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Pseudomonas species isolated via high-throughput screening significantly protect cotton plants against verticillium wilt

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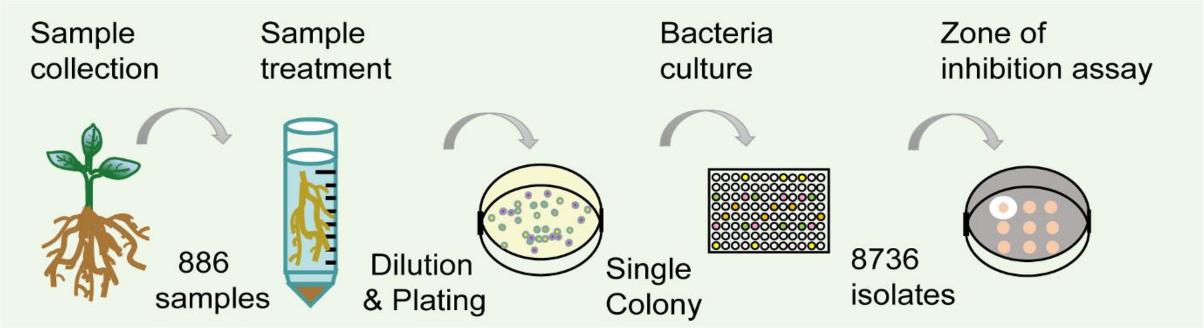

Figure S1. Schematic diagram showing work flow for high-throughput screening.

