4. GENETIC ASPECTS ON RESISTANCE TO BACTERIAL LEAF BLIGHT IN RICE AND VARIATION OF ITS CAUSAL BACTERIUM.

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Introduction

Breeding of rice for resistance to bacterial leaf blight in Japan, which has long history —the first recognition of varietal difference in resistance to the disease dating back to 1909— may be considered successful in a most practical sense. In Kyushu where the disease is most prevalent, ca. 40% of the acreage planted to rice in 1965 was covered by the varieties which have in their ancestors Zonsho 26 or Norin 27, both having a single major gene for the resistance to the most prevalent strains of *X. oryzae*. In future, however, difficulties are foreseen in the breeding, for more virulent strains will become prevalent; the phenomenon already encountered in a part of Kyushu. Of three groups of *X. oryzae* in this country, one with the widest virulence actually attacks almost all the cultivated rice varieties. Significance of the damage due to this disease, which has long been considered localized, is being recognized in wider regions covering most rice growing countries in Asia. Prevalence of highly virulent strains in South Asia has also been reported (Wakimoto 1967).

Microbiological studies have revealed that the genus *Xanthomonas* comprises species which have relatively small diversity in physiological traits and in base composition of DNA inspite of diverse host specificity. Comprehensive aspects on the variability of xanthomonades may be required for the breeders who will have to counteract the possible emergence of bacterial strains with higher virulence.

In the present short review are summarized genetic and microbiological findings, mostly obtained at Department of Genetics, National Institute of Agricultural Sciences, on resistance of rice to bacterial leaf blight and variability of its causal bacterium. The topics include resistance to bacterial leaf blight of varieties of cultivated rice, *Oryza sativa* and *Oryza glaberrima*, and several wild rice species, genes controlling the resistance in rice, genetic recombination in *X. oryzae*, and taxonomic relatedness among Xanthomonades parasitic to rice.

Resistance to Bacterial Leaf Blight in Rice

1. Classification of Rice Varieties and Strains of *X. oryzae* in Terms of Host-parasite Relations.

Varietal difference in resistance to bacterial leaf blight in rice has been recognized since before 1910. Kidama, Zensho 17, Zensho 26, and Koganemaru obtained by cross breeding in late 1930's (Kuwazuka 1942, Fujii and Okada, this volume) were the varieties with distinct resistance to the disease besides economical properties and have long been taken as the standard for the disease resistance in rice breeding. In 1958, strains of *X. oryzae* which attack these varieties were reported to have been collected from the field where Asakaze carrying a resistance gene from Norin 27 succumbed to the disease (Kuhara, Sekiya and Tagami 1958). Since then, several sttempts were made to classify strains of *X. oryzae* and

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rice varieties in terms of host-parasite relations (Mukoo et al. 1960). Most of them, however, dealt only with domestic varieties of rice.

Table 1. Synthetic medium for *X. oryzae*.
(By T. Suwa 1962)

Na-glutamate	10 g (2 g)*	Fe (as FeEDTA)	1 mg
$MgCl_2 \cdot 6H_2O$	1 g	Dist. water	1 liter
K_2HPO_4	0.1 g	(Peptone	1 %)

pH is to be adjusted to 7.0.

* Application of 10 g Na-glutamate instead of 2 g in Suwa's original recipe is favorable for the bacterial growth.

Experiments have been conducted at Dept. Genetics, Nat. Inst. Agric. Sci. to establish the classification using exotic as well as domestic varieties of rice and domestic bacterial strains (Sakaguchi, Suwa and Murata 1967). A new synthetic medium (Table 1) devised by Suwa (1962) for *X. oryzae* which enabled the single colony isolation of the organium which had been considered unable to form colonies from single cells facilitated the study. Three groups in each host (Kinmaze, Kidama, and Rantaj-emas groups) and parasite (groups I, II, and III) were recognized as follows.

		Bacterial strains			
		Group I	Group II	Group III	
Rice varieties	Rantaj-emas group	R	R	S	Exclusively exotic var.
	Kidama group	R	S	S	
	Kinmaze group	S	S	S	
		X-17	X-14	X-13	
		X-18	X-30	X-82	
		etc.	etc.	etc.	

