Study on the Bacterial Rot of Sunflower
1 Symptom and Identification of the Pathogens

Gao Jie Yuan Meili Feng Jun Ma Guilong Zhang Jiahuan
(Jilin Agricultural University, 130118)

ABSTRACT It is the first report of sunflower bacterial stalk rot in China. 20 isolates of the bacteria obtained from diseased sunflower plants in different regions of the Northeast China were tested. According to their pathogenic, morphological, cultural, physiological, biochemical as well as serological characteristics, the pathogens were identified to be Erwinia carotovora subsp. carotovora Dye, Erwinia carotovora subsp. atroseptica Dye and Pseudomonas caryophylli (Burkholder) Starr et Burkholder.

Sunflower bacterial stalk rot is a new disease in China. The main breeding and commercial fields were investigated in Shangyang of Liaoning, Kangjinjing of Heilongjiang and Changchun, Baicheng, Changling of Jilin from 1988 to 1994. The results showed that average incidence of the disease in breeding fields was 5.47%, that of some inbred lines was 46% and that in commercial fields is 2.56%. According to the record in Kangjinjing, it was very serious in breeding fields in 1987 and the disease incidence of some strains was as high as 100%.

1. Symptom and Identification of Isolates

From second to last ten days of July, the disease occured in Northeast China as the sunflowers mature and heads form. The organisms may attack stalks and heads, mainly stalks. Infected tissues are brown or black at first, then become wet rot and gradually spread up and down and toward the pith. Brown or black soft rot happens when the organisms have reached the pith. At last, the stalk is hollowed and weakened. Under the weight of maturing head, the stalk is bent and collapsed. When the organisms invade heads, primary lesions are water-soaked, irregular shape and gradually become brown or black. The lesions

develope rapidly and cause brown or black soft rot of the whole head. Finally, the head is hollowed (only connected by cellulose) in dry condition or may rot off in suitable weather.

2. Identification of Isolates

2.1. Materials and Methods

Remove a small part of tissue from the advanced portion of the lesion using a sterile scalpel. The tissue should be washed with sterile water or sterilized for 3 minutes in a dilution(1:10) of household bleach. After rinsing in sterile water, the tissue should be chopped up with a sterile scalpel in a droplet of water in petri dish. After sitting for 2 or 3 minutes, the macerate is streaked on NBY medium with a wire loop. Inoculated petri dishes were incubated at 28 C for two days, well-isolated individual colonies of the bacteria were restreaked to ensure culture. The pure isolates were inoculated to maturing sunflower stalks by wounds. 20 bacterial strains have been proved to be pathogenic to sunflowers, transferred to YDC medium and maintained at4 °C for later use.

Cell morphology, stainning reactions, cultural, physiological and biochemical characteristics were determined by standard microbiologic procedures. Authority of determining the bacteria is "Bergey's Manual of Systematic Bacteriology "1st edition.

Control strains Ecc. and Eca.are got respectively from the Chinese Academy of Sciences and the Minnesota University of the U.S.A.

2.2. Results

2.2.1. Tests of Pathogenicity

All pathogenic strains can cause sensitive necrotic reaction on tobacco. Two strains of isolates which cause slightsoft rot on carrot after inoculating for 48 hours are classified to group SFP. The others of isolates which cause serious soft rot on carrot are classified to group SFE.

2.2.2. Morphology and Stainning Reaction

Group SFP: Two strains are similar, straight rods, 0.65 to 0.71 by 1.60 to 1.70μm, Gram-negative, no capsule, polar flagella (one or more). They can accumulate PHB as a carbon reserve.

Group SFE: 18 strains are similar, short straight rods, o.60 to 0.90 by 1.20 to 1.40μm, Gram-negative, no capsule, pertitrichous flagella.

2.2.3. Bacterial Cultural Characteristics

Group SFP: Two strains form round, convex, grey-white, shiny, smooth, non-opaque colonies on NBY medium. The colonies measure 0.8 to 1.0 mm in diameter after inoculating for 48 hours on NBY medium. They grow vigorously on YDC medium. Old cultures can make the medium brown. The bacteria can not grow on D1 medium, can not produce fluorecent pigment on KB medium and can not form deep pit on CVP medium.

Group SFE: 18 strains form round, grey-white, translucent, convex, smooth colonies which are measured 0.80 to 1.0 mm in diameter on NBY medium after inocubating for 48 hours. The bacteria grow vigorously on YDC medium, can not grow on D1 medium and can not produce fluscent pigment on KB medium. They can form deep-pit on CVP medium.

2.2.4. Physiological and Biochemical Tests

Group SFP: Two strains have the same characteristics of physiology and biochemistry(see table 1).

