*Bot. Bull. Academia Sinica* **13**: 111-117 (1972)

# BACTERIAL LEAF BLIGHT OF RICE PLANT

## V. The nature of rice wilt induced by *Xanthomonas oryzae* polysaccharide[1][2]

H. N. FAN and T. T. KUO[3]

**Abstract**

*Xanthomonas oryzae* produces extracellular polysaccharide which is able to wilt rice cuttings. Since the wilting was produced by very low concentration (10 ppm) of those polysaccharides, it was thought to be caused by toxigenic action rather than by plugging of xylem vessels. The results of present study, however, seem to favor the plugging theory. The polysaccharide blocks the acid fuschine transport of rice cuttings; dextran with a molecular weight of 150,000 can induce similar wilting. Enzyme studies give no sign of the presence of an active site. No damage of cell permeability by the polysaccharide was found.

### Introduction

Previous report (Kuo *et al.* 1970) has shown that *Xanthomonas oryzae* produces polysaccharides capable of inducing wilt in plant cuttings. The substances have molecular weights of 200,000, or bigger with glucose and mannose making up most of their weight. They are antigenic (Lin *et al.* 1969). Rice plant cuttings treated with these polysaccharides show leaf wilting. Since only 10 ppm of the purified substance is needed to wilt the rice cuttings, it is toxigenicaly similar to *Corynebacterium sepedonicum* polysaccharide which induces wilting of tomato cutting (Strobel, 1967). However, *X. oryzae* polysaccharide has a MW 10 times greater than that of *C. sepedonicum* and its effect on rice physiology is still not known. In this investigation the effect of *X. oryzae* polysaccharide on dye transport of rice cuttings, the wilt inducibility of dextran, the water soluble polysaccharide with similar MW, and the effect of these polysaccharides on rice cell permeability etc. were studied.

### Materials and Methods

*Xanthomonas oryzae* strain 604, isolated from infected rice plants from

(1) This work was supported by National Science Council. Republic of China.
(2) Paper No. 124 of the Scientific Journal Series, Institute of Botany, Academia Sinica.
(3) Respectively, Assistant Research Fellow and Research Fellow.

Taichung in 1970, was grown in 800 ml semi-synthetic medium at 25–30°C on a rotary shaker for a minimal of 7 days. The medium contains 5g bactopeptone, 15 g sucrose, $10^{-2}$ M tris buffer pH 7.5, 1 g Ca $(NO_3)_2$, 5 mg $FeCl_3$, 1 mg $MnCl_2$, 1mg $MgSO_4$ and 500mg $NH_4Cl$ per liter of distilled water. The synthetic medium eliminated possible polysaccharide contamination from potato extract in case of PS medium (Kuo *et al.* 1970). It was important to incubate bacteria for more than 7 days. Because the polysaccharide thus prepared could induce rice wilt at a lower concentration and it also gave better yield. Cells were removed by centrifugation at 16,300xg for 10 minutes. The supernatant was concentrated to 200 ml with Buchler's flash evaporator. 95% alcohol was added to the concentrated supernatant until final alcohol concentration was about 75% and let the polysaccharide precipitate in 5°C for over night. The crude polysaccharide was then collected by centrifugation at 16,300xg for 10 minutes and dried between 40–45°C for 24 hours.

The rice-seedlings (Taichung Native 1) grown in the green house about 20 cm in length were used in all experiments. Bioassay followed the method of Kuo *et al.*, (1970), except 2-hour pre-immersion in MKN solution was omitted. The assay was done in Sherer, Model cel 37–14, controlled environment chamber which was set at 30°C, 7,000 lux, and relative humidity at 70%. 0.1% acid fuschin-MKN solution was used in dye transport experiments. Because acid fuschin is water soluble and dose not precipitate the bacterial polysaccharides. Dextran with MW 150,000 was purchased from Pharmacia, Uppsala, Sweden.

Bronwill Biosonik was used for sonication. Commercially purchasced amylase, cellulase, lysozyme, papain, pronase and trypsin were used to digest the bacterial polysaccharides. Infrared spectrum was done by a Perkin-Elmer model 21 double beam infrared spectrophotometer. Conductivity was measured at 28°C by a YSI medel 31 conductivity bridge.