Kinmaze group of varieties of rice which includes majority of vareities now cultivated in Japan are susceptible to any of three groups of the bacterium. Kidama group of varieties are resistant to group I of the bacterial strains but susceptible to the other two groups. Rantaj-emas group of rice varieties exclusively consisting of exotic varieties are resistant to either group I or II but susceptible to group III of the bacterium. For the classification, preliminary test was performed on 39 varieties of rice and 37 strains of the bacterium and the result was confirmed on 20 rice varieties and 12 bacterial strains listed in Table 2. It is worth noticing that group III of the bacterium which attacks Rantaj-emas group of rice, the most resistant group consisting exclusively of exotic varieties was actually isolated in Japan. In fact, 2 out of 3 established strains of this group were isolated from susceptible Kinmaze group of varieties.

2. Two Genes in Rice Controlling the Resistance to Group I and II of *X. oryzae*.

Several genetic studies on the resistance of rice to *X. oryzae* coincide in that there is a single major gene controlling resistance to group I of the bacterium in rice. Sakaguchi (1967) tested 20 vareieties of Kidama group and 7 varieties of Rantaj-emas group for their genes for resistance by crossing them with 17 linkage testers of Kinmaze group; 3 of the testers carrying morphological markers and 14 carrying translocation of chromosomal seg-

Table 2. Origin of *X. oryzae* strains classified into three groups in terms of their virulence (Modified from Sakaguchi, Suwa, and Murata 1967)

Groups	Strains	Places where lesions were isolated	Rice varieties from which the lesion was isolated	Institutions at which the bacterium was isolated	Date of single cell isolation at Dept. Genetics, N. I. A. S.
III	X-13	Fukuoka Prefecture,	Asakaze,	Div. Environ., Hokuriku Agr. Exp. Sta.	1960. 1. 19
II	X-14	Oita Pref.,	Hozakae,	Div. Plant pathology, N. I. A. S.	〃 2. 9
I	X-17	Tokushima Pref.,	Unknown,	〃	〃 2. 17
I	X-18	Iwate Pref.,	Chokai,	Div. Genetics, N. I. A. S.	〃 3. 13
II	X-30	Akita Pref.,	Norin 41,	〃	〃 2. 6
II	X-54	Saga Pref.,	Jukkoku,	〃	〃 2. 19
I	X-57	Yamanashi Pref.,	Kinmaze,	〃	〃 4. 5
II	X-68	Saitama Pref.,	Norin 25,	〃	〃 2. 22
III	X-82	Fukuoka Pref.,	Jukkoku.	〃	〃 2. 25
II	X-95	Akita Pref.,	Dai kei 28	〃	〃 3. 24
II	X-104	Mie Pref.,	Kinmaze,	〃	〃 3. 18
III	X-108	Kanagawa Pref.,	Kinmaze,	〃	〃 3. 23

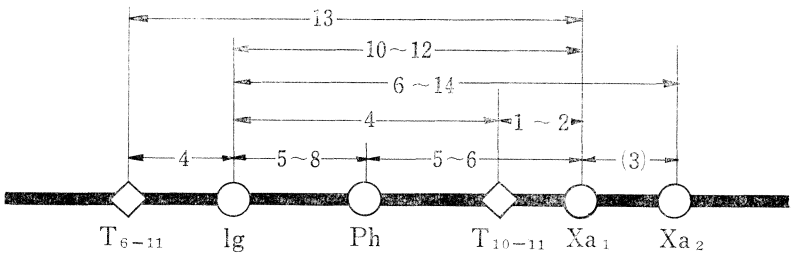
Table 3. Rice varieties used in analysis of genes for resistance to bacterial leaf blight by Sakaguchi (1967)

Rantaj-emas group: Basilanon (P), Chinsurah Boro II (I), Kele (I), Nep Vai (IC), Pinulpot 1 (P), Rantaj-emas 2 (IS), Tokushudaisuishu (IC).

Kidama group: Akamai (J), Bomba (S), Gaisen (J), Gangsale Bhatta (M), Higashi Africa-san Hakumai (E), Jaguary (B), Kaeu N751 (R), Kidama (J), Koganemaru (J), Chian-pei-tao-tsen (C), Futao (T), Nakate Kidama (J), Nihh (IT), Norin 27 (J), Pi 1 (J), Shigadogabo (IS), Sirenteng (IS), Texas Fortuna (U), Zensho 26 (J).

