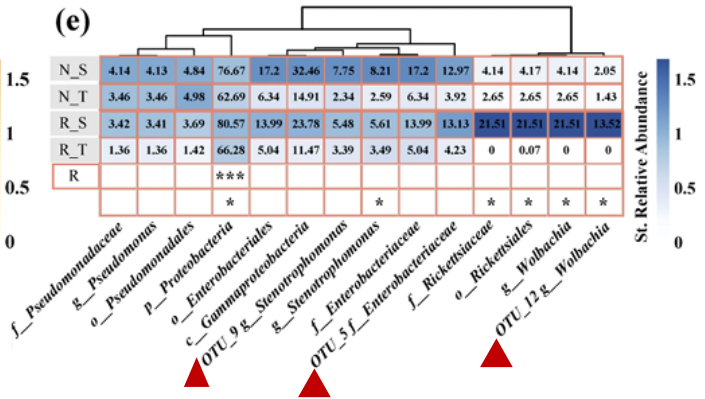
Screening Cadmium-related Endophytic Bacteria





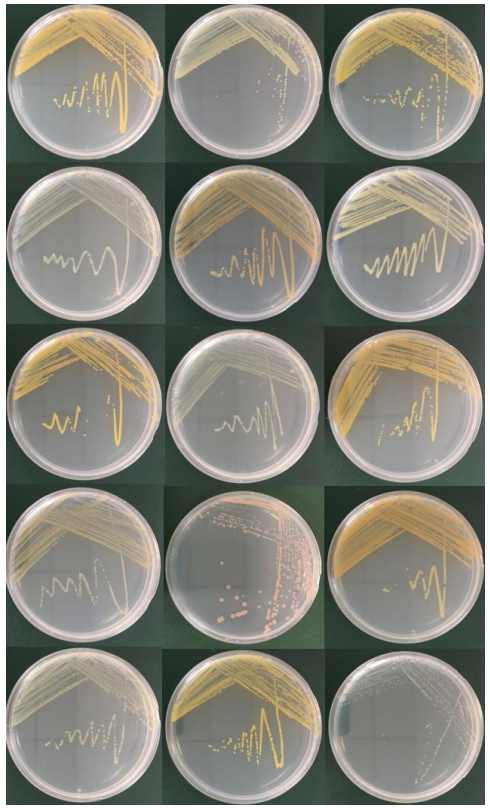




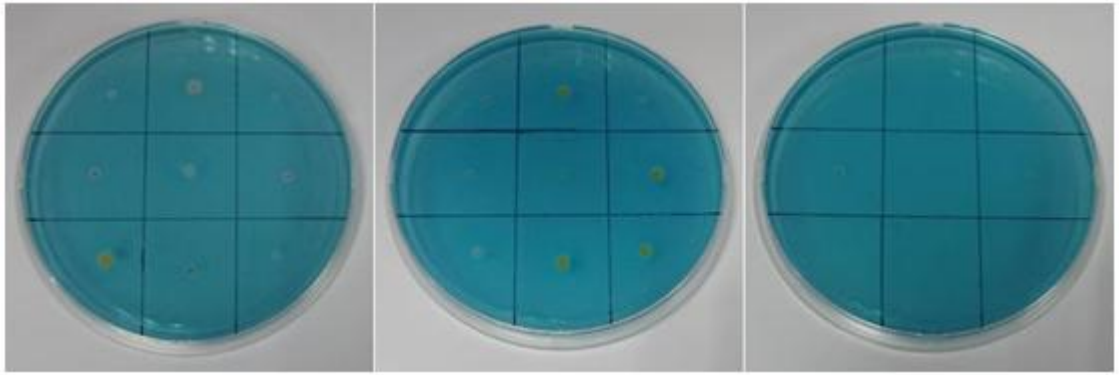


- The key taxa that are resistant to cadmium risks in soil and rice were identified.
- *Stenotrophomonas* significantly accumulated in both cadmium-uncontaminated and cadmium-contaminated farmlands, resulting in rice cadmium levels that did not exceed the acceptable limit.

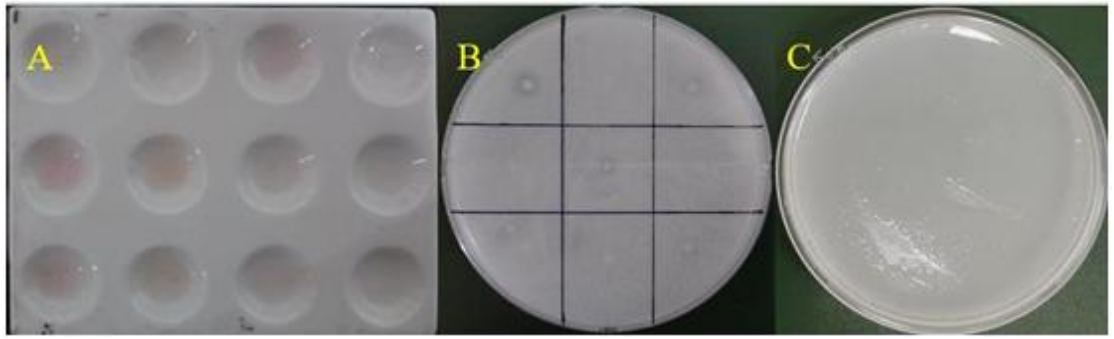
Functional Validation of Candidate Strains



The morphology of endophytic bacteria in rice



Function identification of iron carrier of strain



Qualitative detection of endogenous bacteria growth promotion ability

The ability of endophytic bacteria to produce iron carrier

Number	CD(mm)	HD(mm)	HD/CD	Number	CD(mm)	HD(mm)	HD/CD
D1	1.0	0.0	0.00	N3	1.3	0.0	0.00
D2	1.0	0.0	0.00	N4	1.2	0.0	0.00
D3	0.9	0.0	0.00	N5	1.1	0.0	0.00
D4	1.2	0.0	0.00	R1	2.0	3.0	1.50
L1	1.1	0.0	0.00	R2	1.0	2.0	2.00
L2	1.6	0.0	0.00	R3	1.0	3.0	3.00
L3	2.1	0.0	0.00	R4	1.5	0.0	0.00
L4	2.5	3.0	1.20	R5	1.5	4.8	3.20
L5	0.8	2.5	3.13	T1	1.2	2.0	1.67
L6	1.0	2.0	2.00	T2	1.8	2.5	1.39
N1	1.4	2.0	1.43	T3	1.3	0.0	0.00
N2	1.0	2.0	2.00	T4	0.8	4.5	5.63

Note: "+" : CD represents the diameter of the colony, HD represents the diameter of the yellow halo, and HD/CD represents the iron-transporting ability of the strain.

