イネ白葉枯病菌Xanthomonas oryzae (Uyeda et Ishiyama) Dowson の性状

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著者	Reddy, C R Ou, S H
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Characterization of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson, the Bacterial Blight Pathogen of Rice*

By C. R. REDDY** and S. H. Ou**

C. R. Reddy** and S. H. Ou**：イネ白葉枯病菌 *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson の性状*

Abstract

A thorough study of physiological and biochemical characteristics of 40 isolates of *Xanthomonas oryzae* from Asia revealed that no distinct biochemical groups or types exist in blight pathogen, which would suggest that the isolates even from wide geographical regions are much more homogeneous in their reaction than described in literature. The present description can be considered as norms of this species in identifying it when seperated from host and also to differentiate it from other nomenspecies of *Xanthomonas*.

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Introduction

Conflicting reports exist on physiological and biochemical description of *Xanthomonas oryzae*[2,7,8,18,19,20,27] and they have not always followed Ishiyama[16]. Deviations from Ishiyama's description were noticed first in the reports of Mukoo & Isaka[18] with respect to gelatin liquefaction, production of ammonia and H_2S, acid production from sugars, and reducing ability.

Moreover a distinct biochemical strain of *X. oryzae* was isolated from seed by Chakravarti & Rangarajan[2] which differed from authentic strain in acid production from maltose and lactose, hydrolysis of starch, gelatin liquefaction, and growth in inorganic nitrogen medium. Similarly Shekhawat & Srivastava[20] recognised two distinct biochemical groups among six Indian isolates : one liquefied gelatin slowly, hydrolysed starch partially or not at all, gave alkaline reaction in litmus milk, did not produce H_2S, and highly sensitive to antibiotics as well as highly virulent; the other liquefied gelatin rapidly, hydrolysed starch completely, gave acid reaction in litmus milk, produced H_2S, and less sensitive to antibiotics as well as less virulent. So the studies were undertaken to clarify the existence of distinct biochemical groups or types in blight pathogen. This might be helpful in determining the norms of the species to identify it when isolated from host and to distinguish it from other nomenspecies of *Xanthomonas*.

Materials and Methods

Bacterial isolates.

Forty isolates of *X. oryzae*, used in this investigation, were selected from stock cultures of Dept. of Plant Pathology, IRRI, Philippines. They were representative

* Part of Ph. D. thesis of the senior author submitted to P. G. School, Indian Agricultural Research Institute (IARI), New Delhi-110012.

** Former research fellow and Plant Pathologist, respectively, of the International Rice Research Institute (IRRI), Los Banos, Laguna, The Philippines.

of various Asian countries: Bangladesh (PA3), Burma (BU3), India (H 14, H 100 & H 257), Indonesia (Ind 1-6, Ind 4-1, Ind 7-3, Kb, Kc & Kd), Japan (N 1, N 2, N 3 & N 4), Philippines (B 1, B5, B 14, B 16, B 31, B 33, B 46, B 56, B 65, PXo 2, PXo 10, PXo 17, PXo 25, PXo 35, PXo 37 & PXo 40), Srilanka (C 16), S. Vietnam (Vn 2, Vn 6, Vn 12 & Vn 17), and Thailand (B 17, B 18, B 19 & B 20-6). All the cultures were maintained on Wakimoto's agar[29] slants in deep freezer at -30 C and they were checked for contaminants before testing. All the test media were inoculated with 24 hr old cultures and incubated at 28 ± 1 C.

Biochemical tests.

Unless specifically cited, the methods described in Manual of Microbiological Methods[25] were followed. All those weakly positive and negative reactions were ascertained by repeated testing and *X. translucens* f. sp. *oryzicola*, the leaf streak pathogen was included in certain tests.

Hydrolysis of casein, gelatin, and starch. Casein hydrolysis was noted in milk agar plates[23] after 4 days incubation. While hydrolysis of gelatin and starch were studied in peptone agar[25] with 0.4% gelatin and 1.0% soluble starch, respectively. Liquefaction of gelatin was tested in gelatin stabs with agar.

Litmus milk reaction. The inoculated litmus milk was observed for acid production, reduction of litmus, clearing *etc* after one week of incubation.