Kinmaze group: Murasaki Muyozetsu (J) (Liguleless), Tsutsui-ine (J) (Liguleless), Tareha-chogoei (J) (Lop leaf, long empty glume), 14 linkage tester lines with translocation of chromosomal segments (All J).

P: Philippines, I: India, IC: Indo-China, IS: Indonesia, J: Japan, S: Spain, M: Mexico, E: Egypt, B: Brazil, R: Russia, C: China (mainland), T: Taiwan, IT: Italy, U: U. S. A.



T_{6-11} and T_{10-11}: Positions of exchange of chromosomal segments on chromosome no. 11 in translocation homozygote no. 204 and no. 141, respectively.

Fig. 1. Loci of Xa_1 and Xa_2, genes for resistance to bacterial leaf blight, in rice. (Sakaguchi 1967)

ments (Table 3). Two genes for resistance, Xa_1 and Xa_2, were allocated to the chromosome no. 11 as designated by Nishimura (1961) or Pl-linkage group by Nagao and Takahashi (1963) as shown in Fig. 1. They are closely linked to each other and located near pl (phenol reaction) locus and the position of reciprocal translocation in a tester line. Kidama group of varieties possess Xa_1 which control the resistance to group I of the bacterium. Rantaj-emas group carries Xa_1 and Xa_2 of which Xa_2 is principally for the resistance to group II of the bacterium. When a variety of Rantaj-emas group is crossed with a variety of Kinmaze group, segregants with xa_1 xa_1 Xa_2 Xa_2 genotype are obtained in the progeny, though rare owing to the linkage, which are characterized by susceptibility to group I and resistance to group II of the bacterium. Varieties which show susceptibility to group I and resistance to group II have not yet been confirmed in the cultivated rice. If the gene-for-gene relationship demonstrated in host-parasite relations in rust or mildew holds true in this case, genotypes for the bacterial groups I, II, and III should be A_1a_2, a_1A_2, and a_1a_2 respectively, where A_1 and A_2 are avirulent genes which are epistatic over the alleles of the other locus and resistance reaction takes place when avirulent gene A_1 in the bacterium and resistance gene Xa_1 in rice or A_2 and Xa_2 come together. The hypothesis awaits verification.

3. Resistance to Bacterial Leaf Blight in Cultivated and Wild Rice.

For the resistance to three groups of *X. oryzae* were examined 863 varieties of cultivated *O. sativa*, of which 159 are domestic and 704 are axotic, 50 lines of *O, glaberrima* and

Table 4. Frequencies of resistant varieties (*O. sativa*) in each region where the varieties originated. (Sakaguchi, Suwa, and Murata 1967)

Geographic origin	Total no. of vars. tested	Reaction to								
		group I			group II			group III		
		Vars. tested	Resist. Vars.	%	Vars. tested	Resist. Vars.	%	Vars. tested	Resist. Vars.	%
Japan	159	132	25	18.9	131	0	0	156	0	0
Korea	14	11	1	9.1	9	0	0	14	0	0
China (mainland)	80	74	23	31.1	73	7	9.6	80	0	0
Taiwan	69	54	10	18.5	67	2	2.9	69	1	1.5
Philippines	52	14	4	28.6	49	10	20.4	52	0	0
Malaya	19	2	0	0	19	5	26.3	19	0	0
Indo-China	16	14	7	50.0	14	6	42.8	16	0	0
Indonesia	71	49	17	34.7	67	7	10.4	71	1	1.4
Burma	26	21	7	33.3	25	1	4.0	26	1	3.8
India & Pakistan	127	91	33	36.3	126	18	14.3	124	0	0
Nepal	24	24	1	4.2	21	0	0	24	0	0
Ceylon	36	33	8	24.2	35	7	20.0	36	2	5.6
Iran	10	0	0	–	10	0	0	10	0	0
Africa	16	15	2	13.3	16	0	0	16	0	0
Russia	30	29	1	3.5	22	0	0	30	0	0
Europe	48	47	4	8.5	45	0	0	48	0	0
N. America	41	41	23	56.1	40	4	10.0	41	0	0
S. and C. America	23	15	6	40.0	22	0	0	23	0	0
Australia	2	2	0	0	2	0	0	2	0	0
Total	863	668	172	25.8	793	67	8.4	857	5	0.6