## Results

*The production of polysaccharide in different age of culture and the effect of these polysaccharides in inducing wilting of rice cuttings:* The bacteria were grown in semisynthetic medium at 28°C with shaking. At two-day interval samples were taken, and the polysaccharide was harvested by precipitating with alcohol. After drying the polysaccharide was directly weighted. The effect of polysaccharide in inducing wilt was also assayed. The results are shown in Fig. 1. The production of polysaccharide was low in the first four days but greatly increased after 8th day. The polysaccharide produced in aged culture was

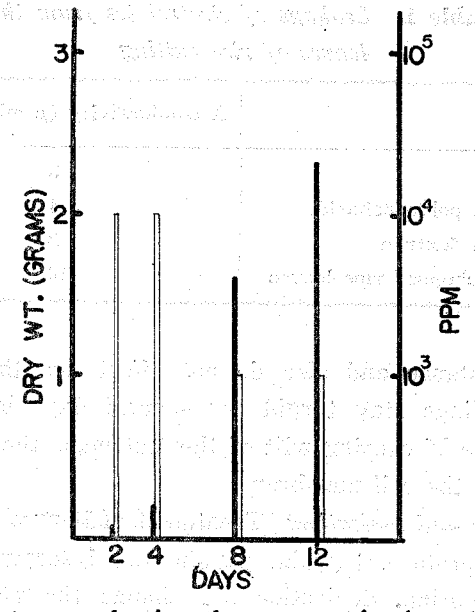


Fig. 1. The histogram showing the amount of polysaccharide produced by *X. oryzae* after different periods of incubation (closed bar in grams) and their effective wilt-inducing concentration i. e. the conc. of polysaccharide to wilt at least 50% of leaves (open bar in ppm).

also more effective in inducing wilt. Because the 50% wilting dose was 10 times less than that of a 4 days culture.

*Dye Transport:* The movement of water in *X. oryzae's* polysaccharide-and dextran-treated cuttings were both studied. A 2-week-old rice cuttings was treated with $10^4$ ppm *X. oryzae* polysaccharide until the leaves were severely wilted. Likewise, a cutting was placed in a dextran solution ($10^5$ ppm) until wilting occurred. Nontreated cuttings were used as control. All of the rice cuttings were transferred to 0.1% solution of acid fuchsin and the time required for the dye to reach the tips of the upper most leaves was recorded. The dye moved to the tip of the leaf in 30 minutes in the case of nontreated rice cuttings, whereas it took 2 hours in the case of dextran-and the polysaccharide-preparation-treatd rice cuttings.

*Leakage of electrolytes:* The tops of rice cuttings treated with polysaccharide, dextran or NMK solution were rinsed in 10 ml of distilled water and the water checked for conductivity. The results are shown in Table 1. There is no significant difference in the amount of electrolyte leakage after the treatments of polysaccharide-preparation, dextran, and NMK solution.

Figs. 2 and 3 showed that the bacterial polysaccharide and dextran do not cause wiltings of intact rice seedlings. This suggests cell membrane are imper-

**Table 1.** *Leakage of electrolytes from the leaves of rice cutting*

| | A conductivity ($\mu$ mhos/cm) |
|---|---|
| MKN | 3 |
| $10^4$ ppm polysaccharide | 4 |
| $10^5$ ppm dextran | 3 |
| MKN+chopped rice leaves | 14 |

meable to these substance and also do not affect on the root cells. Since the intact rice seedlings stay turgid for several days in these solutions at concentration capable of causing wilt of the cuttings, the polysaccharide does not seem to damage the cell membrane.

*Enzyme digestion and sonication:* Treatment of 10 ppm polysaccharide with amylase, cellulase, protase, trypsin, papain and lysozyme did not affect its potency to induce wilting. Sonication did change the viscosity of *X. oryzae* polysaccharide solution, however, the potency to wilt rice cutting was not affected. A dextran sample with a molecular weight of 150,000 was treated with the same way, the viscosity did not change. It seemed that sonication did affect the *X. oryzae* polysaccharide the molecular weight of that was larger than 200,000, but did not affect on the dextran the molecular weight of that

