Table S1 Selective chemical properties of the soil samples used in this study

Sample	Mn	As	Cu	Zn	Mo	Cr	Cd	Fe	EC	pH
	mg/kg							g/kg	μs/cm	
YKS	173	916	21	317	938.9	36	0.40	71	398	4.78
LCS	513	18	19	71	3.1	74	0.19	25	387	7.46
AXS	752	115	124	159	2.4	85	2.73	36	273	8.13
HXS	285	7	10	32	0.3	28	0.08	14	2420	7.75

Table S2 Descriptions of the open reading frames (ORFs) identified from the Cu resistance clones

ID Clones (Length (bp)/ % GC)	ORF No.	ORF Length (aa)	BlastP Best Match			
			Most Similar Protein (organism)	Accession No.	Predicted Function	% Identity
CR1（1450 / 65）	1	271	Uncharacterized protein	ABA53426.1	Glycerone kinase activity and glycerol metabolic process	35
	2	143	Aminoacyl-tRNA hydrolase （Acidobacteria bacterium 13_1_40CM_4_65_8）	OLC81619.1	Hydrolase activity and translation release factor activity	68.2
CR8（1084 / 61）	1	141	Uncharacterized protein （Acidobacteria bacterium）	PYT37751.1	Uncharacterized	55.3
	2	49	Uncharacterized protein （*Streptomyces viridosporus* strain ATCC 14672 / DSM 40746 / JCM 4963 / KCTC 9882 / NRRL B-12104 / FH 1290）	EFE70172.1	Nucleotidyltransferase, RNA-directed DNA polymerase	100
	3	37	Uncharacterized protein （*Lactobacillus crispatus*）	KWU02988.1	Uncharacterized	100
CR9（1786 / 63）	1	270	Uncharacterized protein	ABA53426.1	Glycerone kinase activity and glycerol metabolic process	35
	2	138	Aminoacyl-tRNA hydrolase （Acidobacteria bacterium 13_1_40CM_4_65_8）	OLC81619.1	Hydrolase activity and translation release factor activity	68.2
	3	26	Catalase （*Bacteroides uniformis CL03T12C37*）	EIY72513.1	Integral component of membrane and resistance to heavy metal stress and removal of excess ROS from heavy metal stress	100
	4	49	Uncharacterized protein （*Streptomyces viridosporus* strain ATCC 14672 / DSM 40746 / JCM 4963 / KCTC 9882 / NRRL B-12104 / FH 1290）	EFE70172.1	Uncharacterized	100
CR11（1681 / 67）	1	> 401	Uncharacterized protein （Acidobacteria bacterium）	PYR72484.1	Endonuclease activity ,encoding different polyketone compounds and non-ribosomal peptides	47.8

	2	68	Serine phosphatase RsbU, regulator of sigma subunit （*Streptomyces* sp. DvalAA-14）	SCD93260.1	Adjust serine phosphatase RsbU to activate a bacterial transcription factor and adjust endonuclease activity	50.9
CR18（1440 / 51）	1	> 336	Pyruvate oxidase （*Acidobacteria bacterium*）	PYQ39079.1	Catalytic activity, magnesium ion binding and thiamine pyrophosphate binding	75.5
CR19（1357 / 63）	1	113	Uncharacterized protein （Acidobacteria bacterium）	RPH59784.1	Transmembrane transporter activity	65.5
	2	286	Uncharacterized protein （*Halomonas heilongjiangensis*）	PMR71650.1	Integral component of membrane , L-lactate dehydrogenase activity	47.4
CR21（1434 / 58）	1	272	Uncharacterized protein （Acidobacteria bacterium）	PYV94921.1	Dipeptidyl-peptidase 5 and biopolymer transporter TolR	75.5
	2	56	Uncharacterized protein （*Candidatus Sulfopaludibacter* sp. SbA6）	SPE32068.1	Uncharacterized	64.9
	3	93	[Fe-S]-binding protein （*Bryobacterales* bacterium）	HAX44309.1	Iron-sulfur cluster binding activity	68.1
CR23（1295 / 60）	1	57	Uncharacterized protein （*uncultured alpha proteobacterium* EB080_L06A09）	ADI20086.1	Uncharacterized	100
	2	280	MFS transporter （*Bradyrhizobium* sp. 2 39S1MB）	AWM04175.1	Transmembrane transport protein activity and integral component of membrane	92.5
	3	>59	Uncharacterized protein （Uncultured *Flavobacteriia* bacterium）	CBL87351.1	Putative reverse transcriptase activity	100
CR33（1549 / 63）	1	192	Uncharacterized protein （marine metagenome）	SUZ98599.1	N-acetyltransferase activity and transmembrane transporter activity	44.1
	2	212	Class I SAM-dependent methyltransferase （Acidobacteria bacterium）	RPI53964.1	Methyltransferase activity	66.3
CR38（1541 / 58）	1	262	3-ketoacyl-ACP reductase	KRB39811.1	Reductase activity and reduction of 3-ketoacyl ACP to 3-	75