total 52 lines of wild rice species (Sakaguchi, Suwa and Murata 1967). All but some of the wild species were tested at seedling stage by needle inoculation. Results with cultivated *Oryza sativa* are summarized in Table 4. Resistance to group I of *X. oryzae* was found in the varieties of cultivated *O. sativa* from various regions. Varieties which are resistant to group I of the bacterium form 25.8 % of all the cultivated *O. sativa* varieties tested. The percentage was low with whole domestic varieties (19 %) but high with domestic upland rice (53 %). There are many of this type in varieties from North America (56 %). Variety Fortuna which is found in the ancestors of the resistant varieties in U. S. A. is presumably one of major origins of resistance of these varieties. Resistance to group I is also prevalent in the varieties from Central and South America, South Asia and Mainland China. Resistance to group II of *X. oryzae* was found in 8.4 % of all the varieties, most of which are from South Asia and none are domestic varieties. A selected line from Lead Rice from Burma and few other varieties showed resistance to group III though the reaction was subject to fluctuation. In

Table 5. Reactions of species of genus *Oryza* to bligh's bacterium
(Modified from Sakaguchi, Suwa, and Murata 1967)

Genome	Species	Reaction to					
		group I		group II		group III	
		R	S	R	S	R	S
AA	*O. glaberrima*	0	50	0	50	0	50
AA	*O. perennis*	2 (M 1)	0	0	4	0 (M 1)	3
	O. sativa v. *spontanea*	0 (M 1)	2	0	5	0 (M 2)	5
	O. sativa v. *spontanea* or *perennis*	0 (M 2)	5	0	9	0 (M 1)	10
	O. sativa v. *fatua*	0	3	0	3	0	4
	O. cubensis					0	1
	Unidentified (*O. sativa*)	1	3	2	4	0	11
BBCC	*O. minuta*			1	0	1	0
	O. eichingeri	0	1	3	0	2 (M 2)	0
CCDD	*O. latifolia*			0	1	1	2
	O. paraguaiensis			0	1	0	1
EE	*O. australiensis*					0	1
CC	*O. officinalis*					1	0
?	*O. granulata*					1	0

Table 5 is shown the result with *Oryza glaberrima*, which is cultivated in a part of Africa and wild rice species. All 50 lines of *O. glaberrima* tested were susceptible to any of three groups of *X. oryzae*. The results with wild rice species were not quite comparable with those with cultivated rice as the developmental stage of plants tested was not uniform. Out of 40 lines of *Oryza* species with AA genome, two lines of *O. perennis* and a line which is presumably *O. sativa* were resistant to group I of the bacterium. Among those with BBCC genome, a line of *O. minuta* showed resistance and 4 lines of *O. eichingeri* showed either resistance or intermediate reaction to group III of the bacterium. Of 3 lines of *O. latifolia* with CCDD genome one was resistant but the rest were susceptible to group III. A line of another species with CCDD genome *O. paraguaiensis* was susceptible to either group II or III. A line of *O. officinalis* of which genome is CC and a line of *O. granulata* of which

genome is not konwn were resistant to group III.

4. Measure of Resistance.

The host-parasite relations thus far discussed are based on the specific response discernible by formation of lesions after needle inoculation on seedlings. There may be other categories of resistance based on severe damage or wilting effected on the host plants. Yoshimura (1963) described this type of symptoms occurring in Japan while the severe damage has been reported to be prevalent in the tropics. Response of rice varieties to the severe damage by *X. oryzae* in the tropics (Goto 1964, Srivastava, this volume) is entirely different from the classification stated in the last section. *Japonica* varieties from Taiwan and Japan rather than *Indica* varieties show higher resistance to the damage. Several reports in Japan on the resistanec of rice varieties to blight bacterium put an intermediate group between Kinmaze and Kidama groups of vareities. It is actually practice of rice breeders in this country to utilize the partial resistance (e. g., that of Norin 18 etc.) in their program. Some other varieties (e. g., a selected line of Wase Aikoku etc.) show resistance to a considerable extent at matured stage to all three groups of bacterium though susceptible even to group I at seedling stage. Whether the resistance of these varieties should be classified in the specific stepwise manner is to be solved by further experiments including gene analysis.