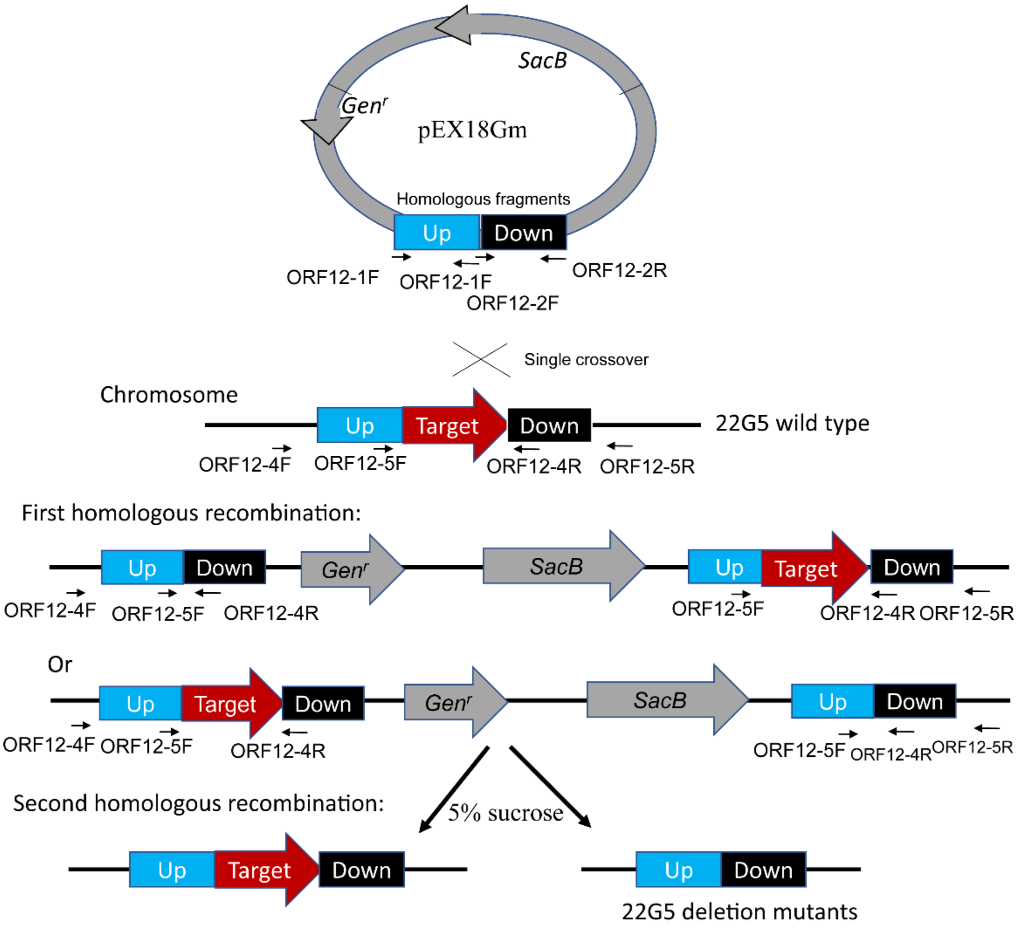

Figure S2. Schematic diagram illustrating the use of the pEX18Gm suicide plasmids to generate a gene deletion in *P. donghuensis* 22G5. Picture was modified from the reference (Wang et al. 2015). Refer to Table S2 for detailed primer sequences.

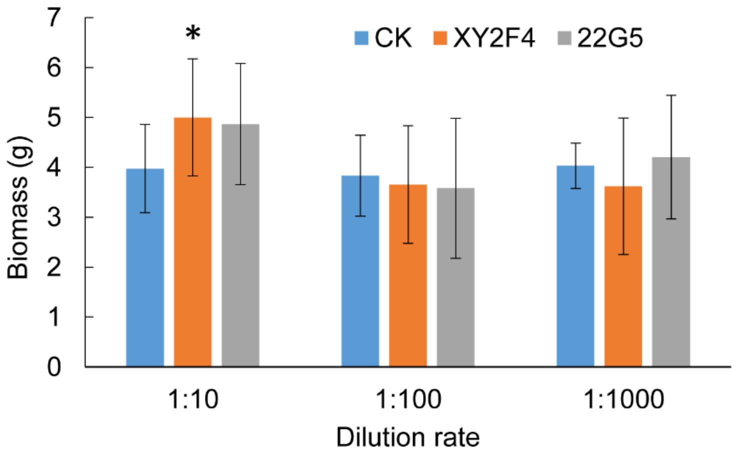

Figure S3. Plant growth promoting and biosafety tests of *P. protegens* XY2F4 and *P. donghuensis* 22G5. 7-day old cotton seedlings with treatments of different concentrations of *Pseudomonas* strains (XY2F4 and 22G5) and the LB control (CK), biomass were recorded three weeks after inoculation. 1:10, 1:10 dilution of overnight culture, ~1×10^7 CFU/mL; 1:100, ~1×10^6 CFU/mL; 1:1000, ~1×10^5 CFU/mL. * $P<0.05$. Student's t test.

Table S1 Informations of plant rhizosphere samples.

Location	Plant type	Collection time (year.month)	Plant rhizosphere samples No.	96 plate No.
Dangtu, Anhui	Cotton & Weeds & Rice	2107.6	1-60	1-7
Dangtu, Anhui	Tea	2017.6	61-77	8-9
Hangzhou, Zhejiang	Soybean	2107.6	78-150	10-14
Hangzhou, Zhejiang	Tea	2017.6	151-156	
Shihezi, Xinjiang	Cotton	2017.7	157-183	15-21
Dangtu, Anhui	Cotton & Weeds	2107.7	184-202	
Baihe, Jilin	Weeds	2017.8	203-217	22-23
Dongyan, Zhejiang	Sorghum, Sesame	2017.8	218-261	24-27
Zhangjiajie, Hebei	Weeds	2017.8	262-275	
Dangtu, Anhui	Cotton	2017.9	276-288	28-29
Zhoushan, Zhejiang	Weeds	2107.9	289-328	30-34
Haerbing, Heilongjiang	Weeds	2017.9	329-361	35-37
Suzhou, Jiangsu	Weeds	2017.10	376-373	38-43
Yancheng, Shandong	Weeds	2017.10	374-385	
Xuzhou, Jiangsu	Cotton & Weeds	2017.10	386-413	
Dangtu, Anhui	Cotton & Weeds & Rice	2017.11	414-846	44-88
Shanghai	Cotton	2018.4	847-886	89-91

Table S2. Primer used in this study.