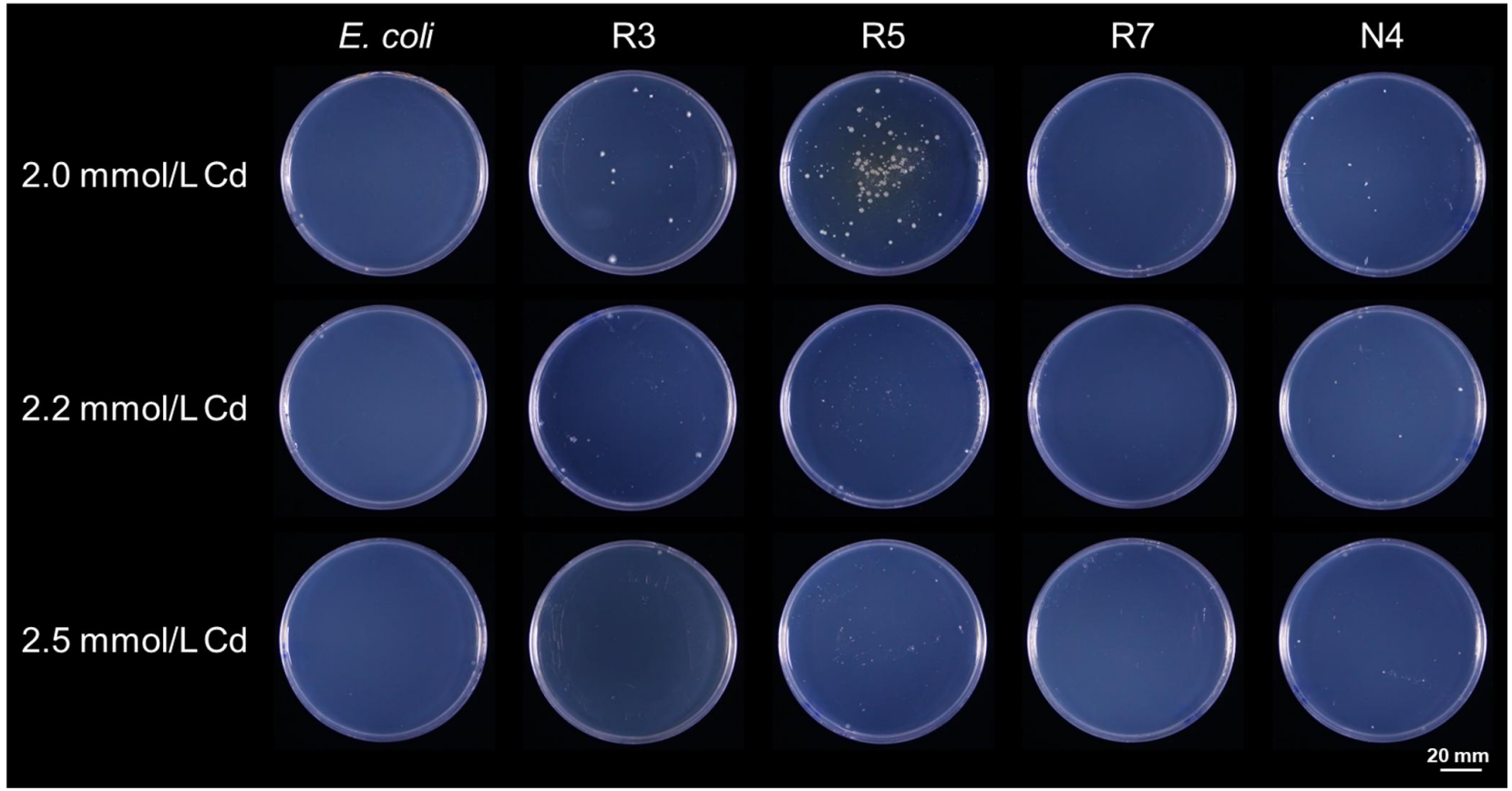
Analysis of the growth-promoting ability of strains

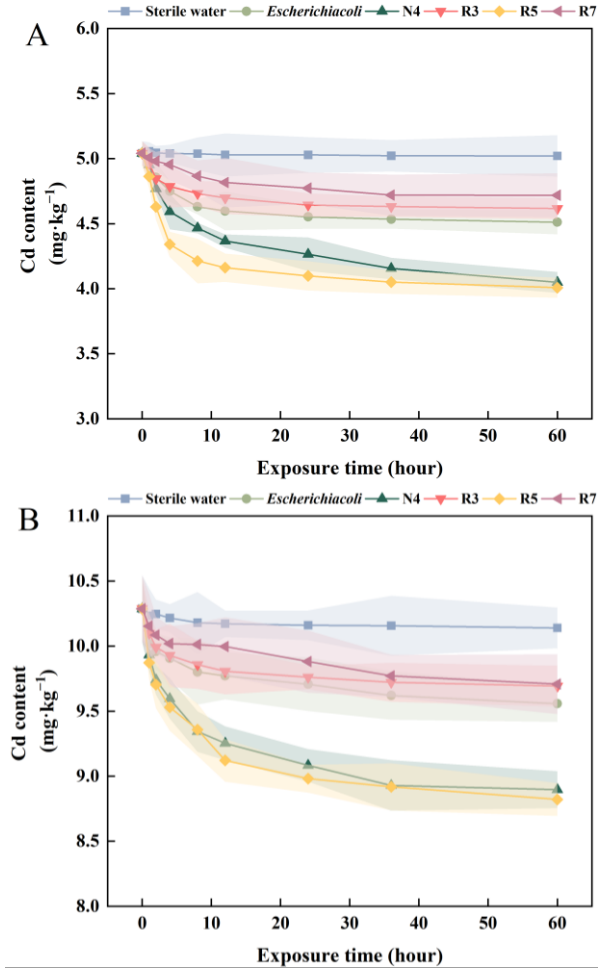
Number	Release IAA	Phosphorus dissolution	Nitrogen fixation	Number	Release IAA	Phosphorus dissolution	Nitrogen fixation
D1	+	-	-	N3	+	-	-
D2	-	-	-	N4	-	-	-
D3	+	-	-	N5	-	-	-
D4	-	-	-	R1	+	1.50	-
L1	+	1.32	-	R2	+	-	-
L2	-	-	-	R3	+	2.01	+
L3	+	-	-	R4	-	-	+
L4	+	-	-	R5	+	1.87	+
L5	-	-	-	T1	+	2.33	-
L6	-	-	+	T2	+	-	-
N1	+	1.33	-	T3	+	-	-
N2	-	-	-	T4	-	-	+

Note: "+" : This function is available, "-" : This function is not available

- Key candidates: R3 (*Herbaspirillum*), N4 (*Chryseobacterium*), R5 (*Stenotrophomonas*) and R7 (*Pantoea*).

Functional Validation of Candidate Strains





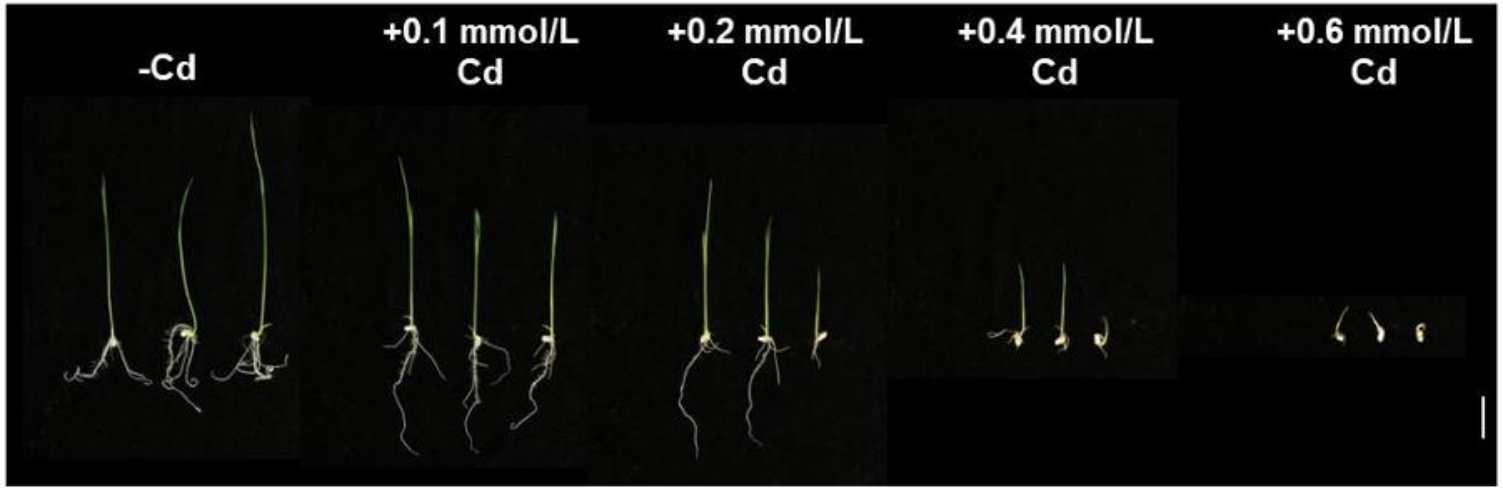
- R3, R5, and N4 exhibit greater cadmium tolerance.
- N4 and R5 can significantly reduce cadmium concentrations in aquatic environments.

