

# The Bacterial Stalk Rot of Maize Caused by *Dickeya oryzae* in Taiwan

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## Abstract

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In 2017, water-soaked brown lesions were found in the stalk of the maize plants in Yuanchang and Huwei township, Yunlin County. The stalks were finally softened by the spreading lesions, resulting in hollowing and lodging of the plants. The bacteria were isolated from the diseased stalk tissues and cultured on nutrient agar. The pathogenicity of the bacteria was verified by Koch's postulates. The 16S rDNA, *gyrB* and *dnaX* gene sequences of the pathogen showed a high identity to *Dickeya zeae*, and multilocus sequence analysis revealed that the pathogen, *D. zeae* and *D. oryzae* were grouped as a clade. However, a publication of a new species of *D. oryzae* reassigned some *D. zeae* strains to *D. oryzae*, and the pathogen was further identified as *D. oryzae* based on the average nucleotide identity nucleotide of whole genome sequences in our study. This is the first report of maize bacterial stalk rot caused by *D. oryzae* worldwide. In the host range test, the pathogen could infect potatoes, carrots, onion bulbs, Welsh onions, rice, and cabbages; it therefore showed a potential threat to the agricultural industry. On screening agrochemicals, the 500-fold-diluted 20% oxolinic acid showed the most effective inhibition of the pathogen growth.

**Key words:** Maize, Bacterial stalk rot, *Dickeya oryzae*.

## INTRODUCTION

Maize (*Zea mays* L.) is an important miscellaneous grain crop, having the largest planting area in Taiwan. It can be used as feed and food. According to the 2022 Taiwan Agricultural Statistics Annual Report (<https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx>), the planting area of feed maize crops was 20,148 ha, and the main production areas

were Tainan City and Chiayi County. The planting area of the edible maize crops was 15,067 ha, with their main production area located in Yunlin County, Tainan City, Chiayi County, and Hualien County.

Many bacterial diseases of maize have been found in Taiwan, including bacterial stripe disease (pathogens *Acidovorax avenae* subsp. *avenae* and *Burkholderia andropogonis*), and bacterial soft rot (pathogens *Erwinia chrysant-*

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*themi* and *E. carotovora* subsp. *carotovora*) (Tzean *et al.* 2019). In addition, Stewart's wilt (pathogen *Pantoea stewartii*), Goss's wilt (pathogen *Clavibacter michiganensis* subsp. *nebraskensis*), bacterial stalk rot (pathogen *Dickeya zae*) (Jardine & Claflin 2016; Kumar *et al.* 2017), etc. were the international bacterial diseases causing maize wilt.

In 2017, some maize plants were observed to appear water-soaked, with brown lesions on the leaf sheath and stalk of the plants in Yunlin County, Taiwan. The brown lesions expanded and eventually softened the stalks. When cutting the stalk tissues of the diseased plant and observing it under a microscope, a large number of bacterial streaming out of the tissue was observed, which is suspected to be bacterial disease. Since the incidence of the disease in the field was about 10%, and there were no recommended agrochemicals to control the disease, the causal agent of this maize disease was identified in this study. In addition, the effects of agrochemicals on inhibiting bacterial growth were evaluated in the laboratory for future application in field control.