Production of ammonia, H_2S, and indole. Peptone broth cultures were tested for ammonia with Nessler's reagent after 2-3 days incubation. H_2S production was noted in slants of peptone agar with 0.01% cysteine by lead acetate paper strips. Indole was detected in tryptone broth cultures by Kovac's reagent.

Nitrate reduction. Peptone broth cultures were tested with dimethyl α-naphthylamine and sulfanilic acid reagents for the presence of nitrite. While unreduced nitrate was detected with zinc pieces.

Methyl red and V. P. tests. Tryptone glucose broth cultures were tested with methyl red for acid production and with α-naphthol for acetoin formation after 3-4 days of incubation.

Mode of glucose utilization. It was studied in Hugh & Leifson's[15] medium.

Acid production from sugars. Starr's medium[26] with 0.05% yeast extract was used. The sugars and related compounds were incorporated at 0.5% using bromocresol purple. All compounds were filter-sterilized except starch, dextrin, glycogen, cellulose, and inulin which were steam sterilized.

Utilization of organic acids. Studied in above medium with 0.2% organic acids using bromothymol blue and pH was adjusted to 6.8 before sterilization.

Lipase. Studied following the method of Sierra[21].

Cellulase (Cx). It was tested in carboxymethyl cellulose agar[9] and plates were flooded with 10% copper acetate solution[4].

Pectolytic activity. Studied by the method of Hildebrand[13].

Phosphatase and sulphatase. Phosphatase was detected by the method of Barber & Kuper[1] and sulphatase was tested with 0.01% phenolphthalein disulphate.

Catalase. Studied by transferring loopful of growth on to a drop of 10 vol H_2O_2.

Oxidase. It was tested using 1% dimethyl-p-phenylene diamine dichloride[6,17].

Amino acid decarboxylases. Studied by Falkow's method as described by Skerman[22].

Arginine hydrolase and asparaginase. Arginine hydrolase was tested by the method of Thornley[28] and aspariginase was detected with 1% L-asparagine.

Phenylalanine deaminase. It was noted in phenylalanine agar[22].

Tyrosinase. Studied following the method of Waksman[30].

Urease. Christensen's agar[3] was used.

Uric acid utilization. Studied in peptone agar with 0.05% uric acid, a modification from Dye[5].

Aesculin hydrolysis. It was tested by the method of Sneath[24].

β-glucosidase. Studied by the method of Hildebrand & Schroth[14]

Results

The results presented in Table 1, revealed that all the isolates of *X. oryzae* hydrolysed gelatin but not casein and starch. However the extent of gelatin hydrolysis greatly varied and among the isolates, PXo 10 hydrolysed gelatin quickly than others. But no correlation was evident between virulence and degree of gelatin hydrolysis. All the 40 cultures failed to produce acid in litmus milk even after 1 week of incubation and the alkaline reaction was evident by the presence of blue colour in undisturbed cultures. Production of ammonia and H_2S was evident with all the cultures, but no indole and nitrite were formed even after one week. However more H_2S production was noted with BU3, Ind1-6, PXo 25, C 16, and B 20-6; and ammonia production was observed with H 100, Ind 7-3, PXo 37, and C 16.

All the isolates of *X. oryzae* did not produced acid from D(−) ribose, L(+) arabinose, L(+) rhamnose, L(−) sorbose, maltose, lactose, cellulose, starch, dextrin, inulin, glycogen, and sugar alcohols like sorbitol, mannitol, dulcitol, and growth was inhibited in above sources. While they readily produced acid in variable degrees from D(+) glucose, D(−) galactose, D(−) fructose, D(+) mannose, D(−) arabinose, D(+) xylose, cellobiose and sucrose. Isolates like B 33 and PXo 37 produced more acid in sucrose. They utilized organic acids of citric acid cycle and did not grow in oxalic acid, tartaric acid, and benzoic acid. Glucose was assimilated oxidatively and negative methyl red and V. P. tests were noted.

The isolates of blight pathogen produced cellulase (Cx) and lipase in different degrees after 3-4 days of incubation. Pectolytic activity was evident in Na-polypectate and pectin gels at pH 4.9-5.1, but at higher pH (8.3-8.5) no acitivity was detected. More cellulase (Cx) and pectolytic reactions were observed with virulent isolates: H 100, and PA 3.