Group SFE: According to the difference in reducing substance from sucrose, acid from α-methyl-glucoside, V.P. reaction, growth at 36°C, the eighteen strains are classified as two subgroup: SFE-I which includes12 strains and SFE-II which includes 6 strains (see table 2).

2.2.5. Serological Reaction

Antigens for making antiserum are strains 88-29, 88-37 which belong to subgroup SFE-I and strain 88-33 which belongs to subgroup SFE-II.

The results show that 12 strains ofsubgroup SFE-I produce aggutination with the antiserum of strains 88-29 and 88-37 at dilution ratio 1:1280 and should have the same source with strains 88-29 and 88-37. Control strains Ecc. produce the same aggutination as the 12 strains of SFE-I. All the strains of SFE-II produce aggutination with the antiserum of strain 88-33

at dilution ratio 1:1280 and should have the same source with Strain 88-33. Control strains Eca. produce the same aggutination as the 6 strains of SFE-II. The strains of SFE-I and antiserum of strain 88-33 produce aggutination at 1:640, so do the strains of SFE-II and antiserum of strains 88-29 and 88-37. Strains 88-29, 88-37 and 88-33 havesome relationship, but not completely the same source.

The results of serological reactions do correspond to that of physiological and biochemical tests (see table 3).

Table 1 Physiological and biochemical tests of group SFP

strains / results	88-3	88-17	Ps. caryophylli *
Diffusible pigment	V. brown	V. brown	V. brown
Arginine dihydrolase	+	+	+
Oxidase	+	+	+
Nitrate reduction	+	+	+
Growth at 40 C	+	+	+
Gelatin liquefaction	-	-	-
Starch hydrolysis	-	-	-
Utilization of carbon source			
D-Arabinose	+	+	+
Cellobiose	+	+	+
m-Tartrate	+	+	+
Mannitol	+	+	+
Sorbitol	+	+	+
L-Rhamnose	+	+	+
Sucrose	+	+	+
Glucose	+	+	+
β-Alanine	-	-	-

* Data come from the "Laboratory guide for identification of plant pathogenic bacteria" 2nd Edition(1988)

3. Conclusions

3.1. Strains 88-3 and 88-17 of group SFP are Gram-negative, polar flagella (one to many), grey-white colonies on NBY medium, oxidase negative and arginine dihydrolase negative. The bacteria can accumulate poly-β-hydroxybutyrate, produce brown diffusible pigment on YDC medium and grow at 41 °C, but can not grow on D1 medium. They can utilize the carbon source as arabinose et al, but can not utilize β-alanine. According to the 1st edition of "Bergey's Manual of Systematic Bacteriology", the present strains

are determined to be Pseudomonas caryophylli(Burkholder).

Table 2 Physiological and biochemical tests of group SFE

Strains / results	Group SFE-1	Group SFE-1I	Ecc*	Eca**
Sensitivity to erythromycin	−	−	−	−
Reducing substances from sucrose.	−	+	−	+
Phosphatase	−	−	−	−
Gas from glucose	−	−	−	−
Pectate degradation	+	+	+	+
Gelatin liquefaction	+	+	+	+
Acid from -methyl-glucose	−	+	−	+
V. P. reaction	+	−	+	−
Aerobic & Anaerobic	F.An.	F.An.	F.An.	F.An.
H2S production	+	+	+	+
Indole	−	−	−	−
Lecithinase	−	−	−	−
Oxidase	−	−	−	−
Growth in 5% NaCl	+	+	+	+
Growth at 36 C	+	−	+	−
Tween 80 hydrolysis	−	−	−	−
Starch hydrolysis	−	−	−	−
Utilization of carbon sourc:				
Glucose	+	+	+	+
Sucrose	+	+	+	+
Arabinose	+	+	+	+
Mannose	+	+	+	+
Rhamnose	+	+	+	+
Lactose	+	+	+	+
Xylose	+	+	+	+
Raffinose	+	+	+	+
Inositol	D	−	D	−
Melezitose	−	−	−	−
Cellobiose	+	+	+	+
Mannitol	+	+	+	+
Sorbitol	+	+	+	+
Dextrin	−	−	−	−
Glycerol	−	−	D	−
Maltose	−	+	−	+
Esculin	+	+	+	+

SFE-1 group includes 12 strains such as 88-6,9,18,19,21,22, 25,28,29,31.
SFE-1I group includes 6 strains such as 88-12,27,30,32,33,3
* Standard control strain.
** Known control strain.