## MATERIALS AND METHODS

### The source of bacterial isolates

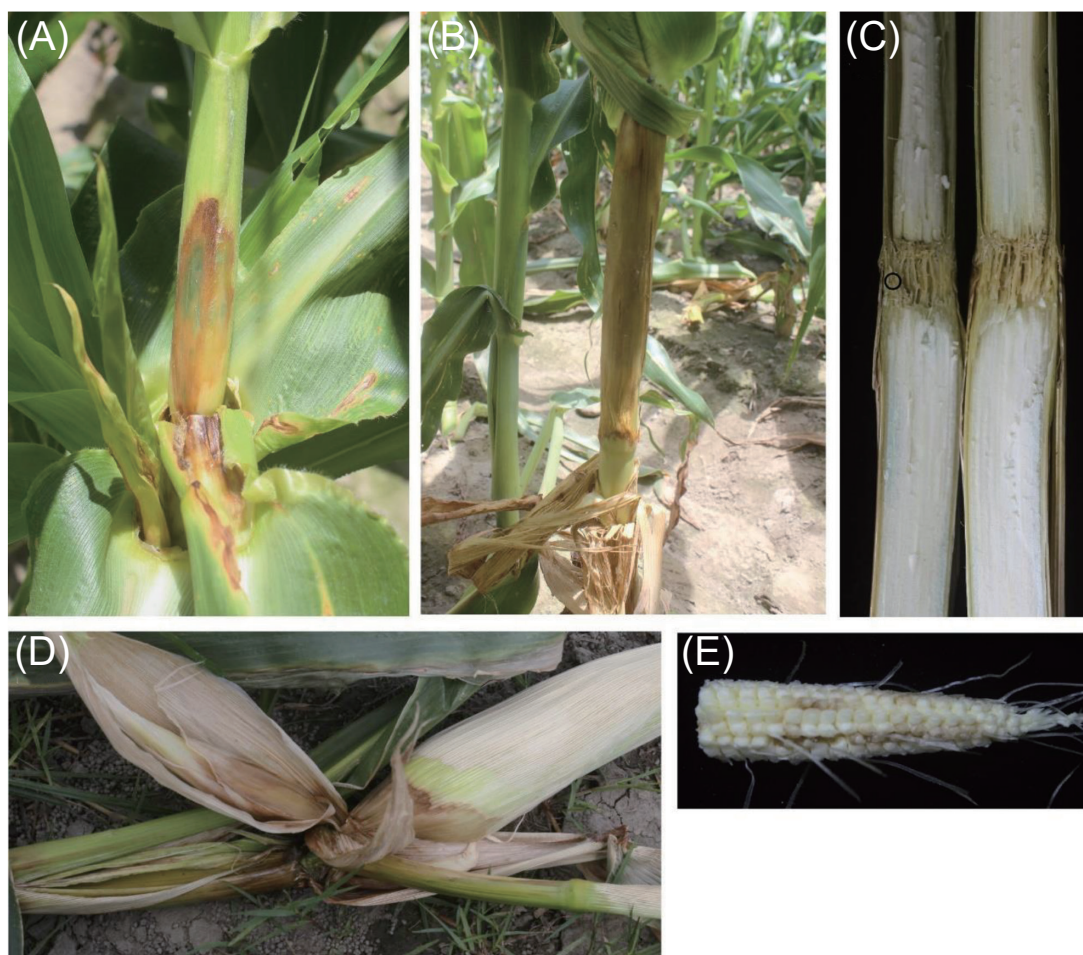
Diseased maize plants were collected from different fields in Huwei Township and Yuanchang Township, Yunlin County. The symptoms of water-soaked and brown lesions were observed on the leaf sheath and stalk of the maize plants (Fig. 1A). Then the brown lesions further expanded and spread on the stalk (Fig. 1B). The vascular bundles showed browning when the diseased stalks were cut longitudinally (Fig. 1C). Later, the stalks of the plants were hollowing, wilt, and toppled down. The infected ears of maize were brown and softened, which would reduce the commercial value of maize (Figs. 1D–E).

The maize plants with water-soaked and softened symptoms were collected from different fields. The diseased tissues of the plant

were cut out and sterilized in 0.5% sodium hypochlorite and then rinsed with sterile water 3 times. The tissues were further minced in sterile water to release the bacteria. The bacterial suspension was dipped with the loop and spread on the nutrient agar (NA) medium (Difco Laboratories; Becton, Dickinson and Company, Le Pont-de-Claix, France). The NA medium containing the bacteria was placed in a 30°C incubator for cultivation. The single colony of cultured bacteria was transferred to a new medium for purification. The purified bacteria were made into a bacterial suspension and injected into tobacco leaves, when the necrotic spots appeared after 1 d, indicating that the bacteria might be pathogenic. Therefore, the pathogenic bacteria from different fields were numbered and preserved for further identification and subsequent experiments. Two bacterial isolates, BSRM01 and BSRM02 were collected from Huwei Township, and the BSRM03 isolate was collected from Yuanchang Township.

### Biolog identification of the bacteria

Three bacterial isolates, BSRM01, BSRM02, and BSRM03, collected from different fields were purely cultured on the BUG™ medium (Biolog Universal Growth Agar, Biolog Inc., Hayward, CA, USA) in a 30°C incubator for 16–24 h. The single colony was picked up with a cotton swab and bacterial cells were suspended in IF-A inoculum (Biolog Inc., Hayward, CA, USA). The concentration of bacterial suspension was adjusted to 90–98% T (turbidity) with a turbidimeter, at a wavelength of 590 nm. The bacterial suspension was inoculated into the Biolog GEN III plates (Biolog Inc., Hayward, CA, USA), and each well of plates was inoculated into 100 µL inoculum. The inoculum GEN III plates were incubated for 8–24 h, and the color change was measured with a spectrometer. The reading values of the plate were analyzed using Biolog MicroLog™ 3 ver.5.22 system (database version 2.6.1).