Genetic Basis of Variation of *X. oryzae*

1. Induction of Auxotrophic Markers in *X. oryzae* and Loss of Virulence Accompanying Auxotrophy.

Auxotrophic markers were induced in *X. oryzae* strains X. 18 and X. 82, each belonging to group I and group III, respectively (Yamasaki, Murata, and Suwa 1964). Some of them were found to be of decreased virulence. Accompanied by loss of virulence was the auxotrophy for arginine, leucine, isoleucine-valine, threonine and phenylalanine. Whereas auxotrophy for histidine and nicotinic acid did not affect the virulence. Some of the tryptophan-requiring mutants were virulent while the others were not. That the loss of virulence was caused by the auxotrophy was confirmed by demonstrating recovery of virulence of leucine-requiring mutants by prototrophic reversion and DNA-mediated transformation to prototrophy.

2. Transformation.

Experiments were conducted to see whether genetic recombination by DNA-mediated transformation is possible in *X. oryzae* (Yamasaki, Murata, and Suwa 1966). DNA extracted by Marmur's procedure from the donor strain was applied to the recipient in Suwa's synthetic medium with half the salt concentration of the original recipe but supplemented with peptone (0.5 %) and CaCl (5×10^{-5} M). Incubation was continued for more than 10 hours. Results are summarized in Table 6. When a his^-leu^-double auxotroph induced from leu^-auxotroph derived from X 18 (group I) was used as recipient and wild type of either X 82 (group III) or X 30 (group II) were used as donor, $his^- leu^+$ transformants were obtained at maximum rate of 2.6×10^{-3}.

By using his^+leu^- strain induced from X 82 as recipient and his^-leu^+ strain induced from X 18 as donor, prototrophic transformants were also obtained. It seemed that competence of recipient culture is higher at an early stage of logarithmic growth. The rate of transformation is dependent on the concentration of DNA applied and the transforming activity of DNA was eliminated by DNase. The decreased virulence of leucine-requiring mutants was recovered when they were transformed to be his^-leu^+ or prototrophic. The host-range of transformants was, however, of the type of the original strain from which the recipient was induced and not the type of the donor. The experiments domonstrated that transformation is possible in this organism and the the loss of virulence in leucine-requiring mutants is due to the leucine-requirement but is independent of the host-range character.

Table 6. Nutritional requirements and virulence of parental strains and transformants of *Xanthomonas oryzae*.

Strains	Nutritional requirements	Virulence to Kinmaze	Virulence to Zensho 26	Virulence to Tadukan	No. of transformant strains tested for virulence
X82 (Donor 1)	his^+leu^+	V*	V	V	
X30 (Donor 2)	〃	V	V	A	
X18	〃	V	A	A	
X18-1505	his^+leu^-	A	A	A	
X18-1505-D128(Recipient)	his^-leu^-	A	A	A	
Transformants obtained by Donor 1	his^-leu^+	V	A	A	9
Transformants obtained by Donor 2	his^-leu^+ his^+leu^+	V V	A A	A A	6 1
X18-2168 (Donor)	his^-leu^+	V	A	A	
X82-1380 (Recipient)	his^+leu^-	A	A	A	
Transformants	his^+leu^+	V	V	V	5

* V: Virulent
A: Avirulent

3. Lysogeny.

It was demonstrated that X 82 strain of *X. oryzae* is lysogenic, producing phage which can be detected on X 18 and some other strains. Significance of the lysogeny in genetic recombination is not known (Suwa unpublished).