Name	Sequence (5'-3')	Usage
27F	AGAGTTTGATCMTGGCTCAG	16S rDNA
1492R	TACGGYTACCTTGTTACGACTT	
ORF12-1F	TGTACGGGTCATGGAAGTGCA	Gene mutation,for upstream fragment PCR
ORF12-1R	ttacctgcaatcacgTCTTTGAACCGGCAG	
ORF12-2F	CTGCCGGTTCAAAGAcgtgattgcaggtaa	Gene mutation,for downstream fragment PCR
ORF12-2R	TGATCGAGCTGGACAAGCAGA	
ORF12-3F	cccggatccTGTACGGGTCATGGAAGTGCA	Gene mutation, for upstream plus downstream fragment PCR
ORF12-3R	cccaagcttTGATCGAGCTGGACAAGCAGA	
ORF12-4F	GCAGCCTCGGGTTTCACCGCCCGCCAGCTG	For first allelic exchange PCR identification
ORF12-4R	TCGAACAAGCGGCCAGTACCGGCAGTGTGC	
ORF12-5F	TCGCCAAAGGCGTGCTCGATGCCGTGCAGA	For first allelic exchange PCR identification
ORF12-5R	TGCGGGCGGATCAGCTCGGTGCGCATCAAC	
ORF12-6F	TGTACGGGTCATGGAAGTGC	For second allelic exchange PCR identification
ORF12-6R	AGATTGAACACTGGCAGGGG	
ORF12-7F	CTGTTTGCCTTACTGCGACG	For second allelic exchange PCR identification
ORF12-7R	CCTGGCGATGCTCTAGTTGT	

Table S3 Genome summary of *Pseudomonas* strains.

	P. protegens XY2F4	*P. donghuensis* 22G5	*P. lini* 25D11	*P. putida* 25E1
Genome size (bp)	6,811,381	5,726,777	7,870,958	6,975,274
Number of contings	50	51	4451	1205
GC content (%)	63.6	62.2	58.7	61.2
CDS	6153	5339	10361	7140
Average CDS size (bp)	969	964	661	823
rRNA	8	4	5	4
tRNA	60	60	71	84

Table S4 RAST Annotation of unique genes in XY2F4.

Feature	Counts
Widespread_colonization_island	10
General_Secretion_Pathway	9
Orphan_regulatory_proteins	5

Methionine_Biosynthesis	4
Rhamnose_containing_glycans	4
Biphenyl_Degradation	3
Butanol_Biosynthesis	3
Cinnamic_Acid_Degradation	3
Cresol_degradation	3
Glutathione:_Non-redox_reactions	3
Lacto-N-Biose_I_and_Galacto-N-Biose_Metabolic_Pathway	3
Lactose_and_Galactose_Uptake_and_Utilization	3
N-linked_Glycosylation_in_Bacteria	3
Polyamine_Metabolism	3
Restriction-Modification_System	3
Universal_stress_protein_family	3
5-FCL-like_protein	2
Bacterial_hemoglobins	2
Biotin_biosynthesis	2
Capsular_heptose_biosynthesis	2
Chitin_and_N-acetylglucosamine_utilization	2
Conserved_gene_cluster_possibly_involved_in_RNA_metabolism	2
D-galactarate,_D-glucarate_and_D-glycerate_catabolism	2
Fatty_acid_metabolism_cluster	2
Fermentations:_Lactate	2
Fermentations:_Mixed_acid	2
Glutamine,_Glutamate,_Aspartate_and_Asparagine_Biosynthesis	2
Glycerate_metabolism	2
Glycerolipid_and_Glycerophospholipid_Metabolism_in_Bacteria	2
Maltose_and_Maltodextrin_Utilization	2
Methylglyoxal_Metabolism	2
NAD_and_NADP_cofactor_biosynthesis_global	2
n-Phenylalkanoic_acid_degradation	2
Pentose_phosphate_pathway	2
Phd-Doc,_YdcE-YdcD_toxin-antitoxin_(programmed_cell_death)_systems	2
Putrescine_utilization_pathways	2
Ton_and_Tol_transport_systems	2
Toxin-antitoxin_replicon_stabilization_systems	2
Two_partner_secretion_pathway_(TPS)	2
Type_I_Restriction-Modification	2
Acetyl-CoA_fermentation_to_Butyrate	1
Allantoin_Utilization	1
Amidase_clustered_with_urea_and_nitrile_hydratase_functions	1
Archaeal_lipids	1
Benzoate_transport_and_degradation_cluster	1
Broadly_distributed_proteins_not_in_subsystems	1
Butyrate_metabolism_cluster	1
carbazol_degradation_cluster	1
Carbon_monoxide_oxidation	1
Cell_division-ribosomal_stress_proteins_cluster	1
Central_meta-cleavage_pathway_of_aromatic_compound_degradation	1
Chorismate_Synthesis	1

