The Causal Pathogen of Bacterial Blight of Mulberry and Its Control

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The first occurrence of bacterial blight of mulberry in Japan was reported by Hori in 1901[1]. This disease has widely distributed throughout silkworm rearing areas in Japan. It is one of the most important diseases that causes serious damage to mulberry trees, and the damage has been gradually increasing. For the control of the disease, investigations on characteristics and ecology of the causal bacteria, varietal reaction of mulberry and chemical control etc. are necessary. In the present paper, the research which has been carried out 1969 to 1975 at the Sericultural Experiment Station, M. A. F. F., in Tokyo is summarized briefly.

Strains of the pathogen

Over 140 isolates of causal bacterium were obtained from diseased mulberry leaves and shoots collected from 32 prefectures distributed from Okinawa to Hokkaido, Japan. Bacteriological properties of the foregoing pathogen were much the same as those of *Pseudomonas mori* (Boyer et Lambert) Stevens reported by Smith (1920) and others. The isolates of *P. mori* collected could be classified into the following strains on the basis of causal symptoms on mulberry, colony types, phage sensitivity, biochemical characteristics, serological properties and drug resistance.

Ordinary-strain and halo-strain[11,12]: The pathogn of bacterial blight infects only young leaves and twings, by invading through stomata and wounds caused by wind, rain and insect attack. The isolates of *P. mori* were classified into 2 strains of ordinary and halo-blight on the basis of the difference of symptoms.

Ordinary-strain causes tissue necrosis at mulberry leaves or shoots. Diseased leaves become wilted, malformed and curled inwards. Brownish black streaks appear along the vein and petiole of the leaves and spotted, withered lesions appear on the leaves. Top necrosis of young shoots is caused occasionally. Black necrotic streaks appear on shoots.

Halo-strain was found out in an area of Shimane Prefecture in 1971. This strain caused necrotic spots with halo on leaves and top chlorosis of young shoots by inducing toxin production. Bacteriological, and serological properties as well as its host range were much the same as those of *P. mori*.

State[4]: In the first, rough colony type (R type) of the pathogen was isolated from a subculture of ordinary smooth colony type (S type) *P. mori* S6914-1 (Hino-shi, Tokyo). The R type strain differed from the wild strain in some characteristics, such as non-motility and non-fragllum. This bacterium grew up long filaments, 11 to 200 nm in length and 0.8 nm in width. Later the R type was isolated accidentally from mulberry tree in the field. Also, this type was obtained from a subculture of motile ordinary, smooth strain by culturing on modified King's medium for a long time. They showed a positive reaction on agglutination test by anti-*P. mori* (S type) serum, sensitivity to seven strains of *P. mori* phage, and pathogenicity to mulberry.

Phagovar[7]: Phage sensitivity of *P. mori* was studied by drop method using 125 isolates of bacteria and 49 isolates of *P. mori* phage. As the result, *P. mori* isolates were classified