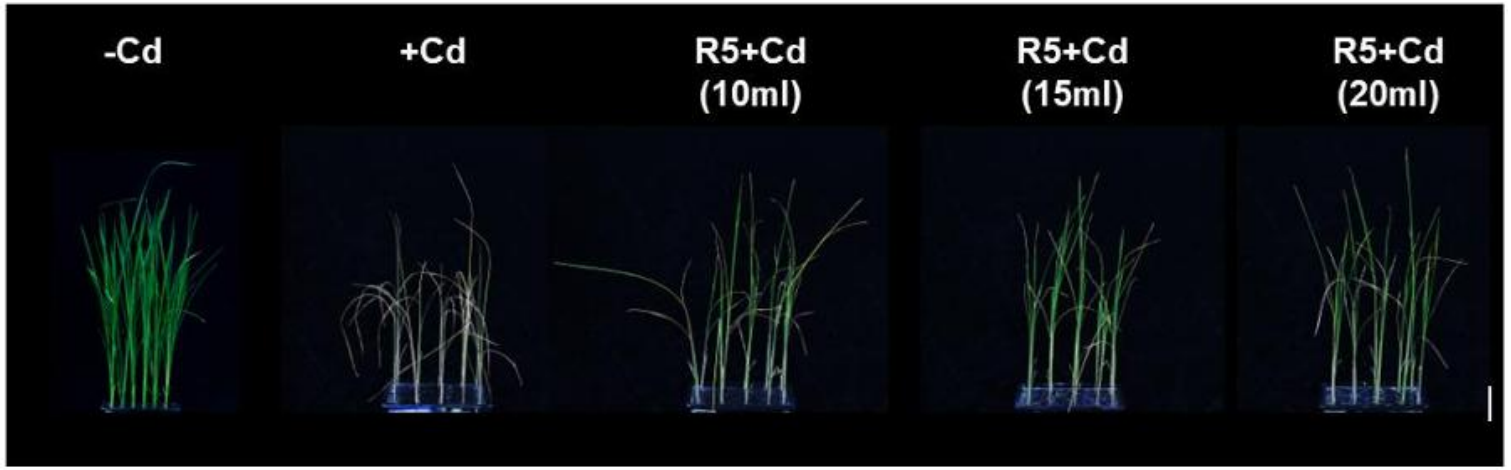
R5 Reduces Bioavailable Cd in Rhizosphere Soil

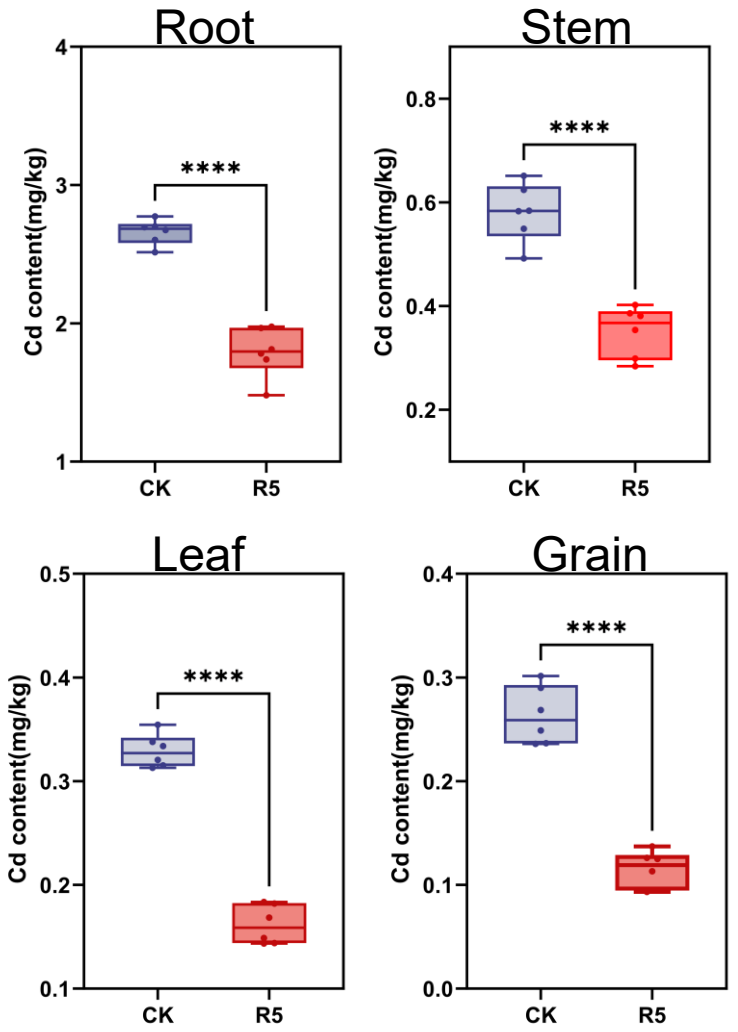
Treatment	Cd content（mg·kg^{-1}）				
	Total Cd	Aci-Cd	Re-Cd	Ox-Cd	Res-Cd
CK	1.28±0.014a	0.68±0.020a	0.24±0.010a	0.09±0.004c	0.27±0.011d
N4	1.27±0.018a	0.58±0.016c	0.19±0.013c	0.11±0.006ab	0.40±0.016a
R3	1.27±0.018a	0.66±0.022a	0.22±0.016b	0.09±0.010c	0.31±0.021c
R5	1.27±0.015a	0.60±0.022b	0.22±0.017b	0.11±0.011a	0.34±0.020b
R7	1.29±0.026a	0.70±0.007a	0.20±0.010c	0.09±0.005bc	0.29±0.012cd

- R5 and N4 significantly decreased acid-extractable Cd (Aci-Cd) in rhizosphere soil.