4. Taxonomy of *Xanthomonas* Species as Seen by Homology of DNA.

Phytopathoginic bacteria in the genus *Xanthomonas*, in spite of a diversity of their host-specificity, show little diversity in physiological traits and G+C ratios of their DNA's fall in rather small range as compared with other bacterial genera. To fully understand the basis of host-parasite ralations and to approach to the variability in *Xanthomonas* species, it may be required to have insight into the infectivity of whole xanthomonades. There are two species of *Xanthomonas* which are parasitic to rice, *X. oryzae* causing bacterial leaf blight and a bacterium causing bacterial leaf streak. The latter which had been called *X. oryzicola* as named by Fang (1957) was identified by Goto (1964) as forma specialis of *X. translucens* and given name of *X. translucens* f. sp. *oryzae*. This organism is prevalent in South Asia but has not been reported in Japan. It is of interest to see the extent of relatedness as determined by DNA homology between *X. oryzae* and *X. translucens* f. sp. *oryzae*, both belonging to the same genus and parasitic to the same host, from the point of view to understand the variability of these organisms. It is also of importance to inquire into the homology of DNA between *X. translucens* f. sp. *oryzae* and *X. translucens* isolated from plants other than rice to confirm the validity of Goto's nomenclature.

Comaprison of DNA was conducted on *X. oryzae* strain X. 82, *X. translucens* f. sp. *oryzae* isolated by Goto in the Philippines, and *X. translucens* isolated from wheat in U.S.A. and maintained at American Type Culture Collection (Murata, in preparation). G+C content of DNA of these organisms was determined by measuring buoyant density in CsCl by density gradient centrifugation. Buoyant density in CsCl of DNA from *X. oryzae*, *X. translucens* f. sp. *oryzae*, and *X. translucens* isolated from wheat were found to be 1.7231, 1.7275, and 1.7277, each corresponding to G+C ratio of 63.1, 67.5, and 67.7, respectively (Table 7).

Homology of DNA was examined by nitrocellulose filter technique (Denhardt 1966) to

Table 7. Buoyant density in CsCl and melting temperature (Tm) in PE buffer of three species of *Xanthomonas*.

	Buoyant density in CsCl	C+C content	Tm in PE buffer
X. oryzae	1.7231	63.1	65.2°C
X. translucens f. sp. *oryzae*	1.7275	67.5	67.4°C
X. translucens (from wheat)	1.7277	67.7	67.6°C

PE buffer: 10^{-3} M. Na_2HPO_4, 10^{-4} M. EDTA, pH 8.0.

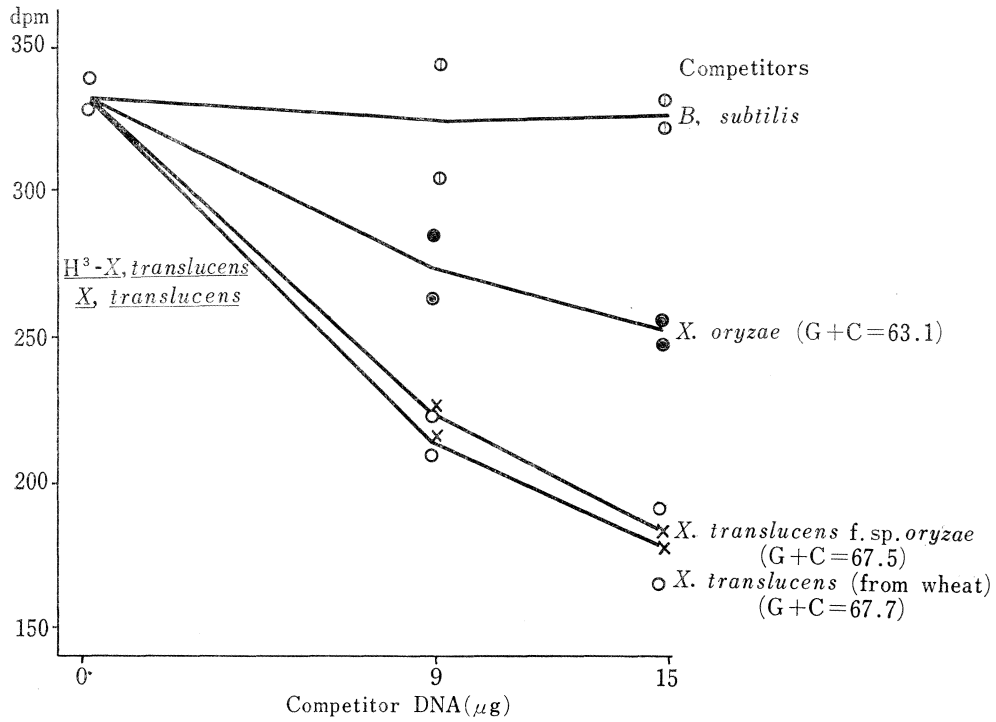


Fig. 2. Homology of DNA's from *Xanthomonas* species as determined by DNA-DNA hybridization on nitrocellulose filters.