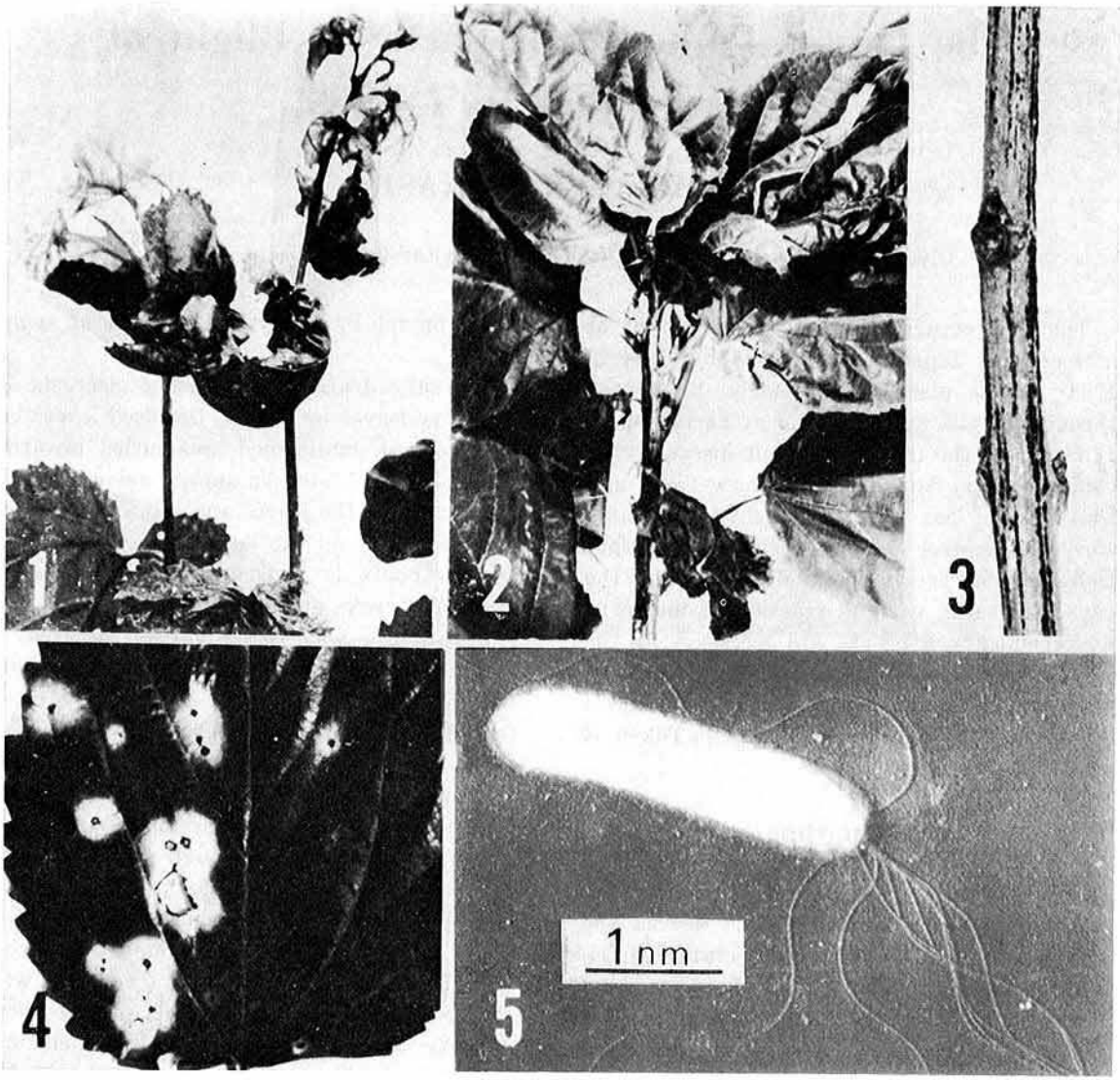

Plate 1. The bacterial blight of mulberry

1–3 Symptoms caused by ordinary-strain. 1. Leaf curl. 2. Top necrosis of young shoot. 3. Black necrotic streak on shoot. 4. Halo blight lesion caused by halo-strain. 5. *Pseudomonas mori* (Boyer et Lambert) Stevens.

into 5 phagovars named A, B, C, D, and E according to sensitivity to 6 phage strains. Of these phagovars, B was most widely distributed in Japan constituting 25.6% of the total isolates. Other phagovars A, C, D, and E were less widely distributed than B. Still others which could not be classified by phage sensitivity showed 19.2% of the total. No regional peculiarity was observed in the distribution of these phagovars in Japan.

Biovar: Strain classification by decomposition of xylose and rhamnose was conducted using 44 isolates of *P. mori*. As the result, fermentative-type capable of hydrolyzing xylose or rhamnose numbered 42 or 24, respectively.

Serological reaction and serovar: Anti-*P. mori* serum was obtained by means of intravenous injection to rabbit or injection using tumorous (Ehrlish's) ascites against mouse with living bacteria of 3 isolates. It showed a positive reaction on agglutination test using some isolates of *P. phaseolicola*, *P. glycinea*, *P. lachrymans*, *P. striafaciens*, *P. tabaci*, *P. eriobotryae* and *P. cichorii* as well as all isolates of *P. mori*, but not against each isolate of another 16 species of *Pseudomonas*, 8 of *Xanthomonas*, 2 of *Erwinia*, *Klebsiella*, and 1 of *Corynebacterium*, *Eschrichia*, *Proteus*, *Serratia*, *Staphylococcus*, *Yorsinia*, *Bacillus* respectivitly.

The antigen analysis of *P. mori* with agar-gel double diffusion test has been carried out using anti-*P. mori* serum obtained by mean of intravenous injection with 3 isolates of *P. mori* to rabbit. As the result, it was classified into 3 groups, serovars a, b, and c.

Drug resistance[9]: Proportions of dihydrostreptomycin (SM) resistant cells in each isolate were assayed on modified King's media added with SM at concentrations of 1,000, 100 and 10 ppm. Usually, the higher the concentration of SM, the fewer the colonies produced. The rates of SM resistant *P. mori* isolates collected from different mulberry cultivating districts were 75% at the concentration of 10 ppm, 59% in 100 ppm and 19% in 1,000 ppm. The proportions of the resistant cells appeared in the isolates were about 10^{-8} to 10^{-9} at the concentration of 100 ppm. Some isolates developed well even on the media containing 5,000, 10,000 and 20,0000 ppm. Usually, SM resistant *P. mori* isolates were susceptible to oxytetracyclin.