**Fig. 1.** Symptoms of maize bacterial stalk rot observed in the field. (A) Water-soaked blotch on the surface of the stalk at the initial stage of the disease; (B) browning on the stem surface; (C) browning symptom in vascular bundle of the stem; (D) water-soaked browning of the diseased maize ears; and (E) browning and soft rot symptoms on maize kernels.

### PCR and gene cloning for sequencing

Three bacterial isolates, BSRM01, BSRM02, and BSRM03, were purely cultured on NA medium. The extraction of the bacterial total nucleic acid procedure described by Wang *et al.* (1993) was slightly modified as follows. The bacterial single colony of 3 bacterial isolates was individually dipped with a loop into 20  $\mu\text{L}$  of sterile water to be bacterial suspension. The 20  $\mu\text{L}$  of 0.4 N NaOH was added to the suspension and mixed well for 10 min, and 40  $\mu\text{L}$  of 1 M Tris-HCl (pH = 8.0) was subsequently added to the mix for neutralization. Then 20

$\mu\text{L}$  of suspension was pipetted for 10 $\times$  dilution with sterile water to serve as a DNA template.

The 16S rDNA, gyrase subunit B gene (*gyrB*), and DNA polymerase III gamma subunit gene (*dnaX*) partial sequences were amplification by PCR using the 16S rDNA universal primer pair f8-27/r1510 (Lipson & Schmidt 2004), the specific primer pair *gyrB*f1/*gyrB*r1 (Pu *et al.* 2012) for *gyrB* and the specific primer pair *dnaX*f/*dnaX*r (Sławiak *et al.* 2009) for *dnaX*. The PCR amplicons were analyzed on 1.4% agarose gel. The obtained expected DNA fragments were cloned with a T&A<sup>TM</sup> cloning

kit (Yeastern Biotech, Taipei, Taiwan), and the successfully cloned DNA fragments were sent to the biotech company for DNA sequencing. The obtained 16S rDNA (NCBI accession number ON430645), *gyrB* (NCBI accession number ON462306), and *dnaX* (NCBI accession number ON462307) sequences were deposited at GenBank and BLASTn in National Center for Biotechnology Information (NCBI).

### Multilocus sequence analysis (MLSA)

The BSRM01 was selected as a representative isolate for MLSA. Partial sequences of the 3 housekeeping genes 16S rDNA, *gyrB*, and *dnaX* of BSRM01 and the gene sequences of reference *Dickeya* sp. downloaded from NCBI were used for MLSA. The DNA sequences were aligned with Clustal W (Larkin *et al.* 2007) and further constructed a phylogenetic tree using neighbor-joining analysis with 1,000 bootstrap replicates by MEGA11 software (Tamura *et al.* 2021).

### Whole genome DNA sequence analysis

The bacterial isolate BSRM01 was purely cultured in 5 mL of nutrient broth for 24 h. The cultured bacterial suspension was extracted with the nucleic acid extraction kit (EasyPure stool genomic DNA kit) for total nucleic acid extraction. The total nucleic acids of bacteria were sent to a biotechnology company (Tri-i Biotech, Inc.) for Illumina MiSeq paired-end sequencing, and the sequences of short fragments were assembled using the SPAdes assembly software (Version 3.15.3) for de novo draft genome sequence. The draft whole genome sequence of bacterial isolate BSRM01 obtained by assembly estimated the average nucleotide identity (ANI) values with *D. zea* EC1 strain (GenBank accession CP006929.1), *D. oryzae* type strain ZYY5<sup>T</sup> (GenBank accession SULL00000000) and *D. zea* type strain NCPPB2538<sup>T</sup> (GenBank accession CM001977.1), respectively, using orthologous average nucleotide identity (OrthoANI) (Lee *et al.* 2016).

### Koch's postulates test

Three bacterial isolates, BSRM01, BSRM02, and BSRM03 were used in this test. After the bacteria were cultured on a nutrient agar medium plate for 2 d, the bacteria cells were suspended in sterile water, and measured with a spectrophotometer (spectrophotometer, Spectronic 70, Bausch & Lomb, Bridgewater, NJ, USA) at a wavelength of 600 nm. The bacterial suspension was adjusted to an absorption value of 0.3 (concentration is about  $10^8$  cfu mL<sup>-1</sup>) as the inoculation source. The test plants "Yumeizhen" maize were planted in a pot about 50 cm high. The inoculation method was to put a drop of bacterial suspension first 10  $\mu$ L on the stalk, and the stalk was then punctured with a sterile needle to create a wound for bacterial infection. Each bacterial isolate was inoculated with 3 plants. The control plants were treated with sterile water. After inoculation, the plants were kept moist in plastic bags for 24 h, and then the plastic bags were opened and placed in a growth chamber at 30°C for observation of disease symptoms. The pathogenic bacteria were re-isolated from the stalks of the diseased plants, and the isolated bacteria were confirmed to be the same as the inoculated isolates.

