A NEW DISEASE (BACTERIAL WILT) OF TAIWAN GIANT BAMBOO[1]

I. Studies on the Causal Organism (*Erwinia sinocalami* sp. Nov.)

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Introduction

According to W. C. Lin (1961, 1963), 35 species, varieties and cultivars, belonging to 11 genera in family Bambusaceae have been recorded in Taiwan. Among them, the widely distributed and economic bamboos are: *Phyllostachys makinoi* Hayata, *Sinocalamus latiflorus* (Munro) McClure, *Bambusa stenostachya* Hackel, *Leleba dolichoclada* Odashima, *Phyllostachys edulis* Houzeau de Lehaie and *Leleba oldhami* Nakai. The total cultivation area of the six species is 75,275 hectares.

The culms of bamboos are used for making building materials, furniture, fishing rods, umbrella handles, and walking sticks. The strings of the culm of bamboos are utilized to produce bamboo baskets, ribs of umbrella and lantern, bamboo curtains and other handicraft products. The leaves of bamboos are used for wrapping rice-dumplings and making rain-hats. The subterrenean stem is used for walking sticks, and pipes. The branches of bamboos are utilized for making brooms and bamboo fences. The bamboo-fibers are slender and long, and are superior to the fibers of conifers and rice-straws for making paper, in Taiwan, 103,000 metric tons of bamboo were used for this purpose each year, from 1961 to 1964. The bamboo shoot is edible, and have been a favorite vegetable for Chinese and Japanese in spring, summer and fall. The shoots of *Sinocalamus latiflorus* enjoy the greatest reputation because of its delicious taste. The bamboo shoot in canned or in dried form, is one of the important export items from Taiwan. According to Summary of Inspection & Quarantine Statistics volumes 10, 11 and 12, 156,446 cases of canned bamboo shoots were exported in 1960, 178,878 cases in 1961, and 180,873 cases in 1962.

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Unfortunately, in recent years a hitherto unrecorded epidemic disease of bacterial wilt of bamboo shoot, has broken out in main bamboo cultivation areas of this island, especially in Nantou, Yuanlin and Chiayi. The disease not only ravages the young shoots, reducing yield and quality, but symptoms of the wilt appear on infected young shoot has become a full grown bamboo and at times the bamboo dies before reaching maturity.

According to the record of a farmer at Luku, Nantou, in his 8 hectares of bamboo field, 917.4 Kg of bamboo shoot in dried form was harvested in 1954, 660 Kg in 1955, 600 Kg in 1956, 540 Kg in 1957, 448.8 Kg in 1958, 444.6 Kg in 1959, 345.6 Kg in 1960 and 240 Kg in 1961. From the data mentioned above, although the reduction of the harvest may be caused by several factors, the disease is considered to be the most important.

The purpose of the study is to identify the causal organism of the new disease of bamboo shoot, and to seek out control measures of the disease.

Materials and Methods

From August to September, 1963, diseased shoots of *Sinocalamus latiflorus* (Munro) McClure, and *Leleba oldhami* Nakai were collected from several cultivation areas at Nantou Hsien, and isolated by Dowson streaking plate method (1949). After pathogenicity test on the shoots of *S. latiflorus* by multiple needle inoculation at Tali, Taichung, from September to October, 1963, 10 isolates were able to induce the typical symptom. The source of the isolates are listed in Table 1.