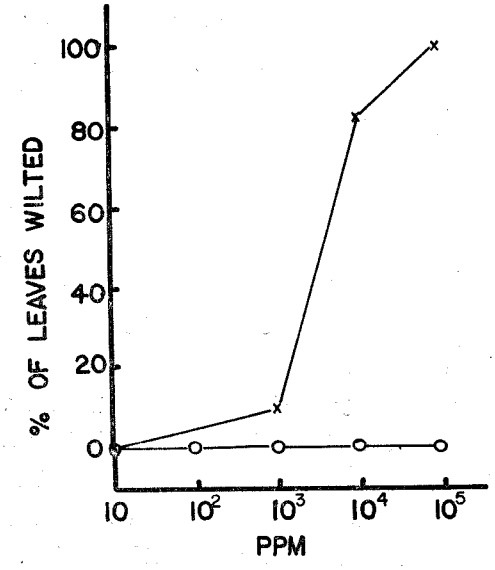


Fig. 2. The effect of *X. oryzae* polysaccharide concentration on the rice cutting wilt (x) and on the intact rice plant (o).

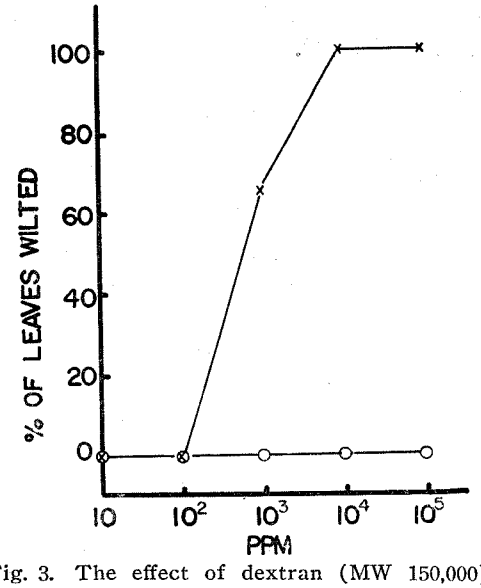


Fig. 3. The effect of dextran (MW 150,000) concentration on the rice cutting wilt (x) and on the intact rice plant (o).

was 150,000. After sonication of *X. oryzae* polysaccharide although the polysaccharide was broken it might still keep its molecular weight above 150,000, which was still in an effective range.

*Infrared spectrum of crude polysaccharide:* The *X. oryzae* polysaccharide was partially purified according to the method used for the partial purification of *C. michiganense* polysaccharide (Rai and Strobel 1969) and a infrared spectum of polysaccharide preparation was taken for comparisom. The result are

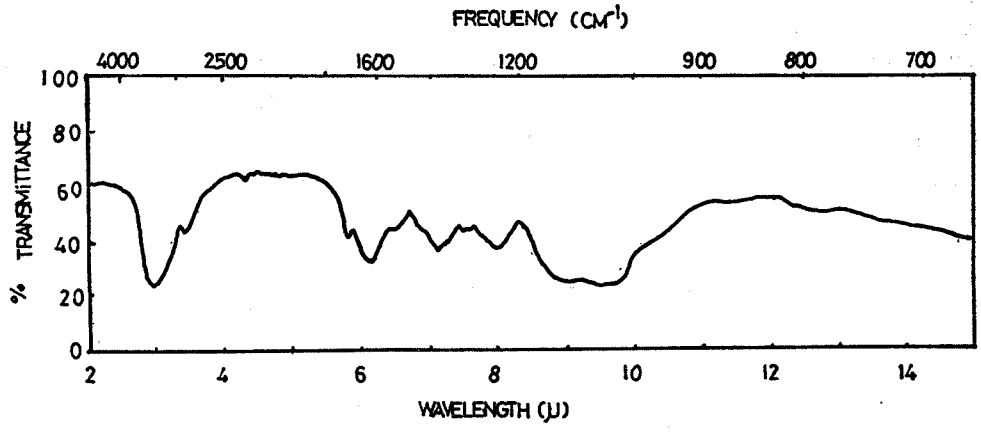


Fig. 4. A typical infrared spectrum of the crude *Xanthomonas oryzae* polysaccharide-KBr disk.

shown in Fig. 4, it differed from spectrum of the crude polysaccharide of *C. michiganense* at 800 and 1250 $cm^{-1}$.