Table 3 Test of serological reactions

Strains		Antiserum of 88-33			Antiserum of 88-37			Antiserum of 88-29		
		1:320	1:640	1:1280	1:320	1:640	1:1280	1:320	1:640	1:1280
SFE-I	6	++++	+++	++	++++	++++	+++	++++	++++	+++
	9	++++	+++	++	++++	++++	+++	++++	++++	++++
	21	++++	+++	++	++++	++++	+++	++++	++++	++++
	22	++++	+++	++	++++	++++	+++	++++	++++	+++
	24	++++	+++	++	++++	++++	+++	++++	++++	+++
	25	++++	+++	++	++++	++++	+++	++++	++++	+++
	28	++++	+++	++	++++	++++	+++	++++	++++	++++
	29	++++	+++	++	++++	++++	++++	++++	++++	++++
	31	++++	+++	++	++++	++++	+++	++++	++++	++++
	37	++++	+++	++	++++	++++	++++	++++	++++	++++
	18	++++	+++	++	++++	++++	+++	++++	++++	+++
	19	++++	+++	++	++++	++++	+++	++++	++++	+++
	Ecc	++++	+++	++	++++	++++	+++	++++	++++	++++
SFE-II	12	++++	++++	+++	++++	+++	++	++++	+++	+
	27	++++	++++	+++	++++	+++	++	++++	+++	++
	30	++++	++++	+++	++++	+++	++	++++	+++	++
	32	++++	++++	+++	++++	+++	++	++++	+++	++
	33	++++	++++	++++	++++	+++	++	++++	+++	++
	34	++++	++++	+++	++++	+++	++	++++	+++	++
	Eca	++++	++++	+++	++++	+++	++	++++	+++	++
SFP	3	-	-	-	-	-	-	-	-	-
	17	-	-	-	-	-	-	-	-	-

Notes: ++++ aggutinate completely, topssolution clear
+++ aggutinate distinctly, top solution turbid slightly
++ aggutinate partly, top solution turbid
+ aggutinate little, top solution turbid
- no aggutination, whole solution turbid

3.2. 18 strains of group SFE are Gram-negative and facultative anaerobic. They have peritrichous flagella, form grey-white colonies on NBY medium and deep pit on CVP medium and cause serious soft rot on fresh vegetable such as potato and sensitive necrosis reaction on tobacco leaves. The bacteria can not produce gas from glucose, or grow on D1 medium. They are not sensitive to erythromycin but can degradate pectate and liquefacte geltin. The organisms produce H_2S from peptone, reduce nitrate, grow in solution of 5% NaCl and produce acid from carbon source such as glucose ; but do not produce indole and acid from melezitose, dextrin and gly-

cerol. From these above, 18 strains can be identified as Erwinia carotovora. Among them, 12 strains of group SFE-I grow at 36°C but can not produce reducing substance from sucrose and acid from α-methyl-glucoside. V.P. reactions are positive. These 12 strains can be identified as Erwinia carotovora subsp. carotovora. The other 6 strains of group SFE-II can not grow at 36°C but can produce reducing substance from sucrose and acid from α-methyl-glucoside. V.P. reactions are negative. They can be identified as Erwinia carotovora subsp. atroseptica. All of the results above have been proved by the serological tests.

4. Discussion

Allen, D.T.(1974) had reported that the pathogen of sunflower stalk rot in Tanzania was Erwinia aroideae. Fucikovsky et al.(1978) proved Erwinia carotovora subsp. carotovora and E. c. subsp. atroseptica to be the pathogens of sunflower soft rot in Mexico. Mazzucchi (1981) identified the pathogen as E. c. subsp. carotovora in Italy. In 1987, Arenijevic,M. et al and Akhtar,M.A. et al had reported the pathogen of sunflower stalk rot respectively in Yugoslavia and Pakistan and identified it as E. carotovora. In China, the present writers identified 3 kinds of bacteria from the sunflower stalk rot in Northeast China: Erwinia carotovora subsp. carotovora Dye, E. c. subsp. atroseptica Dye and Pseudomonas caryophylli (Burkholder) Starr et Burkholder. But Ps. caryophylli (Burkholder) appeared to be an opportunistic pathogen which might act secondarily to increase the symptom.

REFERENCES

1. Allen,D.T. 1974. Plant Disease 58:596-599.

2. Akhtar,M.A. et al. 1987. Proceeding of the 12th International Sunflower Conference. Vol.II. p.150.

3. Arenijevic,M. et al. 1987. Proceeding of the 12th International Sunflower Conference. Vol.II. pp.151-153.

4. Gudmested,N.C. et al. 1984. Plant Disease 68:189-192.

5. Richeson,M.L. 1981. Plant Disease 65:1019-1021.

6. Schaad,N.W. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd Edition.

7. Krieg,N.R. and Holt,J.G. 1984. Bergey's Manual of Systematic Bacteriology 1. Williams & Wilkins, Baltimore.