Table 1. *The source of 10 tested isolates*

Isolate No.	Host	Locality	Date of isolation
6301	Shoots of *S. latiflorus*	Taho	Aug. 22, 1963
6302	Shoots of *S. latiflorus*	Yenshan	Aug. 22, 1963
6303	Shoots of *S. latiflorus*	Chushan	Aug. 24, 1963
6307	Shoots of *S. latiflorus*	Chushan	Sept. 7, 1963
6308	Shoots of *S. latiflorus*	Luku	Sept. 7, 1963
6309	Shoots of *S. latiflorus*	Luku	Sept. 7, 1963
6313	Shoots of *S. latiflorus*	Tayen	Sept. 7, 1963
6314	Shoots of *S. latiflorus*	Hsiangchieh	Sept. 7, 1963
6319	Shoots of *S. latiflorus*	Hsiufeng	Sept. 9, 1963
6320	Shoots of *L. oldhami*	Chushan	Sept. 11, 1963

The procedures and methods for determining bacteriological properties, Manual of Microbiological Methods, edited by the Society of American Bacteriologists (1957) were followed. Special techniques used in individual experiments will be referred in the respective sections.

Symptoms

The disease appears first on the margin of outer sheath at the sprouting stage, the lesions are pink or brown in color and elliptic or irregular in shape, and then bordered by a dark brown margin. The spots are large and form a series of bands across the sheath as the disease progresses. In severely affected ones, infection take place through the low epidermis into the inner sheaths, and the whole shoot decays immediately. Although, in less severely affected ones, the shoot may grow as culm, but a large, brown spots will appears at the end of the culm, and developed downwards, the culm dries within a few months.

The disease chiefly appears on *Sinocalamus latiflorus*, and least severe in *Leleba oldhami*. Up to present time, the disease have not been found in other cultivated species of Bambusaceae in this island.

Causal Organism

I. The Morphology and Staining Properties of the Causal Organism

In order to observe the Gram reaction, acid-fast property, presence or absence of endospores, arrangement of flagella and the size of the bacterium, the isolates which inoculated on the nutrient agar slant and incubated at 30°C for 24 hrs. were stained by Hucker modification, Ziehl-Neelsen's method, Wirtz's method, Löffler's method and Benian's congo red method. Potato agar slant cultures incubated at 30°C for 24 hrs. were stained by Anthony's method for detecting the presence of capsule.

The results indicate that the causal bacterium is a Gram negative, not acide-fast, nonspore-former, nonchain-former, capsulated and 2–8 peritrichous flagella rod, exhibiting size range of 0.72–0.89×1.53–1.87μ.

II. The Cultural Characteristic of the Causal Organism

All isolates give white, circular, entire-margin, convex, smooth, moist, translucent, homogenous colonies on nutrient agar plate at 30°C for 48 hrs.

The test isolates grow moderately in nutrient broth, and are uniform in turbidity, viscid in precipitation, with pellicle on surface.

No growth or poor growth is obtained from all isolates in Cohn's, Uschinsky's and Fraenkel's solutions.

III. The Physiological Characteristics of the Causal Organism

1. The effect of temperature on multiplication of the organism

Nutrient broths were inoculated with 0.5 ml. of various inoculum, and incubated at different temperature, e. g., 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52°C for 48 hrs. The turbidity was detected by photoelectric colorimeter.

The temperature range of growth of organism was 8–44°C, the optimum temperature for growth being 32°C

2. The effect of hydrogen-ion concentration on multiplication of the organism

Portions of nutrient broth were adjusted to pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0. After inoculation with various isolates and incubated at 32°C for 48 hrs., the turbidity was determined by photo-electric colorimeter too, the records show that the pH range for growth is 4.0–9.0, the optimum pH for growth being 7.0.

3. Carbohydrate fermentation by the causal organism

Ayers, Rupp and Johnson's synthetic media were used as a basic medium, in which arabinose, rhamnos xylose, glucose, fructose, galactose, mannose, lactose, sucrose, maltose, starch, inulin, dextrin, glycerol, mannitol and salicin were supplemented for the fermentation test. In addition, 0.1% of a 1.6% alcoholic solution of bromcresol purple was added as indicator. After sterilization in Arnold sterilizer at 100°C for 20 minutes on 3 consecutive days, the prepared media were inoculated with the test isolates and incubated at 32°C for 20 days.

