



# Chico Crop Science Co., Ltd.

## Company Profile:

Chico Crop Science Co., Ltd. is a high agro-tech company specialized in providing comprehensive nutrition for crops, seed treatment and systematic prevention and control of crop diseases, pests and weeds.





## Biological control Huanglong Diseases

# CULAR™

Q: Is there a product **safe,fast effective, no resistance** to **Huanglong diseases** ?

A: There's no doubt that **CULAR™** can do it.





# CATALOG

- ① Introduction
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- ⑤ Field application
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Biological control Huanglong Diseases  
BIOLOGICAL CONTROL HUANGLONG DISEASES





## 1、 Introduction

- **CULAR™** is a beneficial and functional bio fertilizer with Organic Input Certification. The numerous field test performed by the plant protection technology department have shown that **CULAR™** specialised in control huanglong disease , which significantly inhibits the growth of pathogenic bacteria.
- **CULAR™** can enhance photosynthesis, promote crop growth and development, and significantly improve crop fruit setting and fruit quality.





## 2、 Background:

- **Huanglong disease** another name: **Citrus yellow shoot、 Citrus greening disease.**
- **Development process** : Huanglong disease first occurred in Afghanistan, Pakistan, and India, and China was first discovered in Chaoshan, Guangdong around 1919. Now It is distributed in more than 40 countries in Asia, Africa, South America, and 9 states in the US.
- **Transmission vector and pathogen:** It was spread by psyllids and caused by Gram-negative pathogenic bacteria confined to the phloem. It belongs to the phylum Parenchyma and cannot be cultivated artificially, so the research on Huanglong disease is very difficult.
- **Huanglong disease is a devastating disease of citrus production worldwide, commonly known as citrus cancer.**
- **At present, there is no citrus variety that Huanglong is resistant to disease in the world .**



**Pathogens:** Spread by psyllids, it is a worldwide disease that is difficult to prevent from phloem parasites.

Psyllids ↓



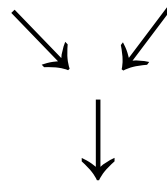
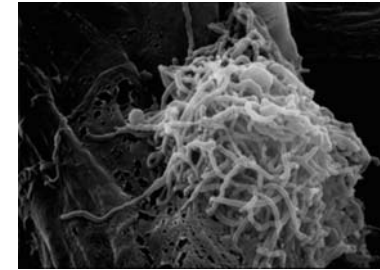
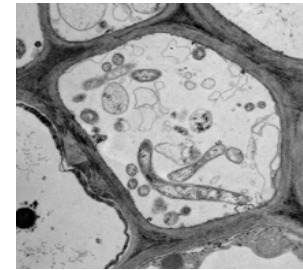
Psyllids ↓



*Candidatus Liberibacter asiaticus (Las)*

*Ca. L. africanus (Laf)*,

*Ca. L. americanus (Lam)*



**Symptom picture**



## 3. Current prevention methods

(1) Healthy Seeding



- Being sick in chance

(3) 7.5M Height Net



- Unavailable for large area

(2) Killing insect



- Killing all psyllids is unrealistic

(4) Antibiotics



- Damage ecological security

5--Replacement & Transplant



- Unavailable for large area





(6) When Huanglong disease broke out seriously, only cut down the trees and burn to remove the pathogens ,which caused heavy economic losses.