			（*Rhodanobacter* sp. Root179）		hydroxyacyl ACP with NADPH as coenzyme	
CR41（1528 / 67）	1	> 488	Uncharacterized protein（*Proteobacteria* bacterium）	PZN28297.1	The twin-arginine translocation (Tat) pathway serves the role of transporting folded proteins across energy-transducing membranes	71.8
CR47（1789 / 64）	1	> 576	HD domain-containing protein（Acidobacteria bacterium RIFCSPLOWO2_12_FULL_67_14b）	OFW46919.1	DNA-binding protein activity	68.2

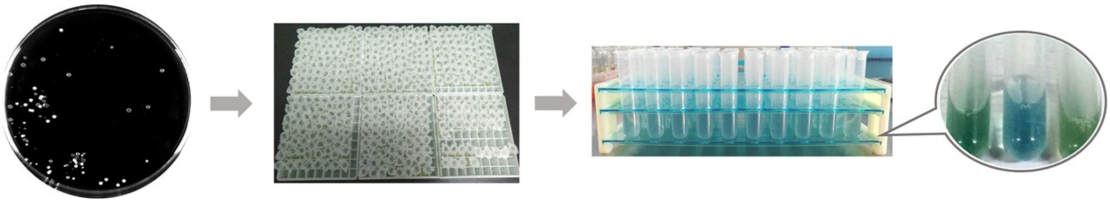

Fig. S1. Screening of positive clones in liquid medium. Common *E. coli* DH5α containing recombinant DNA fragments were incubated in liquid LB medium with 100 μg/mL ampicillin and 4.2 mmol/L Cu. After 6 hrs, the growth rate was measured using a biophotometer (Eppendorf, Germany).