### Pathogenicity of the bacteria to different crops

In order to understand the host range of the bacterial isolate, different crops referred to Lin *et al.* (2016) were inoculated with 3 bacterial isolates, BSRM01, BSRM02, and BSRM03, for the inoculation test of various crops. The inoculated crops included rice plants, potato tubers, Welsh onions, carrot roots, onion bulbs, and cabbages; they were kept moist for 2 d after inoculation, and the symptoms of each crop were observed daily.

### Laboratory agrochemicals susceptibility test

The paper disc diffusion method (Adaska-

veg & Hine 1985) was used to test the effectiveness of several commercially available agrochemicals in inhibiting the bacterial pathogen of maize stalk rot.

BSRM01 (from Huwei Township) and BSRM03 (from Yuanchang Township) were selected as test isolates. The tested agrochemicals were selected from the plant protection handbook, and their testing concentration range was slightly adjusted according to the suggested concentration in the handbook. Four types of agrochemicals were used: (1) antibiotics, such as streptomycin + tetracycline (10.0% water soluble powder; SP), streptomycin (12.5% soluble concentrate; SL), kasugamycin (2.0% SL), oxolinic acid (20.0% wettable powder; WP), and validamycin (10% SL); (2) copper-containing agrochemicals, such as copper hydroxide (53.8% water dispersible granule; WG), copper oxychloride (85.0% WP), and tribasic copper sulfate (27.12% suspension concentrate; SC); (3) zinc-manganese-containing agrochemical such as mancozeb (80.0% WP); (4) mixed agrochemical, such as thiophanate methyl + streptomycin (68.8% WP), kasugamycin + copper oxychloride (81.3% WP).

The agrochemical testing method was as follows: first, prepare a bacterial suspension and adjust the concentration to approximately  $10^8$  cfu mL<sup>-1</sup>, add 0.1 mL of bacterial suspension to 6 mL of water agar, mix well, and then cover it with NA medium. Add 0.12 mL of each agrochemical diluted to different concentrations into a filter paper disk with a diameter of 13 mm (Whatman International Ltd., Chalfont St. Giles, UK), and then place the filter paper disk containing the agrochemicals on the NA medium covered with bacterial water agar. The filter paper disc dripped with sterile water was used as a control treatment, and each treatment was repeated 3 times. Afterwards, the culture plates of each treatment were placed in a constant temperature oven at 28°C for 48 h, and then the diameter of the inhibition zone (minus filter paper diameter 13 mm) was measured to determine the effect of agrochemicals.

## RESULT AND DISCUSSION

### Identification of bacteria by Biolog system

Three bacterial isolates, BSRM01, BSRM02, and BSRM03, from different fields were analyzed by the Biolog identification system to determine the physiological characteristics and the utilization of carbon sources. The bacterial isolates were cultured at 30°C for 16–24 h, and then the color change of the reaction plate was measured with a spectrometer. The measured readings were compared with the database by Biolog MicroLog™. These 3 bacterial isolates, BSRM01, BSRM02, and BSRM03, were most similar to *D. chrysanthemi*. Their similarity values were 0.638, 0.646, and 0.638, respectively, exceeding the critical value of 0.5.

