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BACTERIAL LEAF BLIGHT OF RICE PLANT

VI. Chemotactic responses of *Xanthomonas oryzae* to water droplets exudated from water pores on the leaf of rice plants.[1,2]

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Abstract

Chemotactic response of *Xanthomonas oryzae* toward water droplets collected from water pores of the leaves from non-hosts, resistant and susceptible rice plants was investigated. The degrees of attractive activities of exudates collected from different hosts correlated with the degree of the susceptibilities of hosts to the disease. The exudates collected from non-host and resistant varieties of host plants attracted less bacteria and the exudates from susceptible varieties of host plants attracted more bacteria. The attractive substances were stable to heating and changing of pH value.

Since the synthetic medium for *X. oryzae* used in this experiment was one of the best attractant for this pathogen and had an attractive activity comparable to the exudate collected from susceptible host, the chemotactic response of *X. oryzae* to each components at the concentration used in synthetic medium and various combinations of different components was analyzed. It was found that among the chemicals used, inorganic components were less attractive than organic components and the combination of several components was better than single one. The concentration of attractive substances also played an important role in chemotactic response, the optimum concentration of sucrose was at 1×10^{-5}M.

Introduction

Water pores are one of the most important infection entrance for *Xanthomonas oryzae*, a pathogen of bacterial leaf blight of rice plants. Water pores are found on the hydathodes and are distributed along the upper surface of the leaf near the edges. The bacterium enters the water pore and multiplies in the epitheme, which led to the vessel. When sufficient bacterial multiplic-

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ation has taken place in the epitheme, some bacteria invade the vascular system and some ooze out from the water pores. The later would then drop into irrigation water or would directly transfer to healthy leaves by contact. It is important to acertain how bacterial cells are attracted into water pores, once leaf blades are contaminated by bacteria.

The chemotactic response of bacteria was first reported by Pfeffer (1884, 1888), and recently extensive studies have been done by Adler on *Escherichia coli* (1966, 1973a and 1973b) and other bacteria (Seymour and Doetsch, 1973). The possible role of chemotaxis in plant diseases caused by Phycomycetes has been studied by Zentmyer (1961), Royle and Hickman (1964) and Ho and Kickman (1967). Except for Zentmyer (1961), these authors agree that zoospore accumulation on roots of host and non-host plants is a non-specific phenomenon. Similar conclusion has been made by Chet *et al.* (1973) on the studies on chemotaxis of *Pseudomonas lachrymans* to plant extracts and to water droplets collected from the leaf surfaces of resistant and susceptible plants. For *Xanthomonas oryzae*, Mizukami and Wakimoto (1969) reported that the bacterium is attracted by the broken roots and swarns into them which facilitates infections. These are important courts of infection and are partly responsible for the severe kresek symptoms found in the tropics.

In this investigation the chemotactic responses of *X. oryzae* to various components of the synthetic medium and their combinations were determined, and possible relationship between host resistant and chemotactic response was also studied.

Materials and Methods

Media and washing buffer

For the preservation of bacterium PS medium was used. This medium contained potato extract (from 200 g fresh potato); peptone, 5.0 g; sucrose, 15.0 g; agar, 15 g; $Na_2HPO_4 \cdot 12H_2O$, 2.0 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 g; and one liter distilled water. To prevent microbial contamination, usually 2000 ppm of streptomycin was added to the medium. For the growth of bacterium for chemotactic assay synthetic medium was used, which contained sucrose 15.0 g; L-glutamic acid, 1.0 g; L-cysteine, 0.05 g; $(NH_4)_2HPO_4$, 3.0 g; KH_2PO_4, 2.0 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $Ca(NO_3)_2$, 0.1 g; $MnCl_2 \cdot 4H_2O$, 0.001 g; $FeCl_3$, 0.001 g; tris (hydroxy-methyl) aminomethane 1.21 g and one liter distilled water. Washing buffer contained 0.01M tris-HCl buffer at pH 7.0.

Bacterium and bacterial suspension

The organism used in these experiments was *Xanthomonas oryzre* strain 604, which was isolated from diseased leaf of a rice plant and preserved in

our laboratory. In order to decrease the contamination of other microorganism during colony assay a streptomycin resistant mutant was induced from strain 604. The pathogenicity of this mutant was identical to its parental strain. The bacteria were grown in the synthetic medium overnight with aeration at 28 C. The cells were harvested with centrifugation, washed with tris-HCl buffer three times and finally suspended in same buffer at a concentration of 5×10^7 cells/ml.