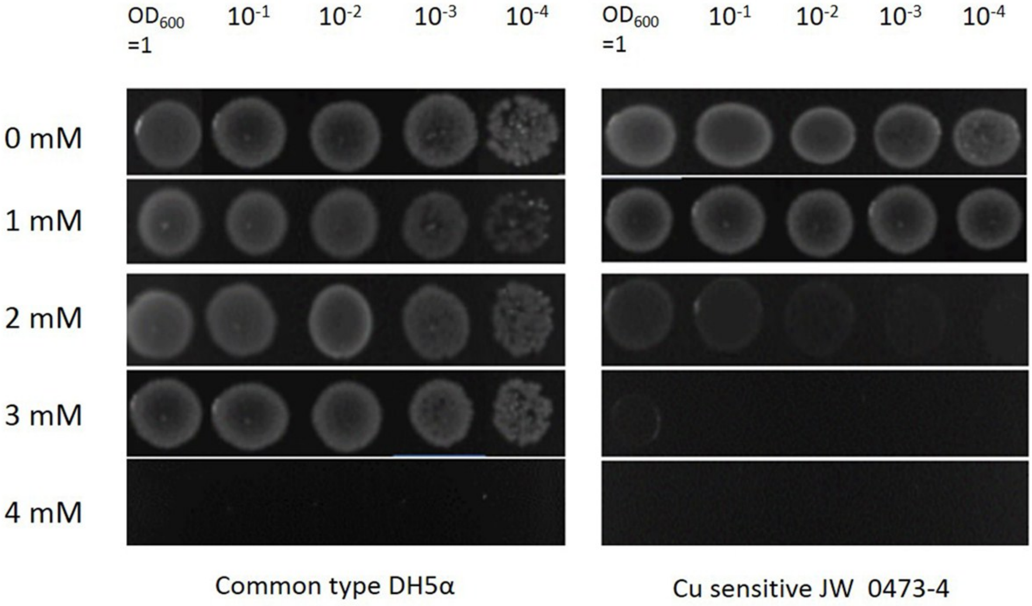

Fig. S2. Drop assay of the Cu-sensitive strain *E. coli* JW 0473-4 (Δ*CopA*) and common *E. coli* DH5α. The *E. coli* cells were subject to a drop assay on LB plates with 0 to 4 mmol/L Cu, and 50 µg/mL kanamycin was added to the plates. They were serially diluted (1–10^{-4}) and incubated at 37 °C for 48h, after which growth curve were measured. This experiment was performed three times.