R5 Improves Rice Growth and Reduces Cd Accumulation

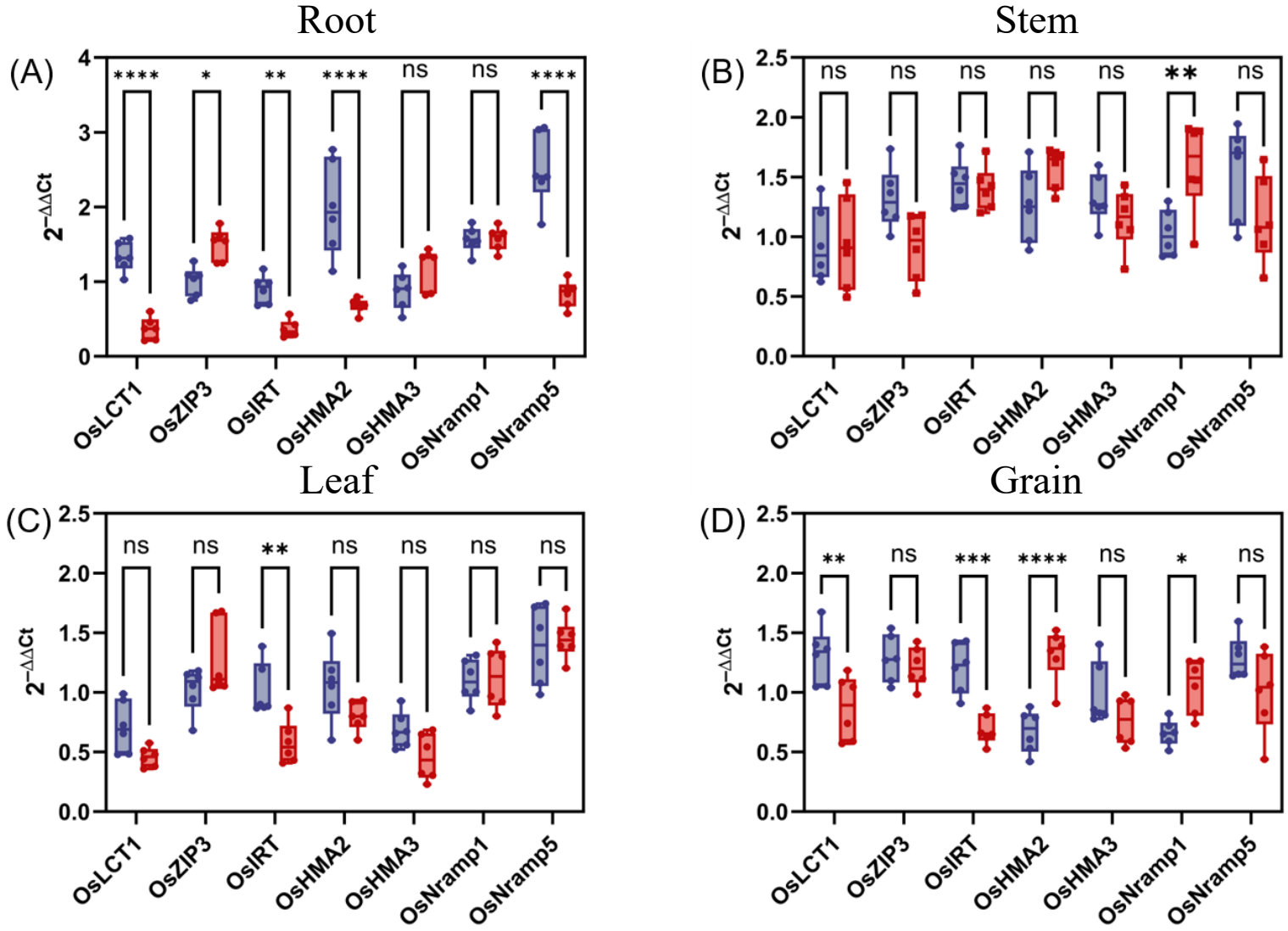






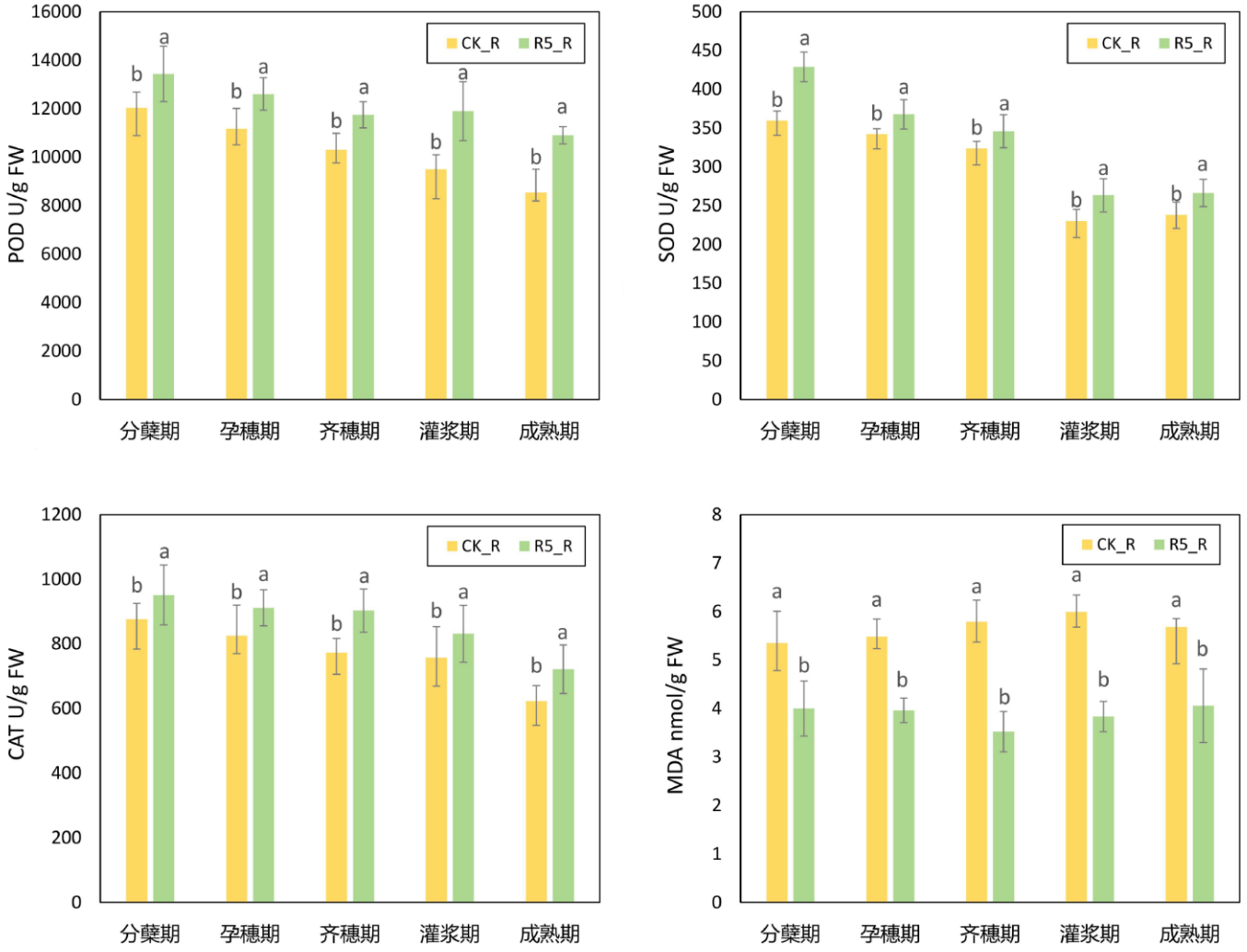
- R5-inoculated seedlings show better root and shoot growth under Cd exposure.
- Grain: 0.26 → 0.11 mg/kg (below national limit 0.20 mg/kg).

R5 Downregulates Cd Transporter Genes



- Significant repression of: *OsLCT1*, *OsIRT*, *OsHMA2*, *OsNramp5* (roots, stems, leaves, grains depending on tissue).

R5 Enhances Antioxidant Defense



- Increased SOD, POD, CAT activities in roots.
- Reduced MDA levels (less membrane lipid peroxidation).

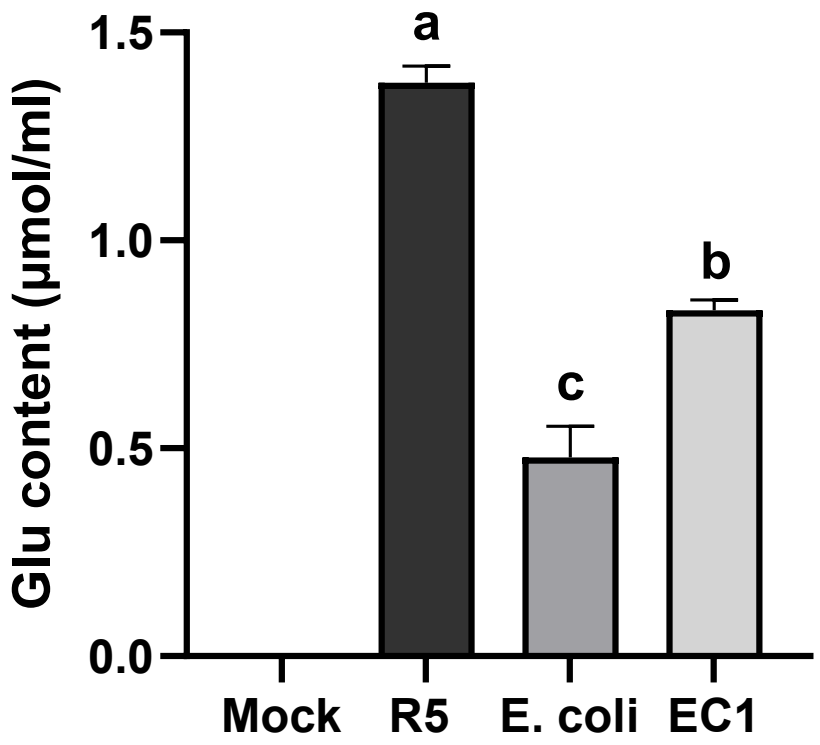
Genomic Insights from Stenotrophomonas sp. R5

Potential Cd-resistant-related genes of the *Stenotrophomonas* sp. R5

Gene/Operon	Copy number	Expression products	Gene/Operon	Copy number	Expression products
Metal ion efflux system			**Oxidation irritable reaction**		
cadAR	2	P-type ATPase	*sodB(Fe)*	1	SOD
czcCBA	3	Cation-Proton Antiporter	*sodC(Cu-Zn)*	1	SOD
czcD	4	CDF	*ahp/Osm/Ohr*	4	AHP
Precipitation reaction			*gltB*	2	**Glutamate synthase**
cysPUWA	1	SulT	*kat*	3	CAT
cysD	1	SAT	*gshAB*	1	GSS
cysH	1	APS reductase	*gst*	9	GST
cysJI	1	SiR	*nqo*	1	NAD
acp	3	ACP	*rdx*	1	Rubredoxin

- Multiple putative Cd-resistance genes (efflux, sequestration).
- Presence of ***gltB*** — glutamate synthase (Glu production).