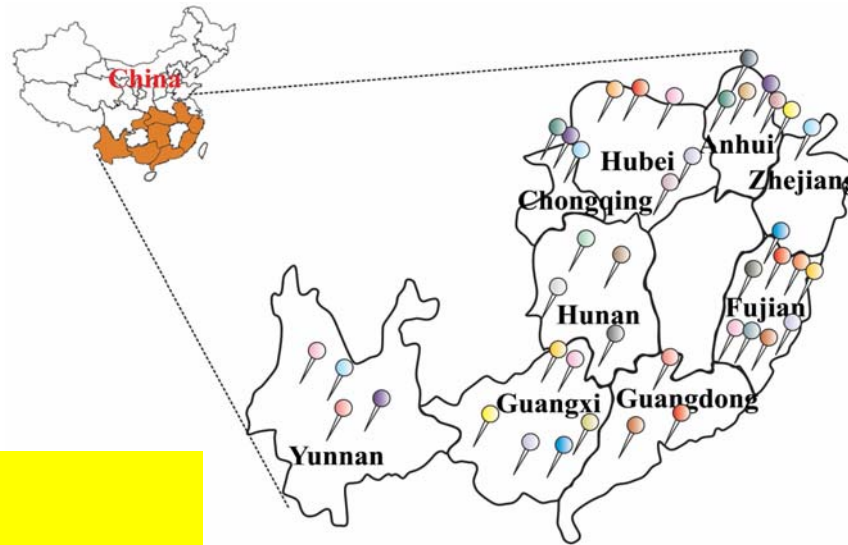
**How can we save them?**





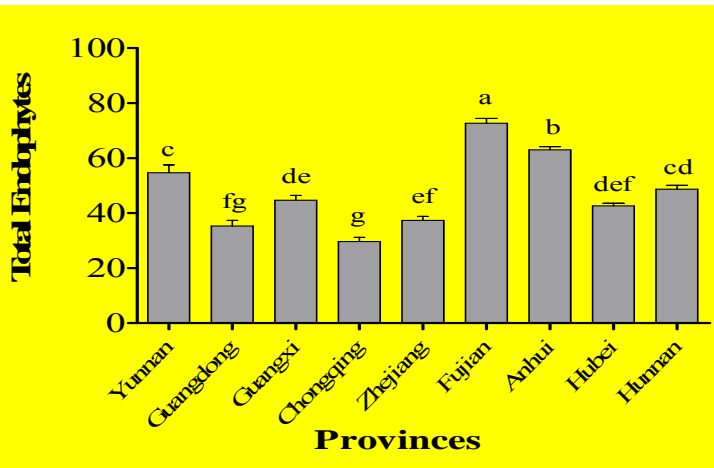


## 4. Experimental procedure



### 1)-Endophyte investigation

- Citrus cultivars
- Citrus reticulata* Blanco
- Citrus sinensis* (L.) Osbeck
- Citrus reticulata* cv. Tankan
- Citrus unshiu* Marcov
- Citrus reticulata* cv. Shatangju
- Citrus maxima* cv. Sanhongmiyou
- Citrus reticulata* Blanco v. Gonggan
- Citrus reticulata*
- Citrus reticulata* cv. Suavissima
- Citrus grandis* (L.) Osbeck cv. Guanximiyou
- Citrus sinensis*
- Citrus tangerina*
- Citrus unshiu* Marc
- Huangyan
- Juhong orange
- Citrus reticulata* (L.)Blanco cv. Nanfengmiju
- Fortunella margarita* (L.) Swingle
- Valencia Orange
- Citrus limon* (L.) Burm. f.
- Citrus reticulata* cv. Ponkan
- Citrus sinensis* Osb. (Naval orange)
- Puzao
- Tezao
- Chishu

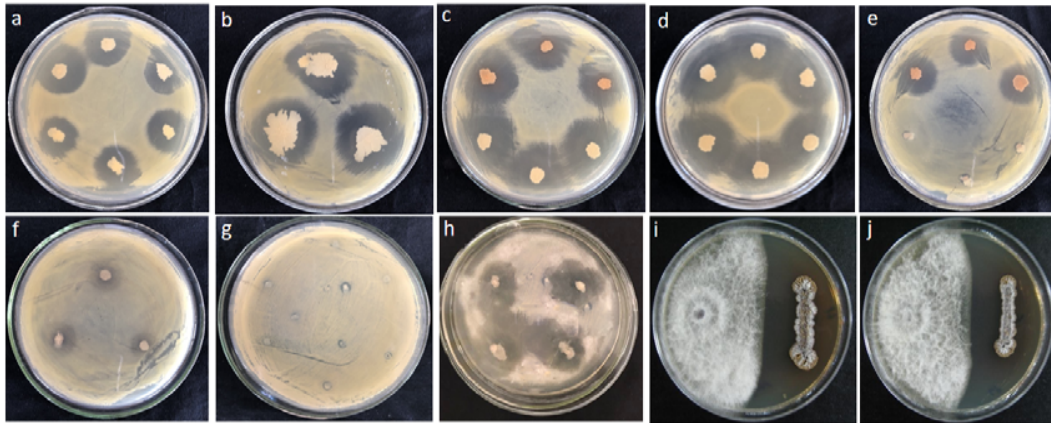


Health Trees : 114 kinds of Endophyte

Sick Trees: 41 kinds of Endophyte

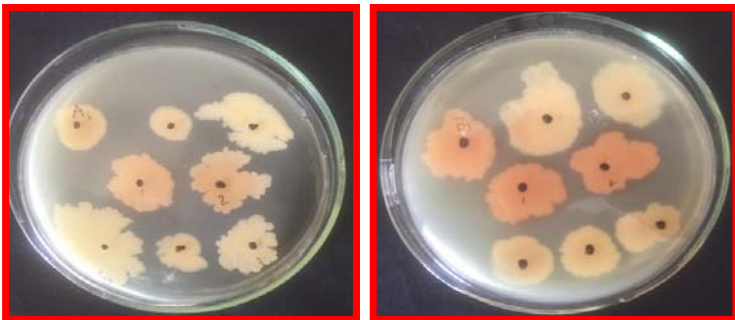
The number of colonies in healthy trees is 5-10 times that of diseased trees.