which was applied competition method. Nitrocellulose filters to which DNA from *X. translucens* isolated from wheat was immobilized by Denhardt's procedure were incubated with tritiated DNA from the same organism. To the above were added various amount of DNA's from *X. oryzae*, *X. translucens* f. sp. *oryzae*, *X. translucens* isolated from wheat, and *Bacilus subtilis* (as control) and allowed to compete with the labeled *X. translucens* DNA in making hybrid with DNA immobilized on filters. The extent of homology between a given DNA and DNA from *X. translucens* isolated from wheat was determined by the efficiency of competition of the former relative to that by the latter. Results are shown in Fig. 2. DNA from *X. translucens* f. sp. *oryzae* gave the competition which is comparable to that by DNA from *X. translucens* isolated from wheat. Thus, *X. translucens* f. sp. *oryzae* was proven to be indistinguishable from *X. translucens* isolated from wheat in respect of overall base sequence of DNA and Goto's nomenclature was substantiated. DNA from *X. oryzae* proved to have ca. 50 % or the homology with that from *X. translucens*. Significance of the partial homology of DNA between the two species in their differentiation is to be solved in future.

Conclusion

Classification of varieties of rice and strains of *X. oryzae* in terms of host-parasite relations, genetics of the disease resistance in rice, possible source of genes for resistance in conducting breeding of rice, and some basic findings related to the variation of *X. oryzae* have been discussed. Three problems may be pointed which are of great importance in protecting rice from bactirial leaf blight by breeding and other means.

1. There seems to be more than one categories of resistance to the disease in rice. One may be resistance to severe symptom accompanying wilting and another may be that by our present classification but there may be still other types. These should be understood in clearer manner and cause of the apparent discrepancy between the host-parasite relations studied in the South Asia and at our department should be elucidated. Bacterial strains concerned may be playing an important role.
2. Gene analysis in host for resistance and that in parasite for virulence should be conducted in parallel, if the technique in the study of the bacterium could be established. Consideration should be given in the study of the parasite to the general feature of host-specificity of whole xanthomonades,
3. Search for the possible source of resistance genes must be continued, in various ecotypes of cultivated rice and also in wild species.

Discussion

M. Goto, Japan: (1) How did you determine "R" and "S" reaction from inoculation tests. (2) For discussing the resistance to so called "Kresek" of japonica and indica type rices, inoculation method must be checked. Some of indica rices easily develop this symptom following leaf infection. I understand this symptom on most the japonica type can be developed through inoculation only in dipping seedlings into bacterial suspension. If this is true, it could be said that indica type rice have higher susceptibility to the bacteria. How about your opinion on this point?

Answer: (1) Inoculation was carried out by needle-method using the bacterial suspension of concentration of 10^9 cells/ml. Reactions were designated "S" or "R" depending on whether diseased plants with lesions exceed 80 % of all the plants tested. Standard varieties were also tested whenever possible to check the reliability of the test. (2) I have no experience in dipping method. What I wanted to stress in the text, referring to your data, was the fact that the varietal response could be different under different conditions and different criteria of reaction. Actually, I need not to have referred to any data, now that Dr. D. N.

Srivastava showed us his original data relevant to this point in the morning.

H. I. Oka, Japan: In relation to the genetic basis of variability in X. species, do you suggest "transduction" due to the phages.

Answer: As I mentioned in the text, X 82 and a few other strains have been found to be lysogenic. Whether or not transduction is possible with these temperate phages is a future problem.

K. Goto, Japan: The variety Kidama you mentioned is to pronounciate rightly "Kogyoku" in Japanese, isn't it?

Answer: I should say both pronounciations are correct. I followed the alphabetical registration of the variety by Dr. H. Ito to FAO. Upon inquiry to an officer of Aichi Prefectural Agricultural Experiment Station, where the variety was brought up, the answer was "Kidama."