R5 Elevates Root Glutamate Levels



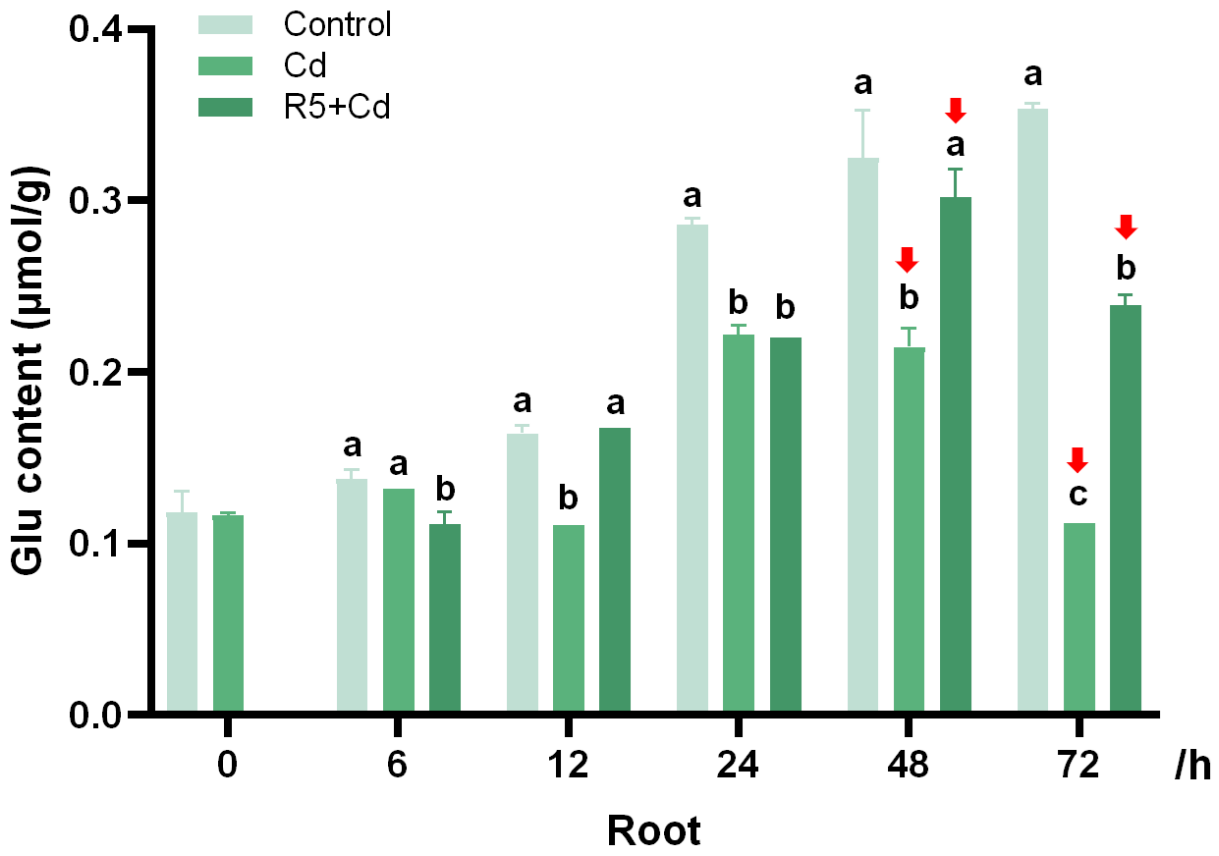
R5: 2.0*10^6 CFU/ml

E. coli: 2.0*10^6 CFU/ml

EC1: 2.0*10^6 CFU/ml

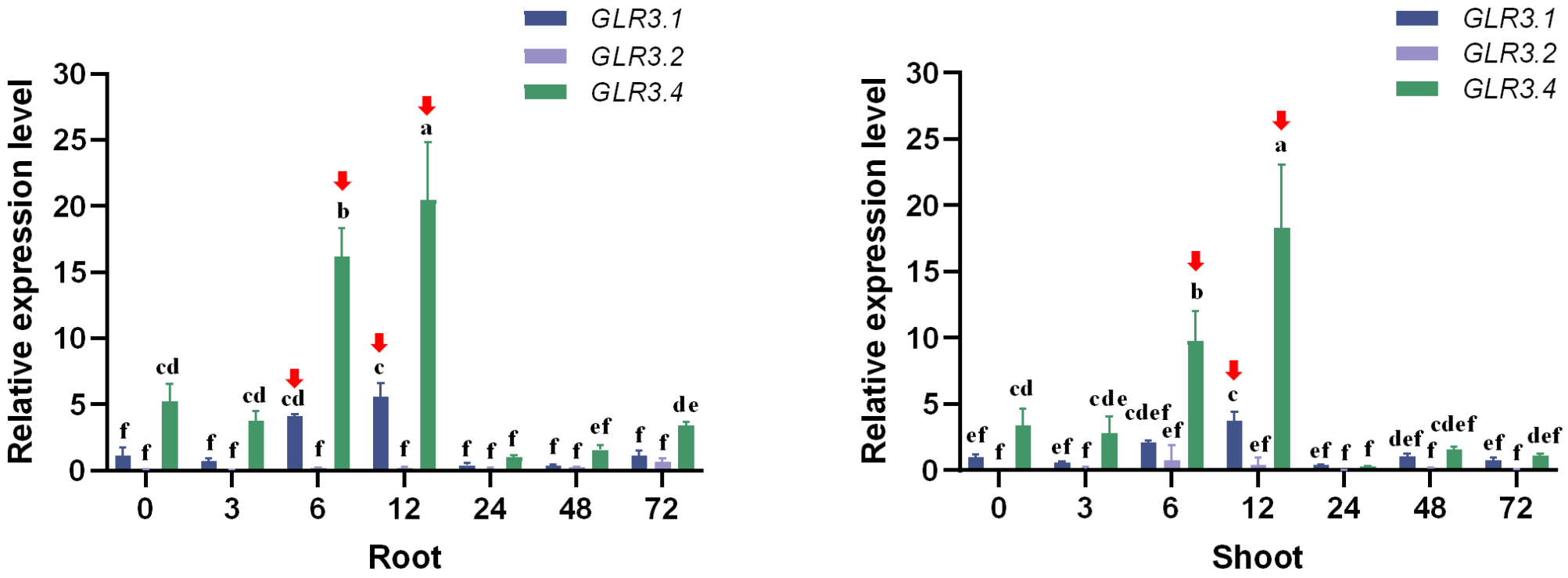
- The R5 strain produces a higher level of glutamate.