The results indicated that all 10 isolates produced acid but no gas was formed from arabinose, xylose, glucose, fructose, galactose, lactose, sucrose, maltose and salicin, no acid and no gas was formed from rhamnose, starch and inulin. The results showed different fermentation abilities for four other carbohydrates, 5 isolates (6308, 6309, 6314, 6319 and 6320) fermented dextrin, 6 isolates (6301, 6302, 6303, 6307, 6309, and 6313) fermented glycerol, 6 isolates (6301, 6302, 6303, 6307, 6313 and 6314) fermented mannitol and 5 isolates (6301, 6302, 6303, 6308, and 6313) fermented sorbitol.

4. Physiological characteristics of the organism other than carbohydrate fermentation

1). Hydrolysis of starch: Inoculated starch nutrient agar was incubated at 32°C for 3 days and when tested by Lugol's iodine method produced a blue color, indicating that starch was not hydrolyzed by test isolate.

2). Reduction of nitrates: Inoculated nitrate broth were incubated at 32°C for 10 days, then, tested by alpha-naphthylamine and sulfanilic acid, no pink or red color occurred. A blue color was obtained by adding diphenylamine reagent, indicating that nitrate was not reduced to nitrite by test isolate.

3). Liquefaction of gelatin: Inoculated nutrient gelatin was incubated at 32°C for 10 days, and tested by Smith's modification method. The colonies were surrounded by a clear zone, indicating that gelatin was liquefied by test isolate.

4). Production of ammonia: Inoculated peptone water was incubated at 32°C for 20 days, and tested by Nessler's reagent method, a yellow-orange precipitate formed, indicating that ammonia was produced by the test isolate.

5). Production of hydrogen sulfide: A strip of filter paper moistened with lead acetate was suspended in the tube of inoculated peptone water, and held in position by the cotton plug, no blackening of the paper could be detected after 20 days at 32°C, indicating that no H_2S was produced by test isolate.

6). Production of indole: An oxalic acid test strip was suspended in the tube of inoculated peptone water. A pink color was obtained after 20 days incubation at 32°C, indicating that indole was produced by the test isolate.

7). Hydrolysis of casein: Inoculated milk agar was incubated at 32°C for 4 days, clear zones surrounding the colonies were not found in this test, indicating that casein was not hydrolyzed by the test isolate.

8). Reduction of litmus: Litmus was reduced in a period from two to ten days in sterile milk.

9). Relation to free oxygen: The tubes of glucose-nutrient agar were inoculated with the test isolates while in fluid condition. The inoculum and the medium were mixed thoroughly, cooled, and incubated at 32°C for 48 hrs. Colonies appeared only on the surface of the agar, indicating that test isolates were aerobes.

IV The Pathogenicity Test of the Causal Organism

Potato, egg-plant, tomato, radish, carrot, sorghum, corn, wheat, rice, barley and apple were inoculated with the suspension of the causal organism by multiple needle method. No lesions or symptoms were revealed on close examination.

Bamboo shoots of *Phyllostachys edulis, P. makinoi,* and *Bambusa stenostachya* were inoculated by injection and multiple needle, no wilt or other symptoms were found. In natural conditions it is also true that the three cultivation species remained unaffected by the causal organism.

Taxonomical Considerations

From consideration of bacteriological properties of five genera of bacterial plant pathogens, e. g., *Xanthomonas, Pseudomonas, Erwinia, Corynebacterium* and *Agrobacterium,* described by Bergey's Manual (1957), Elliott (1951) and Dowson (1949), the causal organism of the bacterial wilt of bamboo shoot is classified as a species belonging to the genus of *Erwinia.*

Although 17 species are recognized in the genus of *Erwinia,* only two species, e. g., *E. amylovora* and *E. carotovora* are similar to the causal organism. A comparative description of the bacteriological properties of the two species organisms and the causal bacterium are given in Table 2.