## Discussion

The plugging hypothesis of toxin action has been held by several researchers (Buddenhagen and Kelman 1964, Spencer and Gorin 1961, Leach *et al.* 1957). Strobel (1967) working on a phytotoxic glycopeptides produced by *Corynebacterium sepedonium* opposed it. His view was based on: (1) amount of toxin taken up by 7.5 cm tomato leaf prior to wilting was only 50 $\mu$g, which was too small an amount to cause plugging. (2) radioautography experiments showed that the $C^{14}$-labeled toxin was distributed all over the plant prior to wilting suggesting that plugging of the petiole is not involved in wilting. (3) when toxin-treated and water-treated plants were placed in acid fuchsin, the dye migrated to the tip of the plants at the same rate, whereas in a dextran-treated plants the movement of dye was obstructed.

Further studies of the effect of toxin on plasmolytic ability of cells, leakage of electrolyte and electron microscopic evidence suggested that toxic glycopeptide induced wilting in plant tissue is caused by the damage of membrane (Strobel and Hess, 1968).

Our data seems to support that the mechanism of the toxic effect of the polysaccharide produced by *X. oryzae* is different from that produced by *C. sepedonium*. First, cell membrane was not damaged by our polysaccharide preparation. Next, it blocks the dye transport of rice cuttings just like dextran. Third, enzymes and sonication studies gave no sign of the presence of an active site. Therefore the plugging hypothesis seems more favourable.

Previous report (Kuo *et al.* 1970) showed that very low amount of purified polysaccharide is required to cause the wilting of rice cuttings, therefore, it was thought that the mode of action of *X. oryzae* polysaccharide might be similar to *C. sepedonicum* toxin, this statement is contrary to our new finding. The possible new explaination for this evidence is that *X. oryzae* polysaccharide has a molecular weight 10 times greater than that of *C. sepedonicum* toxin, it is easier to plug the xylem vessels in a small amount.

## Literature Cited

BUDDENHAGEN, I., and A. KELMAN, 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum.* Ann. Rev. Phytopathol. **2**: 203-230.

KUO, T. T., B. C. LIN, and C. C. LI. 1970. Baterial leaf blight of rice plant, III. Phytotoxic polysaccharides produced by *Xanthomonas oryzae* Bot. Bull. Acad. Sinica **11**: 46-54.

LEACH, J. G., V. G. LILLY, H. A. WILSON, and M. R. PURVIS, Jr. 1957. Bacterial polysaccharides—The nature and function of the exudate produced by *Xanthomonas phaseoli.* Phytopothol. **47**: 113-120.

LIN, B. C., C. C. LI, and T. T. KUO, 1969. Bacterial leaf blight of rice. I. Serological relationships between virulent strain and weakly virulent strain of *Xanthomonas oryzae.* Bot. Bull. Acad. Sinica **10**: 130-133.

RAI. P. V. and G. A. STROBEL. 1969. Phytotoxic glycopeptides produced by *Corynebacterium michiganense.* I. Methods of preparation, physical and chemical characterization. Phytopathol. **59**: 47-52.

SPENCER, J. F. T. and P. A. J. GORIN. 1961. The occurrence in the host plant of physiologically active gums produced by *Corynebacterium insidiosum* and *C. sepedonicum.* Can. J. Microbiol. **7**: 185-188.

STROBEL, G. A. 1967. Pubification and porperties of a phytotoxic polysaccharide produced by *Corynebacterium sepedonicum.* Plant Physiol. **42**: 1433-1441.

STROBEL, G. A. and W. H. HESS 1968. Biological Activity of a phytotoxic glycopeptide produced by *Corynebacterium sepedonicum.* Plant Physiol. **43**: 1673-1688.

# 水稻白葉枯病的研究

## V. 病原菌多醣類產物引起水稻萎凋的性質

范華年 郭宗德

水稻白葉枯病病原菌能產生體外多醣類產物，此種產物在很低之濃度下能使水稻萎凋，因此曾猜測萎凋之原因可能是由此物質的毒性所引起。但進一步的由各種生理分析結果發現，引起萎凋的原因定全是一種物理作用，是單純之阻塞導管所引起的一種缺水現象。其所根據的理由是此多醣類物質，（一）能阻塞 acid fuschine 的運輸，此現象與大分子的 dextran 的阻塞現象相同，（二）不影響細胞之滲透性，（三）經各種可能破壞此物質之酵素處理後不影響其活性，（四）分子量大於200,000在很低濃度下就可引起導管的阻塞。，