R5 Elevates Root Glutamate Levels



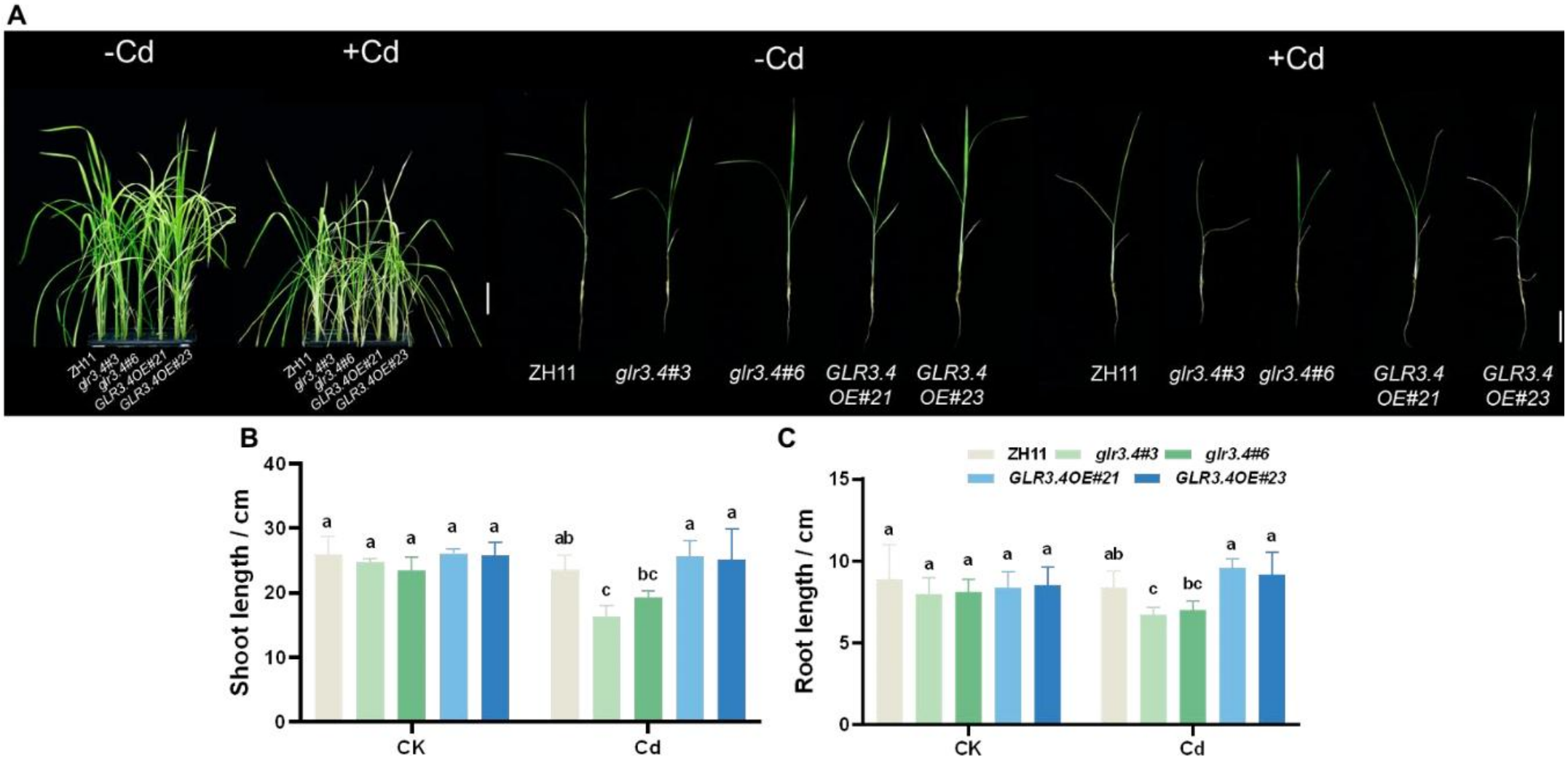
- Cd stress reduces root Glu after 24h.
- R5 inoculation restores/increases root Glu levels.

Glutamate Receptor Genes Are Induced by Cd



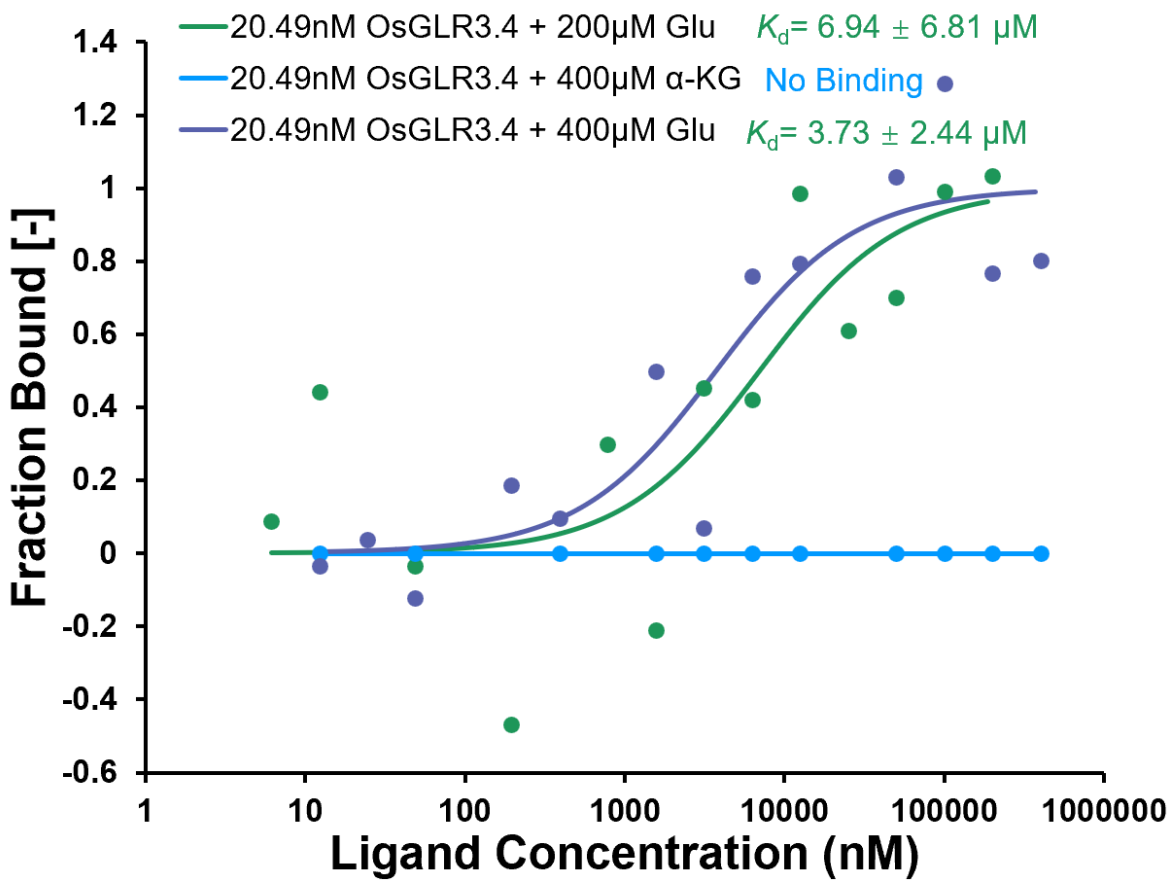
- ***OsGLR3.1 a***nd ***OsGLR3.4*** are induced at 6–12 h after Cd exposure.
- ***OsGLR3.4*** induction > ***OsGLR3.1***, root expression > shoot.

OsGLR3.4 Positively Regulates Cd Tolerance



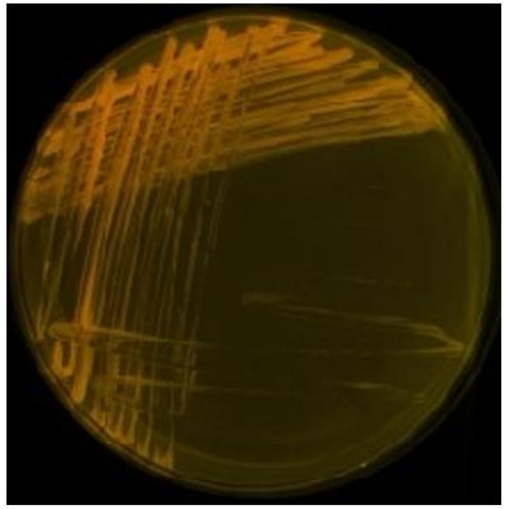
- GLR3.4 mutant: more sensitive to Cd.
- GLR3.4 overexpression: enhanced Cd tolerance