## 2) Antibacterial activity determination, screening of strains with application potential

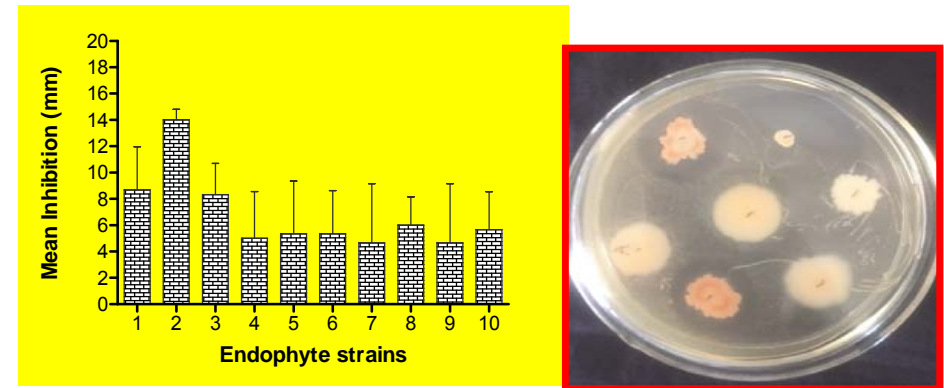


(a-g) Endophytes against the different bacterial phytopathogens including *Xanthomonas citri* (G), *Erwinia carotovora* (B2), *Erwinia* sp. (HBYL), *Pseudomonas syringae* (JB), *Xanthomonas oryzae* (LKF), and *Ralstonia solanacearum* (WL).

(h-j) Endophytes inhibition against *F. verticillium* and *F. oxysporum*.



L1-21 have good inhibitory effect on pathogens, but without inhibition on citrus endophytes



Endophyte strains with dominant inhibition effect (mm) against several plant pathogens.

*B. amyloliquefaciens* S11-1, *B. subtilis* L1-21, *B. licheniformis* J15, *B. methylotrophicus* J16, *B. siamensis* S12, *B. subtilis* CA52, *B. amyloliquefaciens* CC12, *Paenibacillus amylolyticus* CH87, *Terribacillus* sp CF01, and *Curtobacterium citreum* CH91.



## 5. Field Application

### 1) Molecular test results

2016-9 Binjun Town--30 Trees with 16 ages

Sampling Time	Sample quantity	Positive	Negative	Efficiency %
March 22	156	156	0	-
July 8th	156	43	113	72.44
November 8th	156	37	119	76.28