Plants and collection of exudate

Non-host plants such as tomato, avena, zizania were used. Resistant rice varieties of host plants were Chianan 8, Tainan 5, Tainan 3, and Taitung 25. Moderately resistant varieties were Taichung 65, Taipei 306, and Taichung 178, Suscsptible varieties were Taichung native 1, Taichung native 2, Kaushun Native 2, IR-480 and IRI-529-53-2. All plants were grown in a greenhouse for 4 to 5 weeks. For the collection of exudate, test plants were moved to a sterile room, and washed with sterile distilled water. The water left on leaves was completely removed by clean cheese cloth, then whole plants were incubated in plastic moist chember at room temperature. The water exudated out from the water pores of leaf surface was collected with a sterile syringe and directely used for the chemotactic assay. For the studies of general properties of the attractant, the exudates collected from a susceptible variety, Taichung Native 1 was used.

Chemotactic assay

Chemotactic experiments were carried out according to the method of Adler (1973a). A small chamber was formed by laying a U-shaped tube (bend from a 5 cm length of melting point capillary tube) between a microscope slide and a cover slip. The chamber was then filled with about 0.2 ml of the above bacterial suspension. The capillary tubes containing the attractant were 1 μl disposable micropipettes. (3 cm long with an internal diameter of 0.2 mm "Microcaps" from Drummond Scientific Co., Broomall, Pennsplvania, U.S.A.) The capillaries were handled with forceps. One end was sealed in a flame, the capillary was then passed through the flame several times and quickly plunged the open end down into a 10 ml beaker containing about 1 ml of attractant dissolved in chemotaxis medium. As the capillary cooled, liquid was drawn in about 1 cm. After about 10 min the capillary was then inserted open end into the chamber containing bacterial suspension. All experiments were conducted at room temperature. The whole slide was then placed in petri dish and incubated at 28 C. After 60 min incubation, the capillary was removed. Its exterior was rinsed with a thin stream of water from a wash bottle. The sealed end was broken off over a test tube containing tris-HCl buffer and the contents were squirted into tris-HCl buffer with the aid of a

rubber bulb supplied with the micropipettes. Dilutions were made in tris-HCl buffer, samples from each dilution were mixed with 3 ml of soft PS agar medium at 45 C and then poured on a PS agar plate. The colonies were counted after 4-day incubation at 28 C.

Infection method and scales for measuring degree of resistance

The needle inoculation method described by Muko and Yoshida (1951) was used. For measuring degree of resistance, a standard scales of 10 units was used (Ou, 1972). However, 1,2 and 3 were considered as resistant, 4, 5 and 6 were considered as moderately resistant, and 7, 8 and 9 were suscepitible. The plants were grown in green house and the degrees of the disease were measured at 20 days after inoculation.

Results

Attraction of bacteria by exudate collected from host

Chemotactic response of bacteria to exudate from susceptible rice plants and tris-HCl buffer was compared. The results are shown in Fig. 1, when the capillary contained exudate the bacteria were attracted into the capillary at a more or less constant rate for about 60 minutes until a plateau was reached. Under this condition the rate of bacterial movement was about 7×10^3 cells per hour. However, when the capillary contained no attractant, a relatively small number of bacteria entered the capillary and following the incubation time, the number of bacterium did not increase. Evidently there is an attractive substance in the exudate of rice plants.

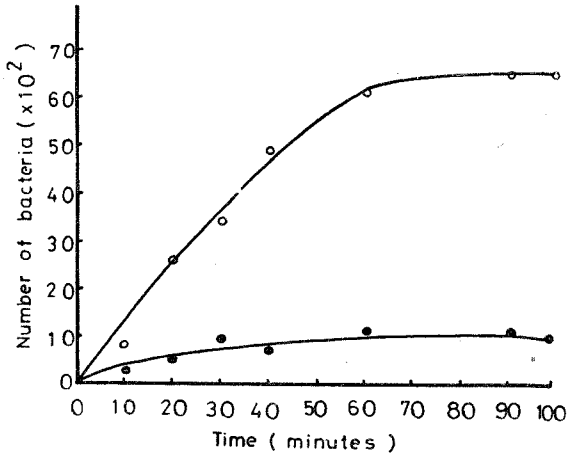

Fig. 1. Chemotactic response of *X. oryzae* to exudates cellected from water pores and buffer solution. ○—○ leaf exudate collected from Taichung Native 1. ○—○ 0.01M tris-HCl buffer at pH 7.0.