All the isolates of *X. oryzae* recorded positive results with catalase, phosphatase, uric acid utilization, and aesculin hydrolysis, and negative reactions for sulphatase, arginine hydrolase, amino acid decarboxylases, phenylalanine deaminase, tyrosinase, β-glucosidase, asparaginase, and urease. No oxidase reaction was detected in 24 hr old cultures grown on Wakimoto's agar, when tested in 5-10 sec.

Discussion

The present study revealed no distinct differences in physiological and biochemical reactions among 40 isolates of *X. oryzae* even from wide geographical regions. From this investigation it is evident that the bacterial blight pathogen is much more homogeneous than reported in literature[2, 19, 20].

A comparison of the data of various workers (Table 2) showed that the present results confirm the reports of Mukoo & Isaka[18] and Goto[7, 8] with regard to gelatin liquefaction, production of ammonia and H_2S, starch hydrolysis, litmus milk reaction and acid production from sugars. So Ishiyama's[16] original description should be revised with respect to above characteristics and can be extended to include several other properties described in the results reported here.

However the present results contradict the existence of distinct biochemical

Table 1. Physiological and biochemical characteristics of 40 isolates of *Xanthomonas oryzae* from Asia.

Test	Bacterial PA 3	BU 3	H 14	H 100	H 257	Ind 1-6	Ind 4-1	Ind 7-3	Kb	Kc	Kd	N 1	N 2	N 3	N 4	B 1	B 5
Hydrolysis of																	
a) galatin	+ *a)*	±	±	±	±	+	+	+	±	±	+	+	+	±	±	+	±
b) casein	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
c) starch	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H_2S production	+	++	+	+	±	++	+	+	+	+	+	+	+	±	±	+	+
Ammonia production	+	+	±	++	±	+	+	++	+	+	+	+	+	±	+	+	+
Reduction of nitrate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Acid production from																	
1) D(−) arabinose	++	++	++	+	++	+	++	++	++	++	++	++	++	++	+	++	++
2) D(+) xylose	+	++	+	++	++	+	+	+	±	±	±	+	±	±	±	±	±
3) D(−) galactose	++	++	++	+	++	+	++	++	++	++	++	++	++	+	+	++	++
4) D(+) glucose	++	++	++	+	++	++	++	++	++	++	+	++	++	+	+	++	++
5) D(−) fructose	++	++	++	+	++	+	++	++	++	++	++	++	++	++	+	++	++
6) D(+) mannose	++	+	++	+	++	++	+	+	++	++	+	++	++	++	+	++	+
7) cellobiose	+	++	+	++	+	+	+	+	+	+	+	++	++	+	+	+	+
8) sucrose	±	±	±	±	±	±	±	+	±	±	±	±	±	±	±	±	±
Cellulase (Cx)	++	±	++	+	+	+	+	+	+	+	+	+	±	±	±	+	+
Lipase production	++	+	++	+	++	++	++	++	++	++	++	++	+	+	+	++	++
Pectolytic activity	++	+	++	+	±	+	+	+	+	+	+	+	±	±	±	+	±
Catalase	+	±	++	+	±	+	+	+	±	+	+	+	±	±	±	+	+
Oxidase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Hydrolysis of aesculin	++	±	++	+	±	+	++	++	+	+	+	+	±	±	±	+	+
Uric acid utilization	+	±	++	+	+	+	++	++	++	++	+	+	±	+	±	±	±

a): Degree of reaction: ++ = strongly positive; + = positive; ± = weakly positive. − = negative reactions.

isolates

B 14	B 16	B 31	B 33	B 46	B 56	B 65	PXo 2	PXo 10	PXo 17	PXo 25	PXo 35	PXo 37	PXo 40	Vn 2	Vn 6	Vn 12	Vn 17	Cl 6	B 17	B 18	B 19	B 20-6
±	+	++	±	±	±	±	±	++	+	±	+	+	+	±	+	±	+	+	±	±	±	+
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	±	+	+	+	+	+	+	+	+	++	+	+	+	±	+	+	+	++	+	+	+	++
±	+	+	+	±	+	+	+	+	+	+	++	+	+	+	+	+	+	++	±	+	+	+
−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+	++
++	±	±	+	++	++	±	+	±	+	+	++	+	+	+	+	+	±	+	+	++	+	+
++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
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+	+	+	+	+	+	+	++	++	+	+	++	++	+	+	++	++	+	+	+	+	++	+
++	±	++	++	±	±	±	±	+	±	±	±	++	+	±	±	+	±	±	±	+	±	+
±	+	+	±	+	+	±	+	+	+	+	+	±	+	+	±	+	+	+	±	+	+	++
+	++	+	++	++	++	++	++	+	++	++	++	++	++	++	+	++	++	++	++	++	+	++
++	+	+	+	+	+	+	+	±	+	+	+	++	+	+	±	+	+	±	±	+	±	+
+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+	++	+	±	±	±	++
±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−	±	+	+	+	+	++	++	++	++	++	++	++	++	++	+	+	+	±	++	++	+	++
++	+	±	++	++	+	++	++	+	++	++	++	+	++	++	++	±	++	+	++	+	++	++