Table 2. *The comparative characters of the causal bacterium, Erwinia amylovora and E. carotovora*

Character \ Pathogen	Causal bacterium	*E. amylovora*	*E. carotovora*
Size of the rods	0.72-0.89×1.53-1.87μ	0.7-1.0×0.9-1.5μ	0.6-0.9×1.5-5.0μ
Flagella	2-8 peritrichous	Peritrichous	1-6 peritrichous
Gram reaction	Gram negative	Gram negative	Gram negative
Capsules	Capsulated	No capsules	No capsules
Endospores	No	No	No
Agar colonies	Circular, white-yelow or white, entire-margin	Circular, grayish white, irregular margin	Circular, gray-white entire-margin
Hydrolysis of starch	−	−	−
Reduction of nitrates	−	−	−
Liquefaction of gelatin	+	+	+
Production of ammonia	+	+	−
Production of indole	+	−	−
Reduction of litmus	+	+	+
Relation to oxygen	aerobes	aerobes	aerobes
Production of H_2S	−	−	−
Acid without gas from carbohydrates	arabinose, xylose, glucose, fructose, galactose, lactose, sucrose, maltose and salicin, acid production from dextrin, mannitol, sorbitol and glycerol variable.	arabinose, fructose, glucose, sucrose, maltose, mannose, cellobiose, raffinose and salicin, acid production from lactose and glucose variable.	arabinose, xylose, glucose, fructose, galactose, lactose, sucrose, maltose, rhamnose, glycerol, mannitol and salicin.
Pathogenicity	*Sinocalamus, Leleba*	Rosaceae	Carrot, cabbage, celery, cucumber, egg-plant, iris, muskmelon, onion, pepper, tomato, potato, radish and turnip.

From the above comparative description, three organisms are similarly in their staining properties, cultural and physiological characteristics, but it should be noted that the indole was produced by the causal organism, which is contrary to the description of *E. amylovora* and *E. carotovora* in Bergey's Manual. The difference of the pathogenicities are also distinct.

Hino (1961) reported that 43 families, 219 genera and 454 species of Ascomycetes, 20 families, 50 genera and 97 species of Basidiomycetes, and 10 families, 106 genera and 195 species of fungi imperfecti had been found as the causal organism of bamboo in Japan. He also pointed out that certain species of bacteria isolated from diseased sugarcane were shown to pathogenic to *Leleba vulgaris* Nakai in the artificial inoculation experiments, though the bacteria have never been known to infest the bamboo under natural conditions.

It is also true that no record of any bacterial disease of bamboo or any *Erwinia* on *Sinocalamus*, *Leleba* or any other bambusaceae have been found in Taiwan or in other countries. Therefore, the authors intend to describe this bacterium as a new species.

Technical description of the bacterium:

Erwinia sinocalami sp. nov.

Rods, 0.72–0.89×1.53–1.87μ, Gram negative, not acid fast, capsules, no spores, motile by 2–8 peritrichous flagella. Nutrient agar colonies, circular, white, entire margin, smooth. Starch and casein not hydrolyzed, nitrates not reduced, gelatin liquefied, litmus reduced, ammonia and indole produced, no hydrogen sulfide. Acid but no gas from arabinose, xylose, glucose, fructose, galactose, lactose, sucrose, maltose and salicin, no acid, no gas from rhamnose, starch and inulin, acid production from dextrin, mannitol, sorbitol and glycerol variable.

Summary

The bacterial wilt of bamboo shoot, a hitherto unrecorded disease in Taiwan was found at Chushan in 1954. The disease is characterized by appearing the pink or brown, elliptic or irregular lesions on the margin of outer sheath at the sprouting shoots. The spots are large and form a series of bands across the sheath as the disease progresses.

