

# A promising bacterial suicide gene against citrus Huanglongbing and canker

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## LasLYS2 is resistant to HLB

Line	Bacterial growth [ $\log_{10}$ ( <i>CaLas</i> cells $\mu\text{g}^{-1}$ of citrus DNA)]					
	25 MAI		27 MAI		29 MAI	
	Leaf	Root	Leaf	Root	Leaf	Root
WT	7.52±0.09 <sup>a</sup>	6.92±0.03 <sup>a</sup>	7.33±0.35 <sup>a</sup>	7.55±0.53 <sup>a</sup>	7.15±0.15 <sup>a</sup>	7.73±0.62 <sup>a</sup>
L1-1	7.17±0.21 <sup>b</sup>	6.86±0.46 <sup>a</sup>	6.46±0.22 <sup>b</sup>	6.95±0.10 <sup>b</sup>	6.76±0.26 <sup>ab</sup>	7.06±0.01 <sup>b</sup>
L1-2	7.19±0.16 <sup>b</sup>	6.44±0.29 <sup>ab</sup>	6.25±0.18 <sup>bc</sup>	6.84±0.21 <sup>b</sup>	6.32±0.22 <sup>b</sup>	7.11±0.13 <sup>b</sup>
L1-4	6.75±0.05 <sup>c</sup>	6.03±0.02 <sup>b</sup>	5.94±0.15 <sup>c</sup>	6.29±0.11 <sup>c</sup>	5.81±0.18 <sup>c</sup>	6.35±0.09 <sup>c</sup>
L2-1	5.71±0.21 <sup>d</sup>	4.58±0.19 <sup>c</sup>	4.83±0.04 <sup>d</sup>	5.32±0.08 <sup>d</sup>	4.66±0.44 <sup>d</sup>	5.21±0.21 <sup>d</sup>
L2-3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
L2-6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

  

The image shows four photographs comparing WT and L2-6 citrus plants. The top-left photo shows a healthy L2-6 plant. The top-right photo shows a WT plant with 'Leaf curling' indicated by red arrows. The bottom-left photo shows the roots of a WT plant with canker symptoms. The bottom-right photo shows the roots of an L2-6 plant, which appear healthy and free of canker.

LasLYS2<sup>?</sup>L2-#<sup>?</sup> has dual resistance to Huanglongbing and citrus canker. Credit: *Horticulture Research*

Huanglongbing (HLB), caused by *Candidatus Liberibacter asiaticus* (*CaLas*), is the No. 1 killer of citrus trees worldwide. HLB causes

billions of dollars in annual economic losses to the global citrus industry.

At present, no HLB-resistant cultivars and no effective therapeutic agents is available for citrus farmers. Citrus canker disease, caused by *Xanthomonas citri* pv. *citri* (Xcc), is another devastating disease in the citrus industry, which can harm almost all citrus cultivars and is currently on the outbreak trend. Breeding disease-resistant varieties is the fundamental way to prevent HLB and citrus canker.

Bacteriophages are the natural enemies of bacteria. They mainly secrete endolysins to the host cell periplasm through the Holin-endolysin lysis system and break the cell wall to kill bacteria. Therefore, endolysin is also considered to be a bacterial suicide gene. Bacteriophages and their lytic genes are a powerful means to control disease by triggering bacterial "suicide" to suppress pathogen growth.

In August 2023, *Horticulture Research* published a research paper entitled by "An endolysin gene from *Candidatus Liberibacter asiaticus* confers dual [resistance](#) to Huanglongbing and citrus canker." The researchers found that an endolysin encoded by the CaLas prophage has dual resistance to Huanglongbing and citrus canker.

In this study, two endolysin genes LasLYS1 and LasLYS2 were cloned from the CaLas genome. In vitro antibacterial assays show that LasLYS2 had visible bactericidal activity against several rhizobium bacteria and Xcc. More than two years of greenhouse testing confirm that LasLYS1 and LasLYS2 had durable resistance to HLB. Importantly, LasLYS2 transgenic plants do not show symptoms such as leaf chlorosis and root rot, but also LasLYS2 can completely clear CaLas from infected plants.

LasLYS2 [transgenic plants](#) with enhanced HLB resistance also exhibit resistance to [citrus canker](#). Ectopic expression of LasLYS1 and LasLYS2 in citrus promote the proliferation of beneficial bacteria

Burkholderiaceae and Rhodanobacteraceae but inhibit the growth of Xanthomonadaceae and Rhizobiaceae in roots, which favors citrus defense response.

Further, the research team reveals that Lyz domain 2 was the key bactericidal motif of LasLYS2. Based on the structures of LasLYS1 and LasLYS2, researchers successfully synthesize four new endolysins with killing activity against CaLas and Xcc.

In summary, the research provides insights for engineering bactericidal proteins or peptides against citrus HLB and canker pathogens, but also for breeding new citrus germplasm with broad-spectrum resistance.

**More information:** Lanzhen Xu et al, An endolysin gene from *Candidatus Liberibacter asiaticus* confers dual resistance to Huanglongbing and citrus canker, *Horticulture Research* (2023). [DOI: 10.1093/hr/uhad159](https://doi.org/10.1093/hr/uhad159)

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