D. N. Srivastava, India: What is the basis of including *Bacillus subtilis* in your comparision of *X. oryzae* and *X. oryzicola* (*X. tr.* f. sp. *oryzae*) and *X. tr.* from wheat?

Answer: I used *B. subtitis* as the control simply because I happened to have *B. subtilis* DNA prepared for other purposes and did not think there is any reason to suspect homology of DNA between *Bacillus* and *Xanthomonas* species. In another experiment, coliphage T 4 was also used as a control. Comparison of the organisms investigated in the present study with other *Xanthomonas* species is planned for future.

A. Alim, Pakistan: It appears that under Pakistan condition indica type are more resistant. So it is perhaps worthwhile to study the reaction under different environment.

Answer: Thank you for the information. I think, as I mentioned in the text, regional difference in the reaction of rice varieties to blight bacterium is one of the major problems to be solved.

References for Paper 4

1. Denhardt, D. T. (1966): A menbrane-filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Com. 23, 641–646.
2. Fang, H. T., H. C. Ren, T. Y. Chen, Y. K. Chu, H. C. Faan and S. C. Wu (1957): A comparison of the rice bacterial leaf blight organism with the bacterial leaf streak organisms of rice and *Leersia hexanda* Swarzen. Acta Phytopath. Sinica, 3, 99–124.
3. Goto, M. (1964): Nomenclature of the bacteria causing bacterial leaf streak and bacterial stripe of rice. Bull. Fac. Agr. Shizuoka Univ. 14, 3–10.
4. Goto, M. (1964): On the resistance to bacterial leaf blight and leaf streak of cultivated rice varieties (*Indica* and *Japonica*) and wild rice species. (Abstract in Jap.) Ann. Phytopath. Soc. Jap. 29, 292.
5. Kuhara, S., N. Sekiya, and Y. Tagami (1958): On the causal bacterium of bacterial leaf blight outburst in the area widely planted to resistant rice varieties. (Abstract, in Jap.) Ann. Phytopath. Soc. Jap. 23, 9.
6. Kuwazuka, K. (1942): Bacterial leaf blight of rice. (In Jap.) Nogyo 741, 64–70.
7. Muko, H., T. Kusaba, M. Watanabe, S. Wakimoto, and Y. Yamazaki (1960): On the virulence of strains of *Xanthomonas oryzae*. 3. (Abstract, in Jap.) Ann. Phytopath. Soc. Jap. 25, 5.
8. Nagao, S. and M. Takahashi (1963): Trial construction of twelve linkage groups in Japanese rice. J. of Fac. of Agr., Hokkaido Univ., LIII, 72–130.
9. Nishimura, Y. (1961): Studies on the reciprocal translocations in rice and barley. Bull. Nat. Inst. Agr. Sci. (Japan) D16, 171–235.
10. Sakaguchi, S. (1967): Linkage studies on the resistance to bacterial leaf blight, *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, in rice. Bull. Nat. Inst. Agr. Sci. (Japan)

D16, 1–18.
11. Sakaguchi, S., T. Suwa, and N. Murata (1967): Bull. Nat. Inst. Agr. Inst. Sci. D 18, 1–29.
12. Suwa, T. (1962): Studies on the culture media of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. Ann. Phytopath. Soc. Jap. 27, 165–171.
13. Wakimoto, S., T. Uematsu, and T. Mizukami (1967): Virulence of strains of *Xanthomonas oryzae* collected from India and the Philippines and resistance of *indica* rice varieties to these strains. (Abstract, in Jap.) Ann. Phytopath. Soc. Jap. 33, 110.
14. Yamasaki, Y., N. Murata, and T. Suwa (1964): The effect of mutations for nutritional requirements on the pathogenicity of two pathogens of rice. Proc. Japan Acad. 40, 226–231.
15. Yamasaki, Y., N. Murata, and T. Suwa (1966): Restoration of lost virulence of leucine-requiring mutants of *Xanthomonas oryzae* by DNA-mediated transformation. Proc. Japan Acad. 42, 946–949.
16. Yoshimura, S. (1963): Diagnostic and ecological studies of rice bacterial leaf blight caused by *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson. Bull. Hokuriku Agr. Exp. Sta. 5, 27–182.