Ecological study of the pathogen

In order to determine the overwintering habitats of the pathogen, the following experiments have been made[2]. The pathogen was successfully isolated by the ordinary dilution plating method from all the diseased shoots, but it was usually difficult to isolate from the soils. Furthermore, *P. mori* phage was well isolated from the soil samples of the diseased field in February and March, although not from a non-mulberry farmland soil. Of all 11 isolates of the phage collected, 5 showed a specificity for *P. mori*, but the remainder made an attack on *P. phaseolicola* and *P. glycinea* beside *P. mori*. The pathogen could survive for a long time of more than 150 days in the non-sterilized soil controlled by some factors as mentioned later. On the other hand, the pathogen was also isolated from the specimens such as fallen leaves and buds collected during winter. In view of the results so far achieved, it was concluded that *P. mori* overwintered mainly within the diseased shoots and soils, sometimes within the fallen leaves.

Some facters conducive to the survival of the pathogen in soils were studied[3]. The duration of survival in sterilized soils was influenced by the size of soil particles, that is, the bacteria in a soil composed of particles smaller than 3 mm in diameter survived longer than those in a soil consisting of particles smaller than 0.59 mm. The pathogen decreased more rapidly in the non-sterilized soils than in the sterilized soils and this tendency was remarkable at 20° or 30°C. The duration of survival in the non-sterilized soil held at 5°C was varied by the bacterial concentration added into the soil. It was 20 days when a bacterial suspension of about 10^8/ml was added, and 53 days when a bacterial suspension of about 10^{10}/ml or a bacterial colony was added. When the bacterial suspension was added into each supernatant of both homogenates, mulberry leaves and mulberry roots, the bacteria survived for 100 days in the former and more than 150 days in the latter. The duration of survival in air-dried, sterized soil was much the same as that in moist soil, but it was somewhat longer in the dried soil than in the moist soil under the non-sterized conditions.

The duration of survival of the pathogen in mulberry tissues was studied[6]. The pathogen in mulberry leaves and shoots which were packed in a paper bag survived more than 7

years when the bag was kept in a refrigerater (5°C). The duration of survival was from 40–290 days (in paper bag) to 143 days—7 years (in desiccator) at the room temperature showing shorter period than in the refrigerater.

Effects of some physical factors on growth and survival of the pathogen were studied[5]. The pathogen was killed almost completely by exposing it to sunlight for about 30 min in May and October. It was also killed when exposed to UV light (15 W, 40 cm) for 40 sec., although the survival rate was markedly increased when the concentration of bacterial suspension was higher than 10^8/ml. The pathogen was able to grow at a temperature range of 2.5 to 34°C, showing the optimal growth at 28 to 32°C. Thermal death point in water was 46°C and 52°C, respectively, when the concentration of the bacteria was 10^3/ml and 10^9/ml, and that in dry air was 110°C. The pathogen survived longer in sterilized distilled water when the bacterial concentration was high and the temperature of incubation was low. Furthermore, the pathogen dried on a cover glass survived for 37 days in a desiccator, and able to grow at pH 4 to 10.

The author and his co-workers investigated the mode of occurrence of this disease in the field (Hino-shi, Tokyo) from 1969 to 1973. As the result, onset of the disease was observed from April to May, then the disease increased markedly during the rains in early summer. In August, the disease tended to decrease gradually. On the other hand, the rate of latent infection of the pathogen in that particular field was 20% in April, 70% in May to June, and extremely low in later seasons. This result was thought to represent the seasonal changes in bacterial population in the field.

Reaction of mulberry varieties[12]

Most of mulberry varieties in Japan can be classified into the following three groups: Yamaguwa-type (*Morus bombycis* Koidz.), Karayamaguwa-type (*M. alba* L.) and Roguwa-type (*M. latifolia* Poiret). Generally, Yamaguwa-type cultivars distributed in northern area of Japan and Roguwa-type cultivars in western area of Japan are resistant to the bacterial blight. The Karayamaguwa-type cultivars in all prefectures in Japan are susceptible. Both cultivars, 'Ichinose' and 'Kairyo-nezumigaeshi' occupied respectively 55% and 26% of mulberry fields of Japan in 1977. These cultivars belonging to the Karayamaguwa-type group were pointed out to be extremely susceptible to bacterial blight. However, newly improved cultivars, 'Shinichinose' and 'Minamisakari' (Karayamaguwa-type × Roguwa-type) which were suitable to western area of Japan or warm area showed a high resistance to bacterial blight. In the other hand, pathogenicity of isolates collected from resistant varieties infected in fields showed stronger than that of isolates collected from susceptible ones. Therefore the improvement and extension of cultivars must be considered as an important problem for disease control.

Control of bacterial blight[10]

During the utilization of agricultural chemicals, we must be cautious to their residual toxicity to silkwarm larvae. Thus the following antibiotics, streptomycin-oxytetracyclin mixture (Agrimycin-100) which is harmless to the insect, was sprinkled at a 500 fold dilution over leaves and young shoots at three times or more.

Chemical control using antibiotics is effective to some extent. Other procedures to diminish the damage are as follows: planting of resistant cultivar, cutting off overwintered branch deseased, removal of waste shoots from field soil, insect pest control and good field management, suitable application of nitrogenous fertilizer and efficient utilization of sunshine etc.

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