Chemotaxic response of X. oryzae to the exudate collected from various host and non-host plants

The results are shown in Table 1, the exudates prepared from all plants attracted bacteria. The exudate collected from non-host and resistant varieties of host plants attracted less bacteria and the exudates from suceptible varieties of host plants attracted more bacteria. The difference in the number of bacteria attracted by the susceptible host and non-host or resistant host was significant.

Table 1. *Chemotactic response of X. oryzae to leaf exudates collected from susceptible and resistant varieties of rice plants and non-host plants*

Plants	Degrees of disease infection	Number of bacterial cells attracted ($\times 10^2$ cells/tube)	Statistical data
Blank (distilled water)	—	7	a (2)
Synthetic medium	—	60	e
Tomato	NH(1)	14.8	a
Avena	NH	10	a
Zizania	NH	19.6	b
Rice varieties			
Chianan 8	R	10	a
Tainan 5	R	11	a
Tainan 3	R	14.7	b
Taichung 65	M	12.5	b
Taipei 306	M	24	c
Taitung 25	R	12	b
Taichung 178	M	27.8	c
Taichung Native 1	S	64	e
Taichung Native 2	S	54.8	d
Kaohsiung Native 2	S	52	d
IR-480	S	70	f
IRI-529-53-2	S	61	e

(1) NH=non-host. R=resistant. M=intermediate. S=susceptible.
(2) The same letter within each group is not significantly different at the 5% level by multiple range test. LSD 5%=4.95.

General properties of attractant in exudate

The exudates collected from the susceptible variety, Taichung Native 1 were used for the study of general properties of the attractant. The attractant in exudate was rather stable, it could be stored at 4C for at least 6 weeks without losing activity, and was also heat stable. When it was autoclaved

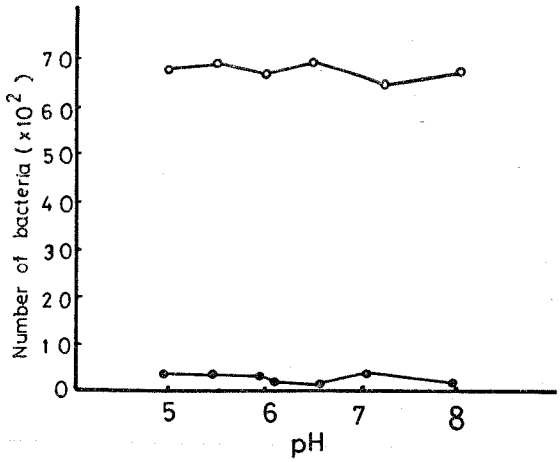

Fig. 2. Effect of pH value of leaf exudate on chemotactic response of *X. oryzae*. ○—○ leaf exudate collected from Taichung Native 1. pH adjusted with 0.1M citrate-phosphate buffer. ○—○ citrate-phosphate buffer 0.1M.

Table 2. *Effects of autoclaving and dialysis of leaf exudate on chemotactic response of X. oryzae*

Substances	Treatments			
	Origin	Filtration	Autoclaving	Dialysis
Distilled water	6*	6	6	7
Exudate	60	62	56	20

Filteration: exudates filterated throught 0.22 μ milipore filter paper.
Autoclaving: autoclaved for 10 min at 120 C.
Dialysis: dialyzed aganist distilled water at 5 C for 24 hr.
* Data represented by number of bacterial cells attracted ($\times 10^2$ cells per tube)

at 121 C for 20 minutes the activity did not change (Table 2). The pH around 5 to 8 did not effect on the activity of attractant (Fig. 2). However, when it was dialyzed against distilled water the activity was greatly decreased (Table 2). The results of chemotactic responses of exudate following concentration or dilution are shown in Fig. 3. The concentration of exudate was paralled with the number of bacterial cells attracted. Highly concentrated exudate attracted more bacteria, while diluted exudate attracted less baccteria.

Collection sequences of exudate from same plants and chemotactic response of these exudates

In order to acertain whether the chemotactic response of exudate to bacteria is a constitutional property of host plant, the following experiment was conducted. Firstly, the exudate was collected from a plant kept in a