The causal bacterium was isolated, its bacteriological characteristics also have been carried out. The bacterium is a rod-shaped, 0.72–0.89×1.53–1.87μ in size, capsulated, without endospore, Gram negative, not acid-fast, motile by means of 2 to 8 peritrichous flagella. Nutrient agar colony is circular, white with entire margin and smooth. Starch and casein are not hydrolyzed, nitrates are not reduced, gelatin is liquefied, litmus is reduced, ammonia and indole are produced, no hydrogen sulfide is produced by the causal bacterium. Acid but no gas is produced from arabinose, xylose, glucose, fructose, galactose, lactose, sucrose, maltose and salicin, no acid, and no gas are produced from rhamnose, starch and inulin, acid production from dextrin, mannitol, sorbitol and glycerol variable.

The causal bacterium does not attack the apple, some vegetables and bambusaceae except *Sinocalamus* and *Leleba*.

The causal bacterium can be conceived to be a new species.

臺灣麻竹之一新病害(細菌性萎凋病)

I. 病原細菌 (*Erwinia sinocalami* sp. nov.) 之研究

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蔬竹細菌性萎凋病，爲一未見記載之新病害。自民國四十三年發現以來日趨嚴重，目前本省之蔬竹及綠竹已普遍發生。其病徵，最初於筍籜之邊緣產生紅褐色斑塊，繼之擴大而呈重層之褐色斑紋，因此，竹筍之生長受阻，嚴重時往往招致萎凋死亡，輕則竹筍雖可生長成稈，但常自其梢部形成褐色乾枯之大斑塊，而向下蔓延，終致全株枯死，故對竹筍及竹材之生產影響至鉅。

本病病原，經反復試驗檢定，證明爲一 Gram 氏陰性、非抗酸性、不含內生胞子，但具有莢膜及 2-8 周生鞭毛之桿狀細菌。在肉汁瓊脂平板上爲圓形，黃白色或白色，全緣之菌落。在肉汁培養基中，生長中等，混濁均勻，而有粘質沈澱，其表面具有輪環或表膜。在 Cohn, Uschinsky 及 Fraenkel 氏培養液中，生長不良或無生長。本病原細菌爲一能產生氨及吲哚，液化白明膠及還原石蕊，但不水解澱粉，不還原硝酸鹽，不產生硫化氫之好氣性細菌。在 arabinose、xylose、glucose、fructose、galactose、lactose、sucrose、maltose 及 salicin 上可醱酵，但不能利用 rhamnose、inulin 及 starch。其中部份菌株可在 dextrin、glycerol、mannitol 及 sorbitol 中產生酸。本病原細菌之生長溫度範圍爲 8-44°C，最適溫度爲 32°C，其 pH 範圍爲 4.0-9.0，最適生長 pH 值則爲 7.0。

本病原細菌之病原性，以多針接種法接種於孟宗竹、桂竹、莿竹及長枝竹上皆不發病，另以塗佈法及針刺法接種於馬鈴薯、茄子、蕃茄、紅蘿蔔、蘿蔔、玉米、高粱、小麥、水稻及蘋果上，亦無病徵顯現。

根據病原細菌之周生鞭毛及使 salicin 醱酵之特性，本病原細菌應屬於 *Erwinia* 屬。屬於 *Erwinia* 之植物病原細菌，已知者計 17 種，其中 *E. amylovora* 及 *E. carotovora* 二種在生理性質上雖與本病原細菌較爲相近，但其病原性則甚相懸殊，故證明其爲一新種，而定名爲 *Erwinia sinocalami* sp. nov.

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PLATES

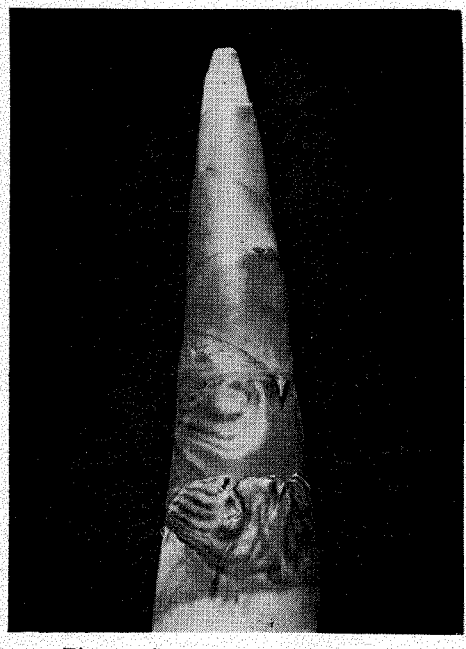

Fig. 1. Symptom on the shoot of *Sinocalamus latiflorus* by natural infection.