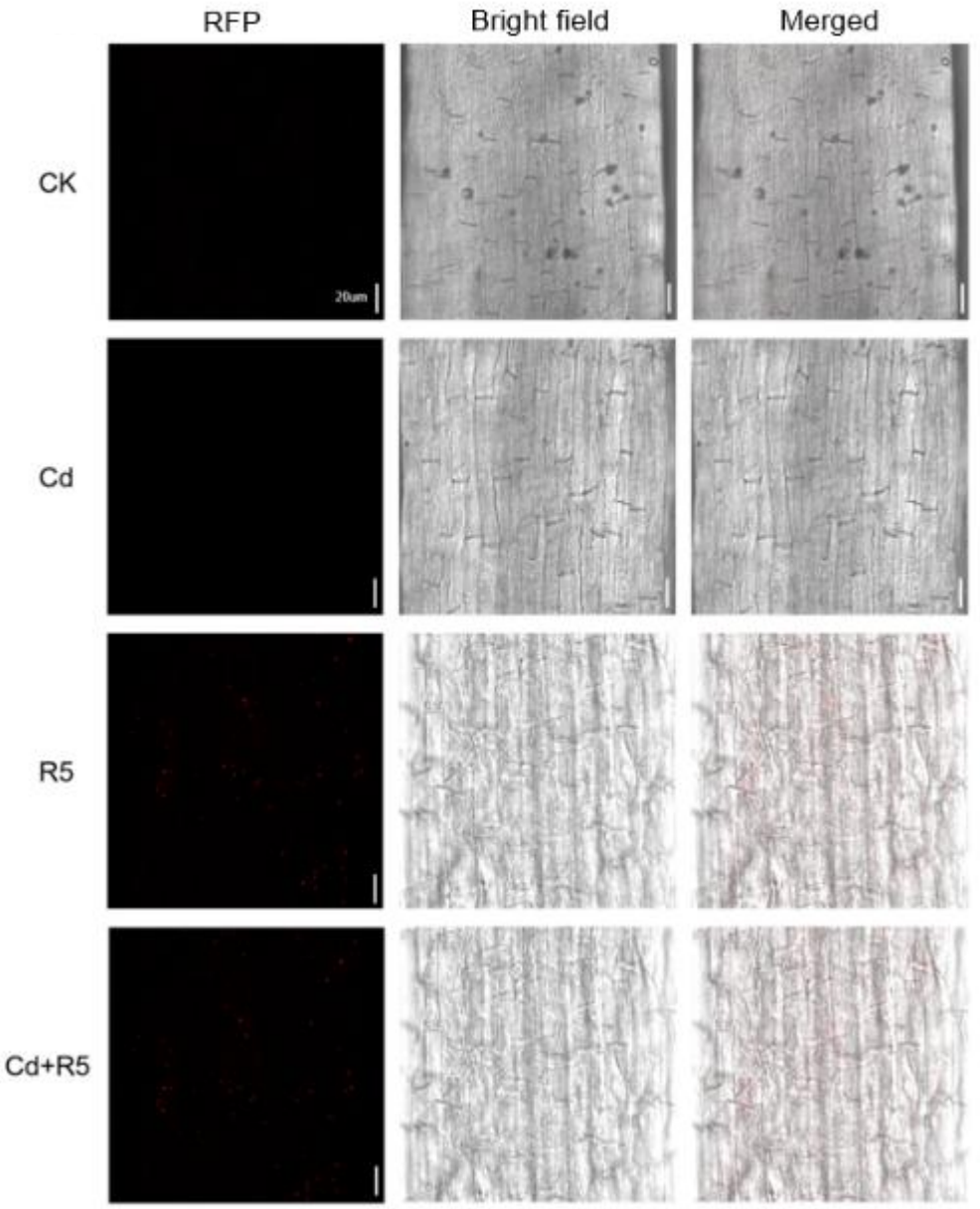
Specific Binding of Glutamate to OsGLR3.4



- MST assay confirms specific Glu–GLR3.4 binding.

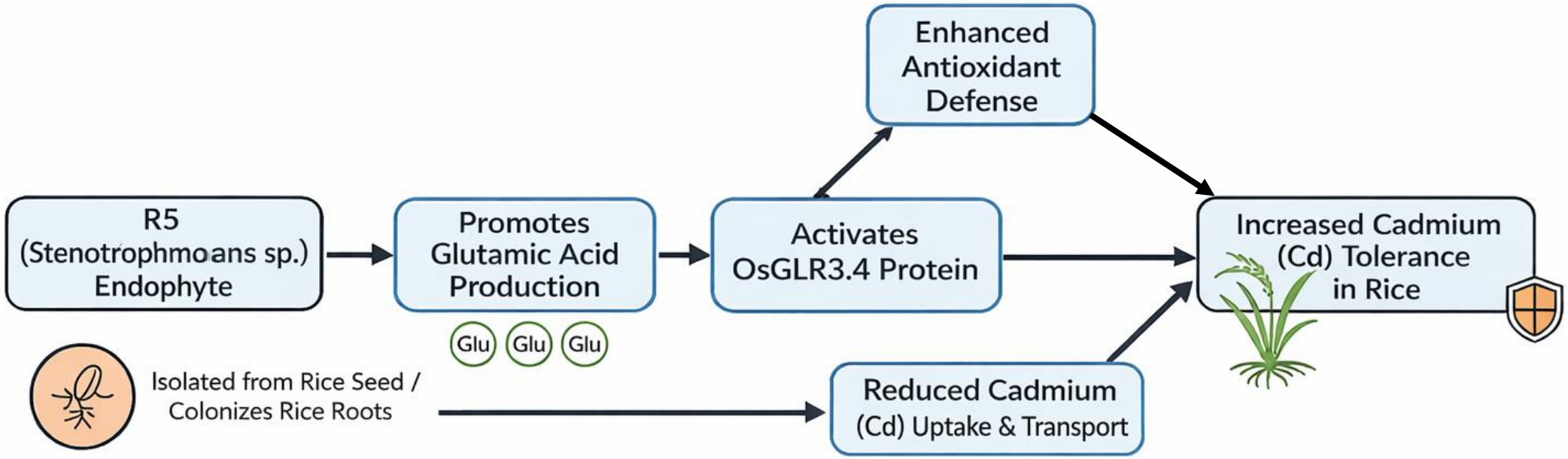
R5 Successfully Colonizes Rice Roots





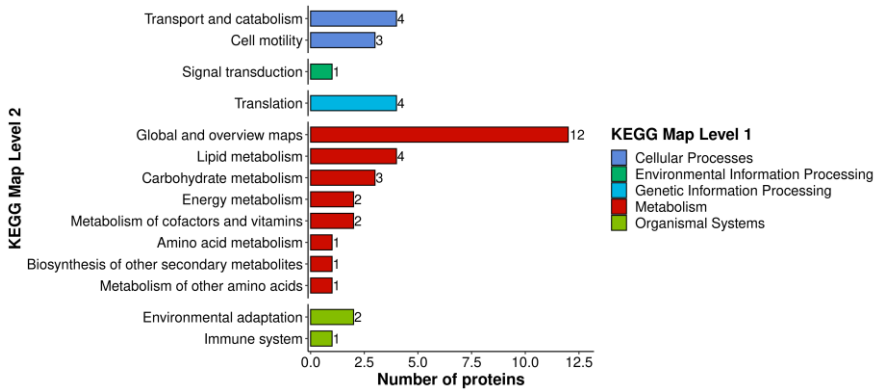
- Fluorescently labeled R5 observed in root tissues by confocal microscopy.