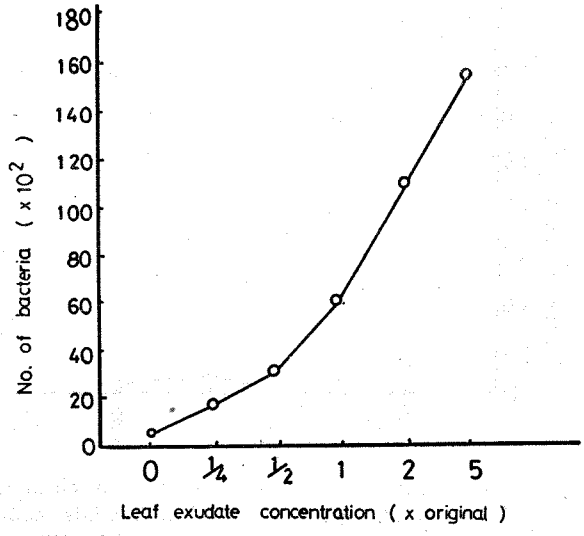

Fig. 3. Effect of diluted exudates on the chemotactic response of *X. oryzae*. Leaf exudate collected from Taichung Native 1 directly diluted with distilled water.

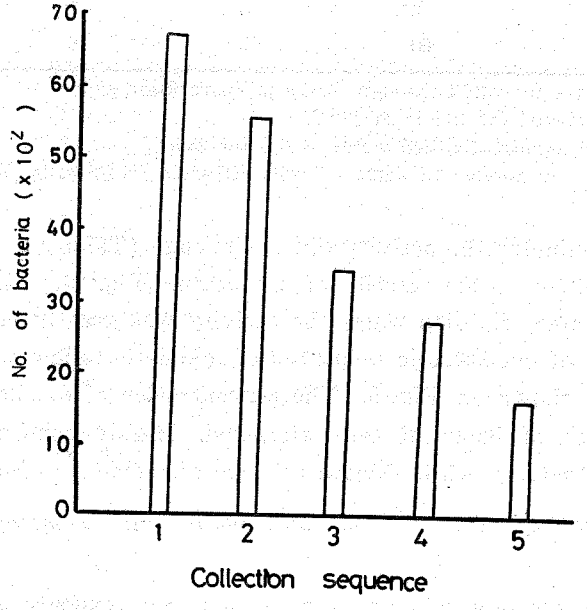

Fig 4. Collection sequence of exudate from the same plant (Taichung Native 1) and the chemotactic response of *X. oryzae* to these exudates.

moist chamber overnight at 28 C. After collection the plant was washed with distilled water. The water left on leaves was removed with clean cheese cloth. After one-hour incubation the exudate from same plant was collected. The collection procedure was repeated five more times. Then sequentially collected exudates were assayed for chemotactic response. The results shown in Fig. 4 indicate that the activity was gradually and proportionally decreased with each further collection of the exudate from the same plant upon continued incubation.

Chemotactic response of pathogen to chemicals

As indicated in Table 3, the synthetic medium for *X. oryzae* used in the

Table 3. *Chemotactic response of* ***X. oryzae*** *towards various chemical and different combinations of these chemicals from synthetic medium*

Substances	No. of bacteria ($\times 10^2$/tube)	Statistical data
Distilled water	7	ab(2)
Synthetic medium complete(1)	61	f
minus L-glutamic acid	44.7	e
minus L-cysteine	48.3	e
minus L-sucrose	28.0	c
minus L-glutamic acid, L-cysteine and sucrose	24.0	c
minus-inorganic elements	45.8	e
minus-$(NH_4)_2HPO_4$ and KH_2PO_4	45.0	e
Sucrose	28.2	c
L-Glutamic acid	12.0	b
L-Cysteine	13.0	b
Sucrose+L-glutamic acid	34.0	d
Sucrose+L-cysteine	36.4	d
L-Glutamic acid+L-cysteine	15.2	b
L-Glutamic acid+L-cysteine+sucrose	45.4	e
KH_2PO_4	16	b
$(NH_4)_2HPO_4$	13	b
$MgSO_4$	10	b
$Ca(NO_3)_2$	2	a
Total inorganic elements	30.6	cd

(1) The concentrations of substances used in this experiment were similar to that in synthetic medium, They are: L-glutamic acid 1g/1, L-cysteine 0.05g/1, sucrose 15 g/1, KH_2PO_4 2g/1, $(NH_4)_2HPO_4$ 3.0g/1, $MnCl_2 \cdot 4H_2O$ 1.0 mg/1, $MgSO_4$ 0.2g/1, $FeCl_3$ 1mg/1, $Ca(NO_3)_2$ 0.1g/1, tris (hydroxy-methyl) aminomethane 1.21g/1.

(2) The same letter within each group is not significantly different at 5% level by the multiple rang test.