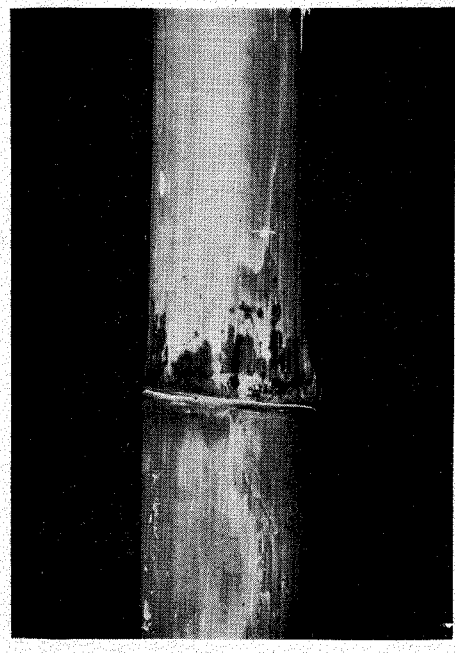

Fig. 2. Symptom on the culm of *Sinocalamus latiflorus* by natural infection.

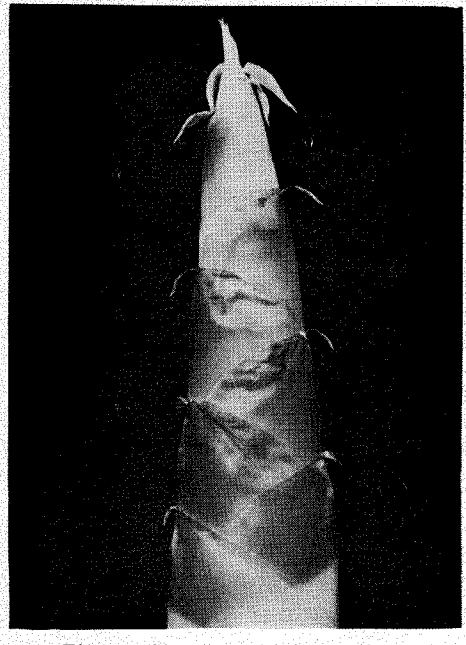

Fig. 3. Symptom on the shoot of *Sinocalamus latiflorus* after inoculation by multiple needle method.

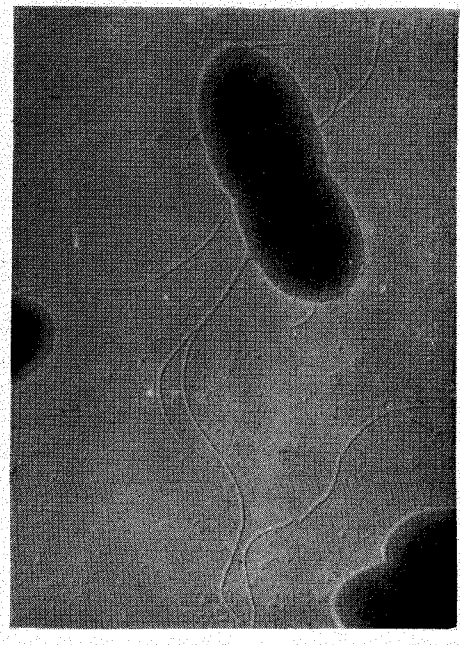

Fig. 4. The electron micrograph of the causal bacterium, *Erwinia sinocalami* sp. nov. (16,000×)