strains in *X. oryzae,* as reported by Chakravarti & Rangarajan[2]. Such strain is doubtful to be *X. oryzae,* since they recovered the colonies on nutrient agar within 24-48 hr of incubation and also fastidious growth characteristics of organism. It seems to appear that biochemical groups, as suggested by Shekhawat & Srivastava[20], did not exist in blight pathogen and might have originated due to culture contaminations. The characteristics of Group 1 isolates as reported by them have mostly agreed with present findings, but however Group 2 isolates are invalid to be considered as *X. oryzae.* The discrepancies encountered in literature[19, 27] can not be considered due to inherent variability of the organism, but created due to artifacts in the methods used.

Table 2. Physiological and biochemical reactions of *Xanthomonas oryzae* as reported by various researchers.

Test	Present Results	Descripition as per source						
		Ishi-yama	Goto	Mukoo & Isaka	Reddy	Chakravarti & Rangarajan	Shekhawat & Srivastava Gr. 1	Shekhawat & Srivastava Gr. 2
Gelatin liquefaction	+a/	−	+	+	−	+	+	+
H_2S production	+	slight	+	+		+	−	+
Ammonia production	+	−	+	+	+	+	+	+
Starch hydrolysis	−		−	−	−	+	V	+
Litmus milk reaction	alkaline	acidic	alkaline	alkaline		alkaline	alkaline	acidic
Acid production from								
a) lactose	−		−	−	+	(+)	−	+
b) maltose	−		−	−	+	+		+
c) dextrin	−		−	−			−	
Reduction of nitrate	−		−	−	+	−		
Lipase activity	+					−		
Pectolytic reaction	+					−		

a/: + = positive ; (+) = delayed positive (reaction after 7 days) ; v=variable (negative or weakly positive) ; − = negative reactions.

This study appears to show that no distinct biochemical types as suggested with *Pseudomonas solanacearum*[10, 12] or *Xanthomonas malvacearum*[11], were present in *X. oryzae.* However it may be noted from the present data that minor quantitative variations do exist among isolates of *X. oryzae,* as might be expected with other microorganisms. If considering a "++" reaction to be more distinguishable from "+"or"±", we can found that one isolate hydrolysed gelatin more quickly than others; 5 isolates produced more H_2S; 4 isolates produced more ammonia; 7 isolates formed more acid in xylose, while 2 isolates produced more acid in sucrose; 3 isolates showed more cellulase and 3 have more pectolytic activity; 4 cultures yielded more catalase and 12 of the 40 isolates hydrolysed aesculin and 13 utilized uric acid more quickly. However the country of origin has no correlation with any of the characteristics of the isolates tested.

The characteristics of blight pathogen reported here will be quite useful to characterize it as well as in bringing about taxonomic relationship with other nomen-species of *Xanthomonas.*

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Mailing address: Dr. S. H. Ou, Plant Pathologist, IRRI, P. O. Box 933, Manila, The Philippines.

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和 文 摘 要

イネ白葉枯病菌 *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson の性状

O. R. Reddy ・ S. H. Ou

アジア各地から採集した *X. oryzae* の40菌株の生理的性質および生化学的性質を詳細に研究した結果，本菌には明瞭な生化学的タイプは存在しないことが明らかとなった。この事実は，さらに広域から菌株を集めてもそれらは，これまでに文献に記載されてきたものよりもはるかに均一な反応を示すことを示唆する。本報告に記載された本菌の性状は，宿主植物を離れて *X. oryzae* を同定したり，*Xanthomonas* 属の他の菌種から *X. oryzae* を類別する際の規準と考えることができる。