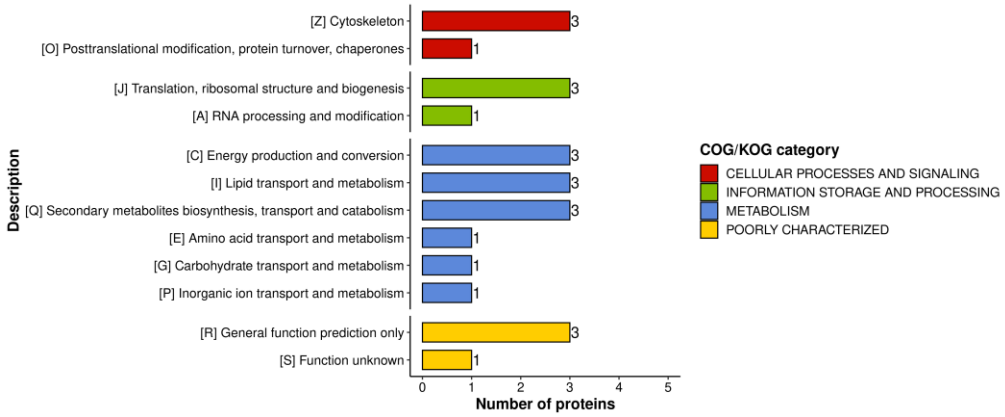
Proposed Mechanism: R5 → Glu → GLR3.4 → Antioxidant Response

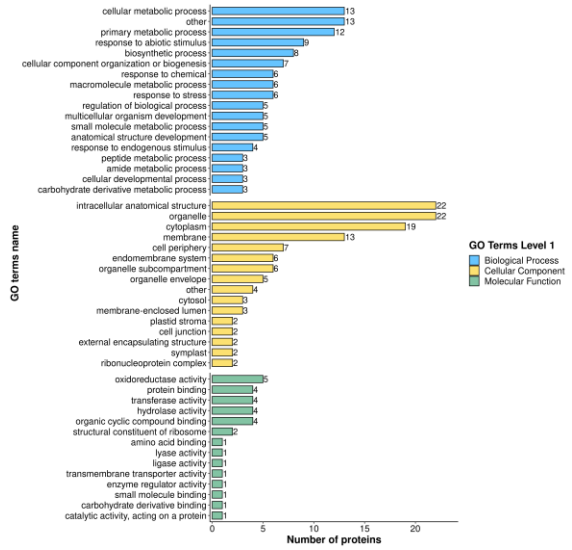


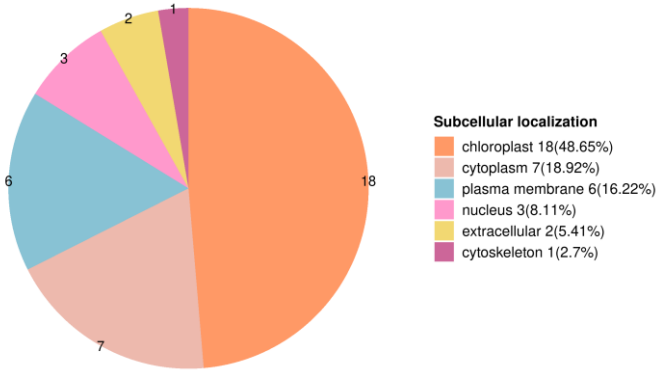
- R5 produces Glu → binds GLR3.4 → activates Ca^{2+}/signal cascades → upregulates antioxidants & downregulates transporters → reduced Cd uptake & damage.

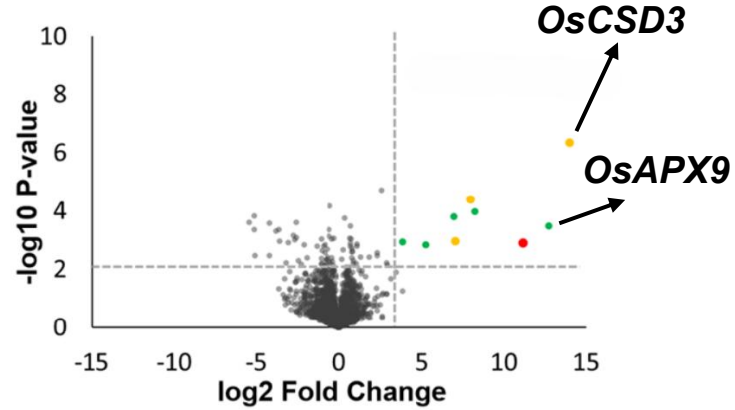
OsGLR3.4 Interacting Proteins (Co-IP/MS)







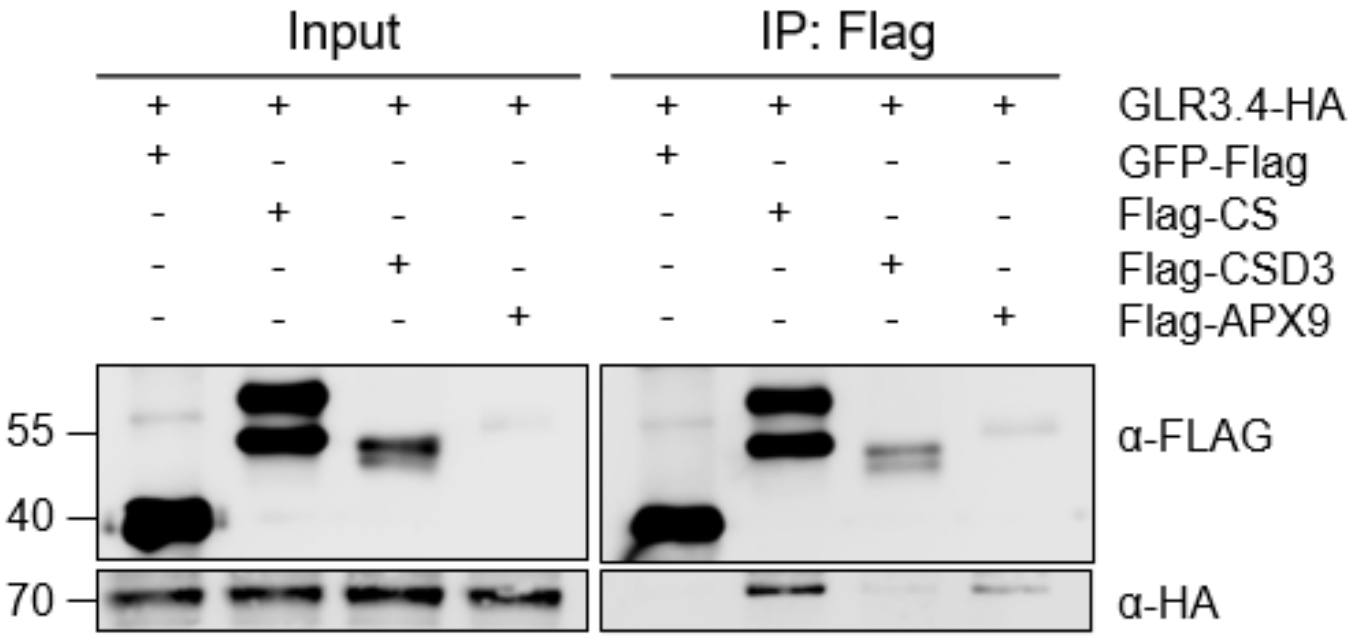




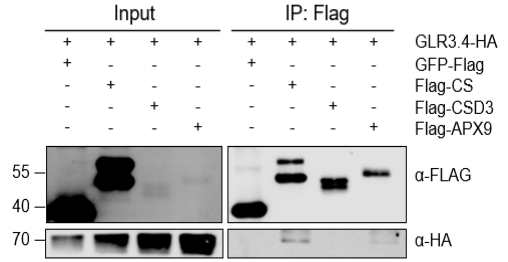
- Co-IP/MS identified candidate interactors — *OsCSD3*, *OsAPX9* (and others).
- Enrichment of redox and signaling-related proteins.

Validation of Candidate Interactors

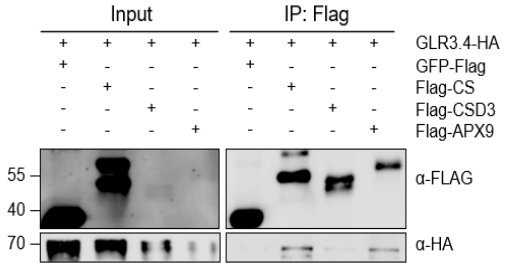
1st



2nd



3rd



- Co-IP confirmed GLR3.4 association with OsCSD3 and OsAPX9.
- BiFC assays are in progress for cellular localization.

Key Findings

1. R5 lowers soil bioavailable Cd and reduces plant Cd accumulation.
2. R5 increases root glutamate, *OsGLR3.4* is induced and functional.
3. Glu–OsGLR3.4 activation links to antioxidant upregulation and transporter repression.

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