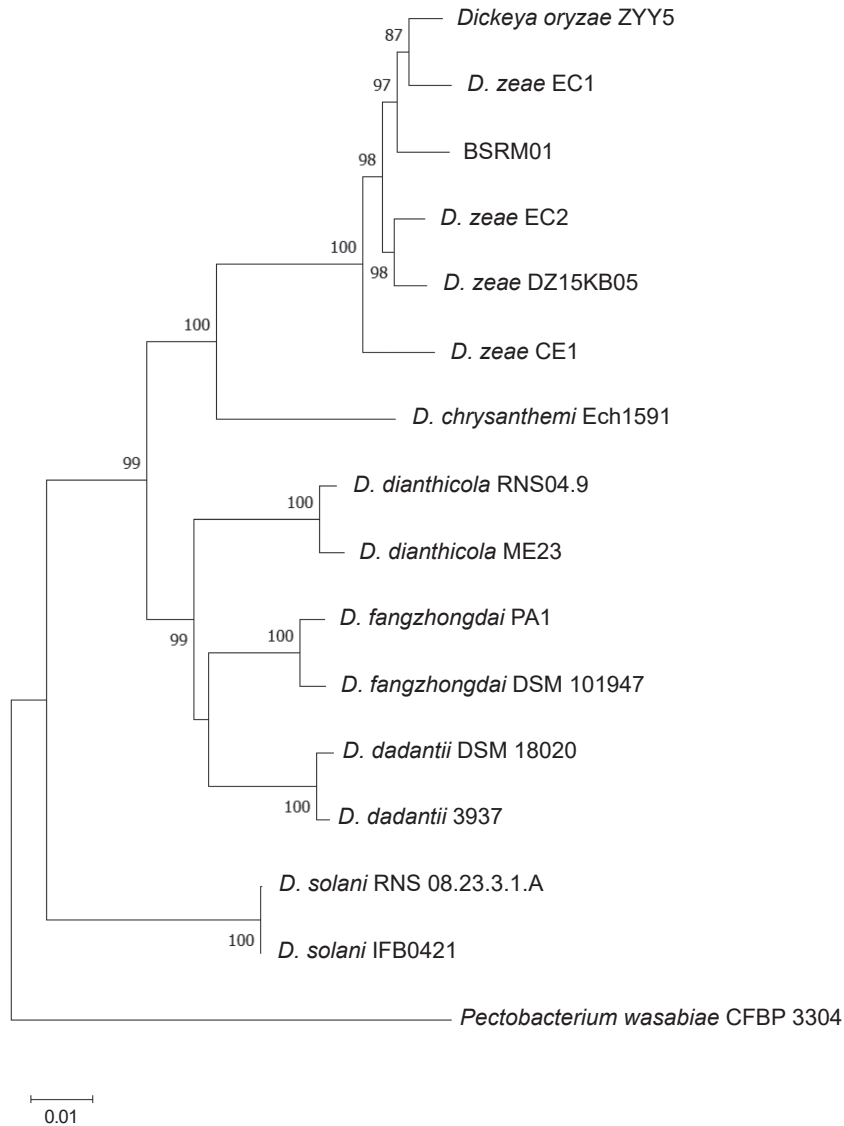
*D. chrysanthemi* was originally *Pectobacterium chrysanthemi*. Samson *et al.* (2005) established the genus *Dickeya* and reclassified *P. chrysanthemi* to 6 new species, namely *D. chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. dieffenbachiae*, *D. paradisiaca*, and *D. zaeae*. Since then, some new species of *Dickeya* have been published including *D. aquatic* (Parkinson *et al.* 2014), *D. solani* (van der Wolf *et al.* 2014), *D. fangzhongdai* (Tian *et al.* 2016), *D. lacustris* (Hugouvieux-Cotte-Pattat *et al.* 2019), *D. undicola* (Oulghazi *et al.* 2019), *D. poaceiphila* (Hugouvieux-Cotte-Pattat *et al.* 2020), *D. oryzae* (Wang *et al.* 2020), *D. parazeae* (Hugouvieux-Cotte-Pattat & Van Gijsegem 2021). However, the database of Biolog MicroLog™ didn't contain all the *Dickeya* sp. It was necessary to further identify the bacteria species.

### Molecular identification of pathogenic bacteria

The 16S rDNA, *gyrB*, and *dnaX* genes of the representative bacterial isolate BSRM01 from the gene cloning were sequenced. The obtained 16S rDNA sequence, *gyrB*, and *dnaX* were deposited to GenBank and BLASTn in the NCBI gene database. The results showed that

these 3 gene sequences had the highest identity with the sequence of *D. zea* strains, reaching more than 99%. MLSA was performed on the 3 genes, and a phylogenetic tree was constructed. The bacterial isolate BSRM01, *D. zea* and *D. oryzae* ZYY5 reference strains were assigned to a clade (Fig. 2).

*D. zea* was originally known as *P. chrysanthemi* pv. *zea*. Samson *et al.* (2005) reclassified *P. chrysanthemi* pv. *zea* to *D. zea*. In 2020, a new species of *D. oryzae* was published according to the differences in the whole genome sequence, and some strains originally belonging to *D. zea*, such as *D. zea* EC1 strain,



**Fig. 2.** A neighbor-joining phylogenetic tree of BSRM01 isolated from maize stalk rot plant and reference strains, based on partial 16S rDNA, DNA gyrase subunit B gene, and DNA polymerase III gamma subunit gene sequences. The number on the branches represents bootstrap values. The scale bar indicates 1 nucleotide change per 100 nucleotides. Bootstrap values are indicated at branch points based on 1,000 replications. Bootstrap values below 60% are not shown.

should be reassigned as *D. oryzae* (Wang *et al.* 2020). The MLSA result indicated that the bacterial isolate BSRM01 was very close to *D. zeae* EC1 and *D. oryzae* ZYY5<sup>T</sup>. The bacterial isolate BSM01 should be further analyzed by whole gene sequencing to clarify its species status.