CMP-N-acetylneuraminate_Biosynthesis	1
Coenzyme_M_biosynthesis	1
Colanic_acid_biosynthesis	1
Common_Pathway_For_Synthesis_of_Aromatic_Compounds_(DAHP_synthase_to_ chorismate)	1
Copper_homeostasis	1
Creatine_and_Creatinine_Degradation	1
Cyanophycin_Metabolism	1
Cysteine_Biosynthesis	1
D-gluconate_and_ketogluconates_metabolism	1
dTDP-rhamnose_synthesis	1
Ethylmalonyl-CoA_pathway_of_C2_assimilation	1
Fosfomycin_resistance	1
Gentisate_degradation	1
Glutamate_and_Aspartate_uptake_in_Bacteria	1
Glutathione:_Biosynthesis_and_gamma-glutamyl_cycle	1
Iron_siderophore_sensor_&_receptor_system	1
Lactose_utilization	1
L-rhamnose_utilization	1
Lysine_degradation	1
Lysine_fermentation	1
Magnesium_transport	1
Mannose_Metabolism	1
Methionine_Degradation	1
O-Methyl_Phosphoramidate_Capsule_Modification_in_Campylobacter	1
Oxidative_stress	1
Phosphate-binding_DING_proteins	1
p-Hydroxybenzoate_degradation	1
Poly-gamma-glutamate_biosynthesis	1
Polyhydroxybutyrate_metabolism	1
Proline,_4-hydroxyproline_uptake_and_utilization	1
Purine_Utilization	1
Quinate_degradation	1
Respiratory_dehydrogenases_1	1
Ribonucleases_in_Bacillus	1
Salicylate_and_gentisate_catabolism	1
Siderophore_Achromobactin	1
Siderophore_Aerobactin	1
Soluble_cytochromes_and_functionally_related_electron_carriers	1
Threonine_and_Homoserine_Biosynthesis	1
Transcription_factors_bacterial	1
Transcription_initiation,_bacterial_sigma_factors	1
Trans-envelope_signaling_system_VreARI_in_Pseudomonas	1
Translation_termination_factors_bacterial	1
tRNA_aminoacylation,_Asp_and_Asn	1
tRNA_aminoacylation,_Cys	1
tRNA_aminoacylation,_Glu_and_Gln	1
tRNA_aminoacylation,_Val	1
Utilization_of_glutathione_as_a_sulphur_source	1
Xanthine_dehydrogenase_subunits	1

Table S5 RAST Annotation of unique genes in 22G5.

Feature	Counts
Type_I_Restriction-Modification	2
Restriction-Modification_System	2
n-Phenylalkanoic_acid_degradation	2
Leucine_Degradation_and_HMG-CoA_Metabolism	2
Iron_siderophore_sensor_&_receptor_system	2
Fatty_acid_metabolism_cluster	2
CMP-N-acetylneuraminate_Biosynthesis	2
Rhamnose_containing_glycans	1
pVir_Plasmid_of_Campylobacter	1
Polyhydroxybutyrate_metabolism	1
Phage_tail_proteins	1
N-linked_Glycosylation_in_Bacteria	1
Lysine_fermentation	1
Lactose_and_Galactose_Uptake_and_Utilization	1
Lacto-N-Biose_I_and_Galacto-N-Biose_Metabolic_Pathway	1
Isoleucine_degradation	1
HMG_CoA_Synthesis	1
Glutamine,_Glutamate,_Aspartate_and_Asparagine_Biosynthesis	1
Glutamate_and_Aspartate_uptake_in_Bacteria	1
Exopolysaccharide_Biosynthesis	1
DNA_repair,_bacterial_RecFOR_pathway	1
DNA_repair,_bacterial	1
Butyrate_metabolism_cluster	1
Butanol_Biosynthesis	1
Biotin_biosynthesis	1
Acetyl-CoA_fermentation_to_Butyrate	1