Biocontrol strains

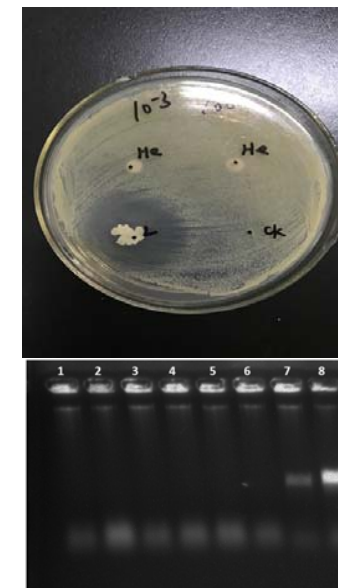


August 8th 2017



September 6th 2017

Citrus canker pathogenic bacteria



September 21st 2017

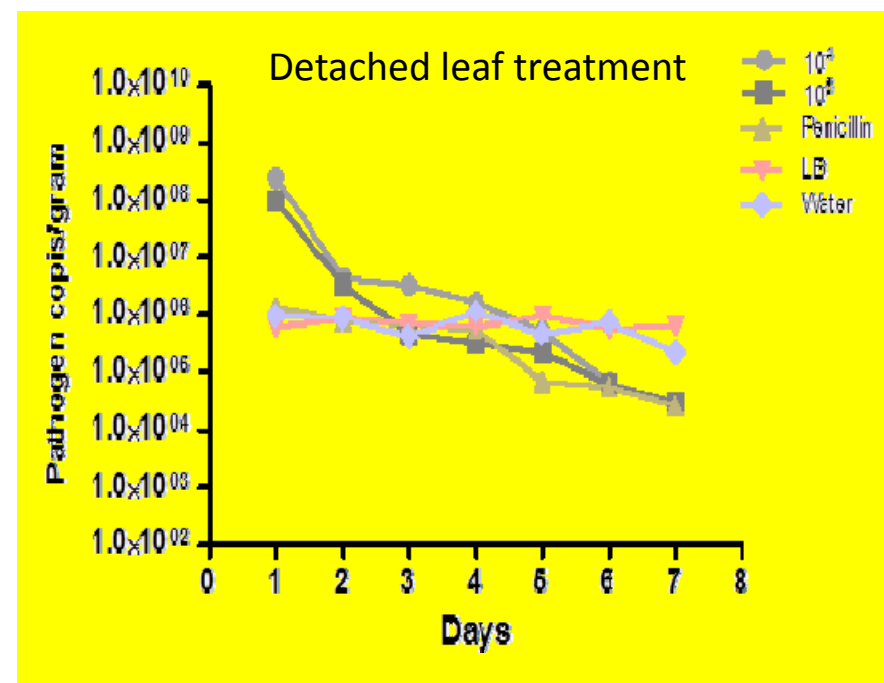


## 2) The number of Huanglong disease bacteria has dropped sharply

Palce: 2018-3 HuaQiao farm 525 trees with 4-5ages

*Conclusion :The germs have dropped by 1-100 million times in 3 months!*

Citrus tree no	Pathogen copies/ gram		
	March	April	June
2	0.00E+00	5.03E+02	1.85E+01
8	1.85E+05	5.63E+04	2.85E+02
12	4.92E+03	3.62E+03	7.88E+01
18	4.94E+06	2.06E+06	4.60E+01
22	8.17E+07	5.13E+07	7.74E+01
27	6.31E+02	1.68E+02	1.62E+02
32	4.29E+02	3.06E+02	3.03E+02
36	5.04E+04	1.54E+05	1.63E+01
41	3.00E+02	4.75E+03	9.17E+01
46	3.11E+03	2.03E+04	2.75E+01
52	8.53E+09	1.51E+04	4.50E+01
62	1.27E+03	1.94E+03	4.71E+01
67	2.86E+03	5.65E+02	3.57E+00
72	1.85E+03	1.91E+03	1.90E+02







## Reduction of Clas pathogen copies in the field

Palce: 2018-3 HuaQiao farm 525 trees with 4-5ages

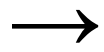
Number of pathogenic bacteria in 2018-2019					
Number of pathogenic bacteria					
Month	I	II	III	Mean	Reduce from April
April 2018	8.34E+08	4.98E+09	3.68E+05	1.94E+09	-
June 2018	4.26E+03	2.24E+04	3.86E+04	2.18E+04	99.9989 %
September 2018	8.91E+03	3.31E+04	4.59E+03	1.55E+04	99.9992 %
January 2019	2.62E+04	1.44E+05	1.57E+03	5.74E+04	99.9970 %
March 2019	2.64E+04	1.29E+05	9.76E+03	5.51E+04	99.9971%

***Each repeats 31 trees, a total of 93 trees. Ten of the trees can no longer detect the huanglong disease***



## Comparance

Before



After

Before



After





## 6. Benefits of CULAR™

- Biocontrol hunglong disease with zero-pesticide
- Highly effective and Non-resistance : Ensure the quality of organic product production and processing
- Organic certificate
- Cost-saving and Labor-saving
- It can improve the micro-ecological environment of the plants, and prompt the crop leaves to quickly turn yellow and turn green.
- It can promote crop growth and development, and significantly improve crop fruit setting and fruit quality



## 7. Usage and recommendation

Usage Target		Dosage	Interval period	Note
Foliar spraying		150--300 Diluted times	The interval in 2 times is 3-4 days; later spraying at an interval of about 7-10 days	1、 Sprayed evenly on the front and back of the leaves 2、 Irrigating roots after activation with water-soluble organic fertilizer the day before use
Root irrigation	Prevention	15000ml --30000 ml /Ha	2-3 times in a year	
	Crop is used in an unhealthy state	100--200 Diluted times	2-3 times in a year; In summer, it can collaborative with foliar spray at 7-15 days interval	



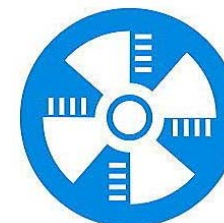


## 8 . Cautions

- **This product must not be in contact with chemical fungicides during use.** There must be a period of time between the last spray of chemical fungicides. Shaking evenly before use.
- Try to spray in the evening, early morning or cloudy.
- Avoid to apply at high temperature, direct sunlight, strong wind and rain day.
- If the solution splashes into the mouth, eyes or other organs, rinse with enough water immediately.