### Whole genome DNA sequence analysis of the bacterial isolates

From the above, the bacterial isolate BSRM01 was very close to *D. zeae* EC1 and *D. oryzae* based on the phylogenetic analysis of MLSA (Fig. 2). However, *D. zeae* EC1 was suggested to be reclassified to *D. oryzae*. In order to clarify the taxonomic status of the bacterial isolates, the extracted total nucleic acid of bacteria was sent to a biotechnology company for whole-genome sequencing. The genome was sequenced using the Illumina MiSeq pair-end sequencing system, and the sequences of short fragments were assembled using SPAdes assembly software (Version 3.15.3).

The assembled genome sequence of the BSRM strain was 4,959,956 bp long, with 42 contigs, an N50 of 324,712 bp, and an average coverage of 177.8.

The assembled draft genome sequence of the bacterial isolate BSRM01 (NCBI accession number JBCGHM000000000) was compared with *D. zeae* type strain NCPPB2538<sup>T</sup>, *D. zeae* EC1 and *D. oryzae* type strain ZYY5<sup>T</sup> for calculation of ANI. The results showed that the identity of the isolate BSRM01 shared 94.61% with *D. zeae* NCPPB2538<sup>T</sup>, 95.58% with *D. zeae* EC1, and 95.8% with *D. oryzae* ZYY5<sup>T</sup>. The ANI values of BSRM01 with *D. oryzae* ZYY5<sup>T</sup> and *D. zeae* EC1 were more similar than BSRM01 with *D. zeae* NCPPB2538<sup>T</sup>.

Wang *et al.* (2020) established new species of *D. oryzae* and reassigned *D. zeae* EC1 as *D. oryzae*. The generally accepted species boundary for ANI values is 95–96% (Lee *et al.* 2016). The ANI value of BSRM01 was 95.8% with *D. oryzae* ZYY5<sup>T</sup> and 95.58% with *D. zeae* EC1, both of which were in the species boundary 95–96%. Therefore, the BSRM01 strain was classified as *D. oryzae*.

### Koch's postulates test

Bacterial isolates BSRM01, BSRM02 (isolated from Huwei Township), and BSRM03 (isolated from Yuanchang Township) were purely cultured on the nutrient agar and made into a bacterial suspension with a concentration of about 10<sup>8</sup> cfu mL<sup>-1</sup>. The bacterial suspension was inoculated on the 'Yumeizhen' maize plant with the puncture stem method. After 2 d of inoculation, the maize plant appeared water-soaked at the inoculation site, and then the stalk showed symptoms of browning and softening. The maize plants gradually wilted and toppled down after 7 d. When the stalk of diseased plants was cut longitudinally, the vascular bundles showed obvious browning. The symptoms of the inoculated plants were the same as those seen in the field. The plants inoculated with sterile water were symptomless, as shown in Fig. 3. The same pathogenic bacteria could be isolated from the stalk of the diseased plants after inoculation.

### Pathogenicity of the bacteria to different hosts

The results of different plants inoculated with the tested bacterial isolates BSRM01, BSRM02, and BSRM03 revealed that the bacteria could infect several economically important crops. Three days after the inoculation, the 3 bacterial isolates caused obvious symptoms of tissue softening and browning on rice, potato, carrot, onion bulb, and Welsh onion (Fig. 4). However, the pathogenicity to Chinese cabbage was relatively weak, and black-brown necrotic spots were limited at the inoculation site (Fig. 4D).

*D. oryzae* previously known as *D. zeae* (Wang *et al.* 2020). *D. zeae* could infect various plants and cause soft rot symptoms in crops and ornamental plants, such as maize, rice, pineapples, chrysanthemums, potato, banana tobacco, tomatoes, eggplants, peppers and clivia (Samson *et al.* 2005; Kumar *et al.* 2017; Hu *et al.* 2018). In Taiwan, Lin *et al.* (2016) reported that *D. zeae* caused rice bacterial foot



**Fig. 3.** Symptoms of the maize plants after artificial inoculation with bacterial isolates BSRM01, BSRM02, and BSRM03. (A) The plant showed wilting symptoms at 7 d after inoculation (left), and plants showed symptomless when inoculated with sterile water (right). (B) Close-up of the vascular browning symptoms.