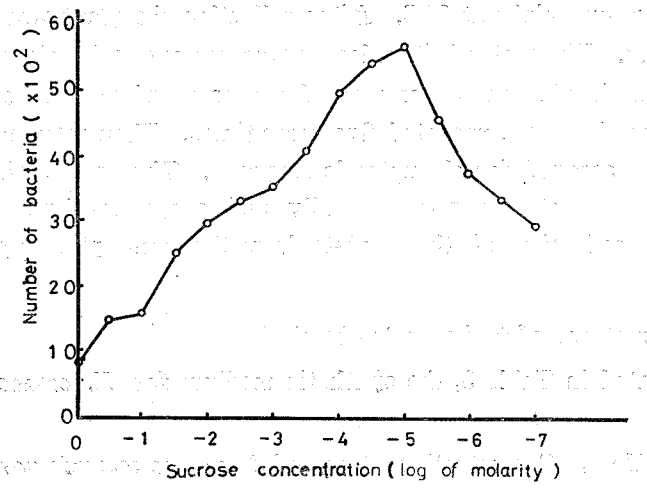

Fig. 5. Effect of sucrose concentration of the chemotactic response of *X. oryzre.*

present investigation is one of the best attractant for this pathogen, and the attractive activity is comparable to the exudate collected from susceptible host, therefore, each components at the concentration used in synthetic medium and various combination of different component were used for chemotactic assay. The results are shown in Table 3. Among the chemicals tested inorganic components were less attractive than organic components. The combination of several components was better than single component. Sucrose seems playing an important role in the attraction of bacteria in the synthetic medium. To understand the relationship between concentration of attractant and chemotactic response, sucrose at different concentrations was assayed for chemtoactic response. As shown in Fig. 5, the optimum concentration of sucrose was at 1×10^{-5}M. Higher or lower concentrations of sucrose caused a decrease of chemotactic response.

Discussion

The results presented above indicated that *X. oryzae* was attracted by the exudate collected from the water pore of rice plants and the exudate collected from the susceptible varieties was more attractive than that from resistant varieites. It was also shown that the attractive substances existed in the host with a good correlation between the magnitude of attractive ability and the degrees of host susceptibility. Based on these correlation it seems that this attractant is specific, however, from the results of the synthetic medium indicate that this deduction may not be true. When synthetic medium was used for chemotactic assay, the attractive activity was comparable to that of

the exudate collected from susceptible host. The components in the synthetic medium were all generally used for the growth of other bacteria and not specific to *X. oryzae*. Among the components used in synthetic medium, sucrose was the best attractant, L-glutamic acid or L-cysteine alone was not a good attractant, however, when they were mixed with sucrose the attractive activity of the mixture was greatly enhanced. Inorganic components: KH_2PO_4, $(NH_4)_2HPO_4$, $MgSO_4$ or $Ca(NO_3)_2$ were all poor attractants each by itself. However, when they were all mixed together the attractive activity was enhanced. From these results it seems that the attractant is not a single substance but possibly a group of substances. Different combinations of these substances gave different chemotactic response. The concentration of each component was also very important, as indicated in Fig. 5, for example the response was greatly affected by the concentration of sucrose. The main components in leaf exudate are inorganic nutrients, low concentration of amino acids and sugars, it may also contain organic acids, therefore the concentration and combination of these substances may play an imporant role in chemotactic response of *X. oryzae*.

The attractant from leaf exudate was heat stable as its activity was not affected by autoclaving at 121 C for 20 minutes. Chet *et al.* (1973) demonstrated that both leaf droplets and plant extracts from cucumber became more attractive to *Pseuodmonas lachrymans* after being autoclaved. These authors suggested that this may have been resulted from the destruction of unknown inhibitor or the release of additional attractant. This apparently was not the case in our present investigation with *X. oryzae* and rice plant.

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水稻白葉枯病的研究

IV. *Xanthomonas oryzae* 對水稻葉部水孔泌液所產生的趨化反應

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實驗結果顯示在水稻葉部水孔泌液中有引誘病原細菌的物質存在。此等引誘物質在酸鹼變化或熱處理之下均甚安定。水稻抗病力的高低與病原細菌趨化反應的强弱有關。高抗品種的葉部泌液對病原細菌的引誘力弱，罹病品種則强。

因爲合成培養液對病原細菌的引誘活性足以媲美罹病性品種的泌液，故進一步以培養液中之各種成分及各成分之組合尋求引誘物的試驗中發現：培養液中有機成分較無機成分更具引誘活性。多數個組成分結合後的引誘活性較各個組成分爲高。引誘力的强弱，除了取決於成分物質的組合之外，組成分的濃度也是一個重要的因子。以蔗糖爲例，其最適濃度在 1×10^{-5}M。