## Storage

- It should be stored in dry, cool, low temperature, ventilated and rainproof place.
- Keep away from fire and heat source and avoid direct sunlight.





# 9. The analysis report of biofungicide

CHICO CROP SCIENCE CO.,LTD



CULAR™

### Certificate of Analysis

Report No.:20210402-SN-013

Product name	Bio fungicide		Test date
Dosage form	AS	Inspection basis	April 2 <sup>nd</sup> , 2021
Test Item(s)	Unit	Specification	Test Result
Viable count (cfu)	a hundred million /ml	≥2.0	26.7
Number of mycobacteria	pcs/ml	≤3×10 <sup>6</sup>	0
Rate of mixed bacteria	%	≤10	0
Rate of spore	%	≥90	91
pH	—	5.0~8.0	7.9
Conclusion	Qualified		

Chief Analyzer: Xia Lang

Examiner: Wang Jiajia

Inspector: Xie Min

Chico Crop Science Co., Ltd.  
For and on behalf of  
CHICO CROP SCIENCE CO., LIMITED  
Signature:

Xie Min  
Date: April 2<sup>nd</sup>, 2021  
Authorized Signature(s)



# 10. Organic certification

# Biological control Huanglong Diseases

## Patent certification

Local Taxation Bureau of Beijing Haidian District  
Stamp Duty  
Withholding Special Seal  
State Intellectual Property Office

Certificate No. 244411

### Certificate of Patent for Invention

**Title of Invention:** A kind of *Bacillus subtilis* strain  
**Inventor:** He Qiuyue, Ma Ping  
**Patent Number:** ZL 2003 1 0104195.6  
**Patent Application Date:** 2003.12.28  
**Patentee:** He Qiuyue  
**Date of Authorization Proclamation:** 2006.1.11

This patent right is granted by State Intellectual Property Office of the People's Republic of China upon examination in accordance with the Patent Law of the People's Republic of China. This certificate of patent is issued to the patentee and the patent is registered on the patent register. The patent right shall come into force from the date of authorization proclamation.

The duration of this patent right shall be twenty years from the date of application. The patentee shall pay the annual fee in accordance with the Patent Law and the Detailed Rules for the Implementation. The patent fee shall be paid 1 month before Dec.28th annually. The patent right shall be terminated from the date of the expiration of the annual fee.

The patent certificate records the legal status of the patent at the time when the patent was registered. The transfer, pledge, invalidation, cessation, recovery of the patent right and the changes of name, nationality, address of the patentee are recorded in the patent register.

Commissioner  
Tian Lipu

State Intellectual Property Office of the People's Republic of China  
Jan 11, 2016

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## Organic certification



ORGANIC FOOD DEVELOPMENT AND CERTIFICATION CENTER OF CHINA  
Certification No:IP0109-953-3\*\*\*

Certificate of Evaluation of Production Materials of Organic Agriculture  
Certify that

This evaluation certificate is used to confirm that the following organic agricultural production materials meet the requirements in Appendix A.1 of GB/T 19630-2019 《Requirements for the Production, Processing, Labeling and Management System of Organic Products》, and can be used for organic agricultural production certified in accordance with the above standards.

Product Name	Product Form	Registration No.	Classification
Microbial agent	Liquid	Microbial Fertilizer (2018) Approved No (3098)	Soil fertilizer and improvement materials

**Date of initial issue:** Feb 21, 2019  
**Date of issue:** Feb 21, 2020  
**Period of validity:** From Feb 21, 2020 to Feb 20, 2022

The organic agricultural production material evaluation certificate is not an organic product certification certificate. This certificate confirms that the product can be used in organic agricultural production that meets the above-mentioned standard certification based on the on-site evaluation and evaluation report, and is not a guarantee of the product quality itself. The quality of the product should be the responsibility of the manufacturer.

(Seal)ORGANIC FOOD DEVELOPMENT AND CERTIFICATION CENTER OF CHINA  
Special seal for certificate

8 Jiangwangmiao Street, Nanjing, 210042 China TEL: 025-85287246

The copy or fax of this certificate is invalid

*Thank you !*

Chico Crop Science Co., LTD

Add: Rm 903, Unit C, Tian An International Bldg, Renmin South Rd, Shenzhen, China

Tel: 0086-0755-22969266 22969199

Fax: 0086-0755-25919993

E-mail: [info@chicocrop.com](mailto:info@chicocrop.com)