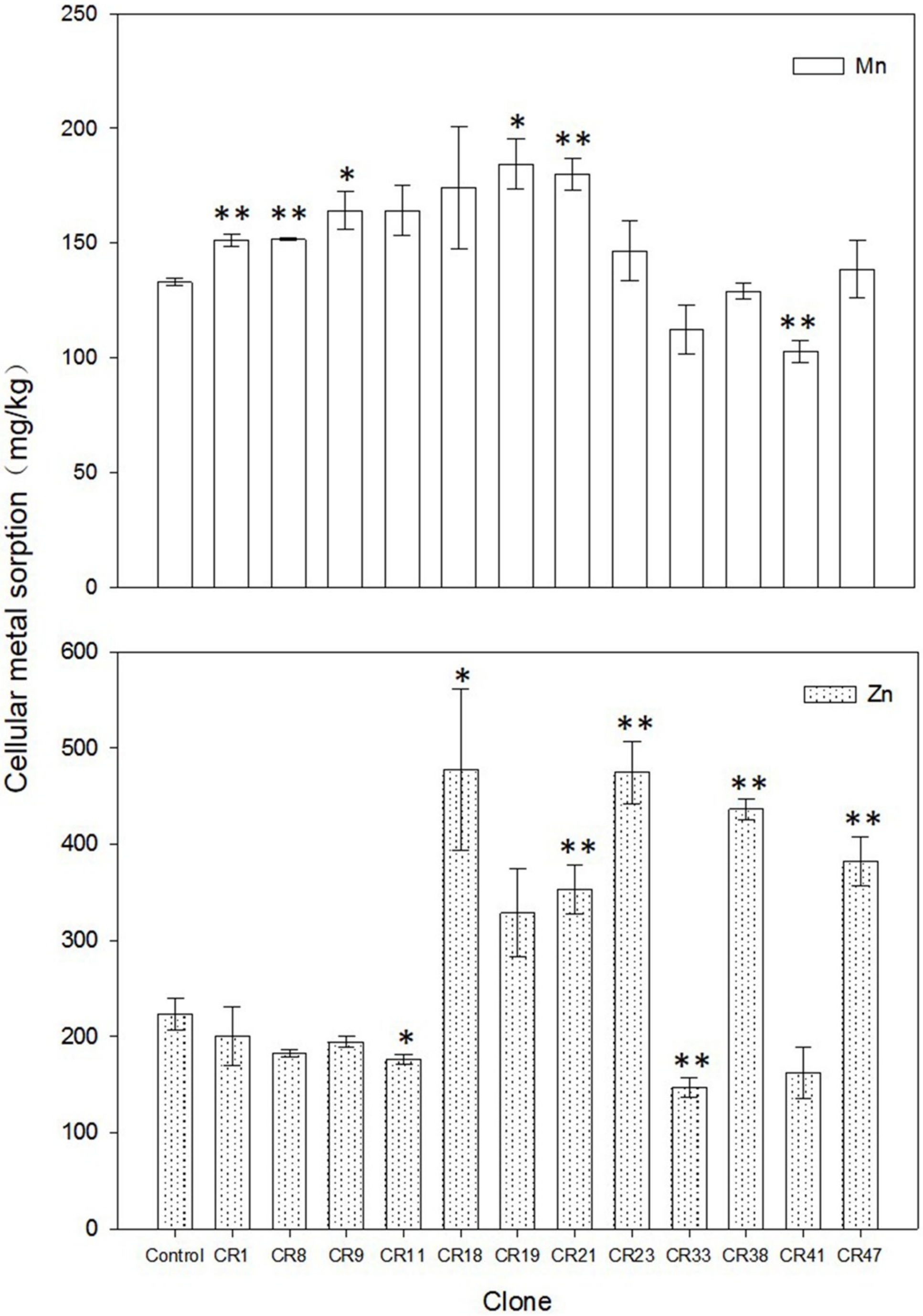

Fig. S3. Manganese and zinc sorption of the 12 clones in Cu-containing culture medium. Common type *E. coli* DH5α containing recombinant DNA fragments were incubated using liquid LB medium with 100μg/mL ampicillin and 1 mmol/L Cu for 6h, followed by the measurement of Mn and Zn content of each strain using an inductively coupled plasma mass spectrometer (ICP-MS)(Thermo Fisher Scientific, USA). Vertical bars indicate standard deviations of the means (n = 3). The asterisk (*) indicates significant difference between the cells harboring DNA fragments and the control harboring empty pUC118 with $p < 0.05$ by t-test.

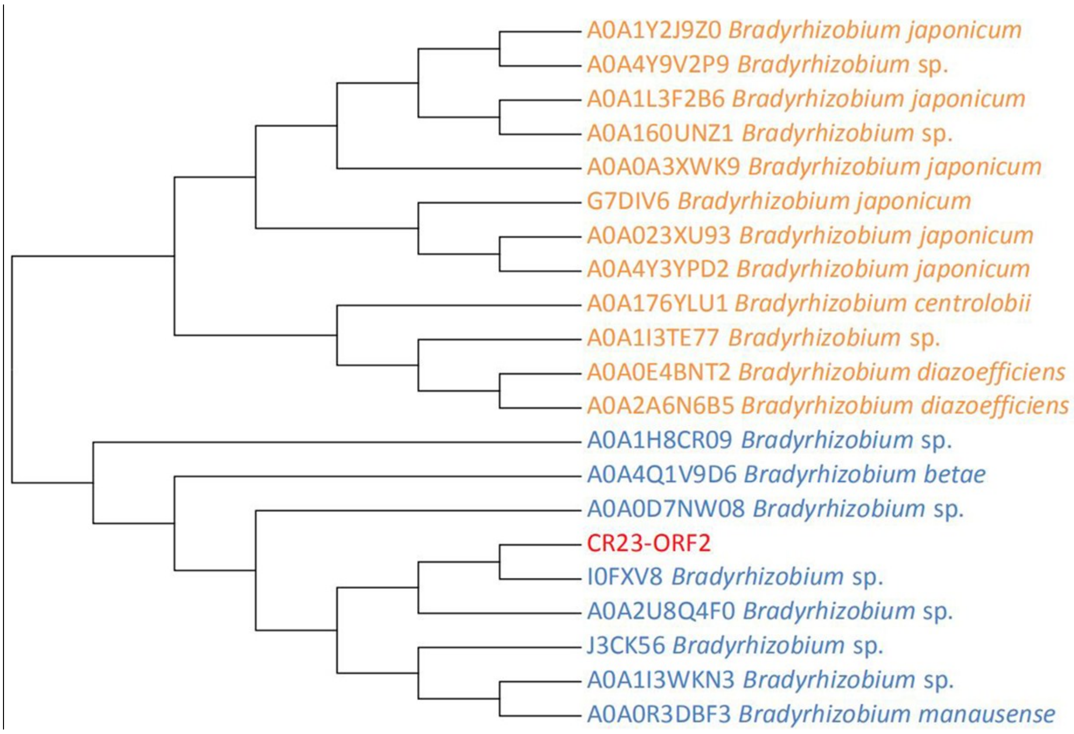

Fig. S4. A neighbor-joining phylogenetic tree to show the evolutionary relationships of CR23-*orf2* and the other homologous proteins.