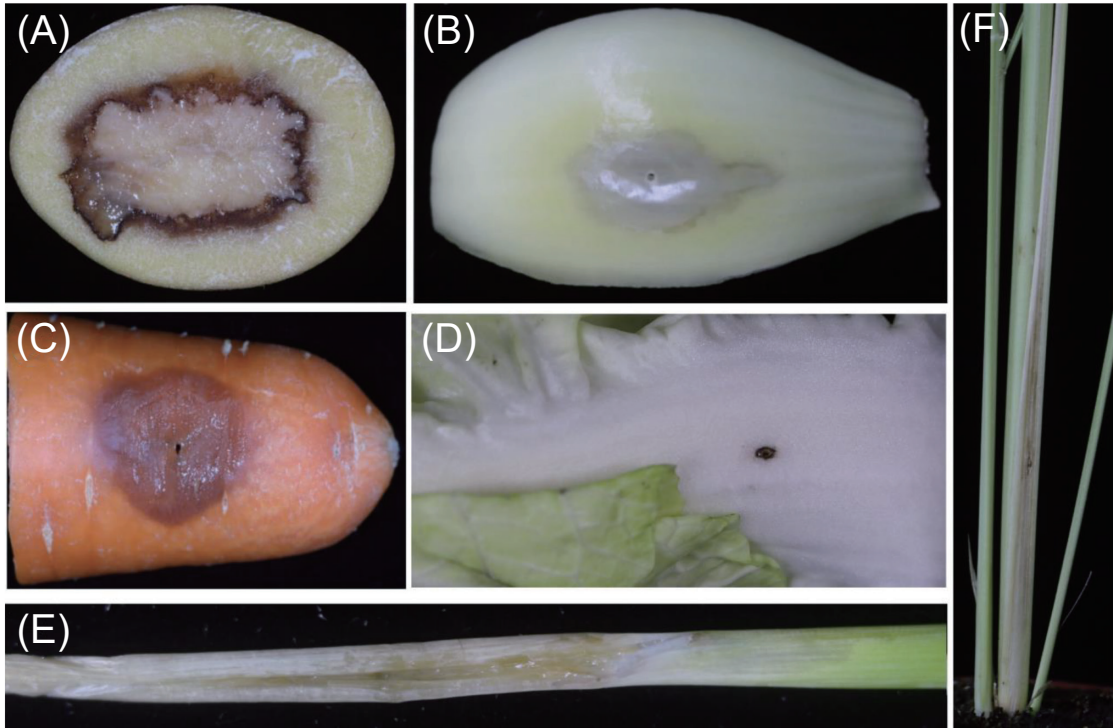
rot and could infect several economic crops through inoculation tests. Our host range test of *D. oryzae* was similar to *D. zea* described by Lin *et al.* (2016), showing that *D. oryzae* is a potential threat to various economic crops in the agricultural industry.

### Susceptibility of agrochemicals tested in laboratory

Disease control of bacterial stalk rot of maize has no recommended agrochemicals at present. However, when the environment is suitable for the occurrence of this disease, it may cause significant losses to farmers. Therefore, it is important

to evaluate those commercial agrochemicals for reference in disease control. The growth inhibitory effects of 10 agrochemicals listed in the plant protection manual were tested by the filter paper disc diffusion method. By measuring the diameter of the inhibition zone, the 4 treatments of agrochemicals, consisting of oxolinic acid, streptomycin + tetracycline, streptomycin, and thiophanate-methyl + streptomycin, yield effects on the growth inhibition of the bacterial isolates. Among these treatments, the oxolinic acid treatment produced the largest inhibition zone (Table 1). These results can be applied in subsequent greenhouse and field control experiments.





**Fig. 4.** Symptoms of different crops inoculated with maize bacterial isolates BSRM01, BSRM02, and BSRM03. (A) Browning soft symptom showed on potato slices at 4 d after inoculation; (B) water soaking and soft rot symptoms showed on onions at 4 d after inoculation; (C) browning soft symptom showed on carrots at 3 d after inoculation; (D) necrotic spot symptoms showed on Chinese cabbages at 7 d after inoculation; (E) soft rot symptom showed on shallots at 7 d after inoculation; and (F) browning symptom showed on rice seedlings at 4 d after inoculation.

## CONCLUSION

In 2017, some maize plants showed water-soaked and soft rot symptoms on stalks in the fields of Yunlin County. These symptoms were similar to bacterial stalk rot of maize described in the literature (Kumar *et al.* 2017). The bacteria isolated from diseased maize tissues were confirmed as the causal pathogen through Koch's postulates test and further identified as *D. oryzae* through gene sequence BLASTn, MLSA and ANI analysis. This is the first report of maize bacterial stalk caused by *D. oryzae* in Taiwan.

*D. oryzae* originally belonged to *D. zeae*. *D. zeae* was known to cause rice bacterial root rot in Taiwan and distributed throughout Taiwan (Lin *et al.* 2016). Our host range test showed that *D. oryzae* was similar to *D. zeae*

and was a potential threat to the agricultural industry. There were currently no recommended agrochemicals for controlling this disease. We tested 10 commercial agrochemicals for their effects in inhibiting bacterial growth and screened out 4 agrochemicals to provide reference for further disease control.

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**Table 1.** Growth inhibition of *Dickeya oryzae* by various agrochemicals at different concentrations.

Chemical <sup>z</sup>	Dilution fold	Inhibition zone (mm in diameter)	
		BSRM01	BSRM03
Oxolinic acid (20% WP)	500	6.93 ± 0.244 a <sup>y</sup>	7.43 ± 0.12 a
	1,000	5.70 ± 0.21 b	5.70 ± 0.20 c
	2,000	5.13 ± 0.12 c	4.97 ± 0.23 d
Streptomycin + tetracycline (10% SP)	500	5.97 ± 0.12 b	7.07 ± 0.03 b
	1,000	3.80 ± 0.36 e	5.53 ± 0.27 c
	2,000	3.37 ± 0.27 f	4.37 ± 0.13 e
Streptomycin (12.5% SL)	500	4.83 ± 0.13 c	4.50 ± 0.31 e
	1,000	3.80 ± 0.17 e	3.53 ± 0.15 f
	2,000	3.33 ± 0.15 f	3.03 ± 0.09 g
Thiophanate methyl + streptomycin (68.8% WP)	500	4.23 ± 0.12 d	3.73 ± 0.12 f
	1,000	2.77 ± 0.33 g	3.07 ± 0.03 g
	2,000	2.57 ± 0.32 g	2.63 ± 0.18 h
Kasugamycin (2.0% SL)	125	0 h	0 i
	250	0 h	0 i
	500	0 h	0 i
Kasugamycin + copper oxychloride (81.3% WP)	500	0 h	0 i
	1,000	0 h	0 i
	2,000	0 h	0 i
Copper hydroxide (53.8% WG)	750	0 h	0 i
	1,500	0 h	0 i
	3,000	0 h	0 i
Copper oxychloride (85.0% WP)	200	0 h	0 i
	400	0 h	0 i
	800	0 h	0 i
Tribasic copper sulfate (27.12% SC)	250	0 h	0 i
	500	0 h	0 i
	1,000	0 h	0 i
Mancozeb (80.0% WP)	250	0 h	0 i
	500	0 h	0 i
	1,000	0 h	0 i
LSD		0.41	0.31

<sup>z</sup> WP: wettable powder; SP: water soluble powder; SL: soluble concentrate; SC: suspension concentrate; LSD: Fisher's protected least significant difference test.

<sup>y</sup> Mean ± standard error ( $n = 3$ ). Means followed by the same letter in the same column are not significantly different at 5% level by LSD test.

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## *Dickeya oryzae* 引起之玉米細菌性莖腐病

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### 摘要

蔡佳欣、黃淑苓、古家榮、林玫珠、林靜宜、關政平、陳金枝。2024。 *Dickeya oryzae* 引起之玉米細菌性莖腐病。台灣農業研究 73(3):169–180。

2017 年在雲林縣元長鄉與虎尾鎮的玉米田區，部分植株出現莖部水浸狀病斑，之後莖部隨著病斑上下蔓延而軟化，導致植株空心與倒伏。從罹病莖部組織可在營養瓊脂上分離出 1 種細菌，該細菌之病原性可經過科霍氏法則得到驗證。該病菌以 16S rDNA、*gyrB* 及 *dnaX* 基因序列比對，與 *Dickeya zeae* 具有高度相同性，以多基因序列分析 (multilocus sequence analysis)，顯示病菌與 *D. zeae* 及 *D. oryzae* 歸屬於同一群。由於 *D. oryzae* 新種的發表，一些 *D. zeae* 分離株被重新歸屬為 *D. oryzae*，因此將該病菌進一步經全基因體序列定序，以平均核苷酸相似度 (average nucleotide identity) 分析，將該病菌鑑定為 *D. oryzae*。本文為世界上 *D. oryzae* 引起玉米細菌性莖腐病之首次報告。在寄主範圍測試，該病菌除玉米外，尚可感染馬鈴薯、胡蘿蔔、洋蔥、青蔥、水稻及白菜，對農產業顯示出潛在的威脅。在室內藥劑篩選試驗，以 20% 歐索林酸稀釋 500 倍對該病菌的生長的抑制效果最佳。

**關鍵詞：**玉米、細菌性莖腐病、細菌性莖腐病菌。

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