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Production of a Specific Bacteriocin by *Xanthomonas campestris* pv. *citri*

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松尾憲総*・松山宣明**・脇本 哲** : *Xanthomonas campestris* pv. *citri* による作用特異的なバクテリオシンの産生

Among phytopathogenic bacteria, some species such as *Agrobacterium tumefaciens*,^{1,2)} *Corynebacterium* spp.,³⁾ *C. michiganense* pv. *michiganense*,⁴⁾ *Pseudomonas* spp.,^{5,6)} *Ps. solanacearum*,⁷⁾ *Ps. syringae* pv. *syringae*⁸⁾ and *Erwinia carotovora*^{9,10)} were reported to produce bacteriocins. In most of these reports, bacteriocins were utilized for taxonomical purposes, namely, the plant pathogenic bacteria were grouped on the basis of the productivity of bacteriocins or susceptibility to them.

In our previous papers^{11,12)}, we have reported two kinds of antibacterial substances produced by *Xanthomonas campestris* pv. *citri* treated with chloroform vapor. Of these antibacterial substances, the one composed of a protein having molecular weight of ca. 300,000¹²⁾ was found to be a kind of specific bacteriocin only produced by *X. campestris* pv. *citri* in this experiment.

The isolates of xanthomonads shown in Table 1 were inoculated on the plate of YPDA medium (peptone 0.6 g, dextrose 3 g, yeast extract 3 g, agar 15 g, pH 7.2) at a distance of 2 to 3 cm from one other and incubated at 30 C for 48 hr. The Petri dish thus prepared was placed upside down and the lid of it was poured with 1 ml of chloroform to sterilize bacteria. The plate was then overlaid with 1 ml of the suspension of indicator bacteria (conc.: ca. $10^7\sim 10^8$ cells/ml) mixed with 5 ml of 0.5% YPDA medium. The plate was incubated at 30 C for 24 hr. The productivity of antibacterial substance was detected by inhibition zone formed around colony.

All of the 19 isolates of *X. campestris* pv. *citri* used in this experiment produced antibacterial substance active against several pathovars of *X. campestris* as shown in Table 2. The antibacterial substance produced clear inhibition zones on the lawns of *X. campestris* pv. *campestris* X1-1-1, *X. campestris* pv. *oryzae* H5809 and H5854, and *X. campestris* pv. *cucurbitae* X1-3-1. Very weak activity was observed on

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Table 1. Bacterial isolates used in this experiment

Bacterial isolate	Origin of isolate
<i>X. campestris</i> pv. <i>citri</i> 19 isolates	Laboratory of Plant Pathology, Kyushu University
pv. <i>citri</i> 29 isolates	National Institute of Agricultural Sciences
pv. <i>oryzae</i> 20 isolates	"
pv. <i>physalidicola</i> X1-8-1	"
pv. <i>glycines</i> X1-7-1	"
pv. <i>hyacinthi</i> X1-4-1	"
pv. <i>pisi</i> 1 isolate	"
pv. <i>cucurbitae</i> X1-3-1	"
pv. <i>vesicatoria</i> 1 isolate	"
pv. <i>vitians</i> N7501, N7509	"
pv. <i>translucens</i> X1-11-2	"
pv. <i>pruni</i> X1-10-1	"
pv. <i>campestris</i> X1-1-1	"
pv. <i>campestris</i> '64, '72, '76	Laboratory of Plant Pathology, Tokyo University of Agriculture and Technology

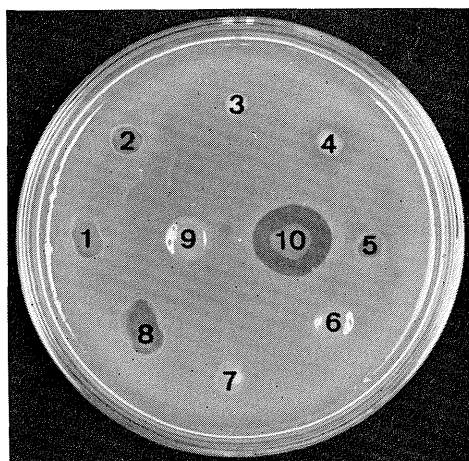


Fig. 1. Inhibition zone formed on the lawn of *X. campestris* pv. *campestris* X1-1-1 by xanthomonads treated with chloroform

4: *X. campestris* pv. *cucurbitae* X1-3-1

10: *X. campestris* pv. *citri* Ku 7503 1~3, 5~9: Other xanthomonads

X. campestris pv. *glycines* X1-7-1 and *X. campestris* pv. *campestris* '64 but not on other pathovars or isolates used in this experiment (Table 2).

All of the isolates of *X. campestris* pv. *citri* with only exception of the isolate Ku 7101 did not show any inhibition zone so far as homologous pathovars were used as an indicator.

The productivity of bacteriocin from many isolates of xanthomonads was tested.

X. campestris pv. *campestris* isolate X1-1-1 was used as an indicator, because the inhibition zone was most clear on this bacterium. The results indicated that all of the 48 isolates of *X. campestris* pv. *citri* produced large and clear inhibition zone (Fig. 1) but all of the other pathovars of *X. campestris* used in this experiment did not (Table 3). This antibacterial substance was considered to be a

bacteriocin similar to that produced by *X. campestris* pv. *citri* Ku 7101¹²⁾ because of their similarity in antibacterial spectrum (Table 2).

Table 2. Activity spectrum of the antibacterial substance produced by chloroform treatment of *X. campestris* pv. *citri*

Indicator	Producer (<i>X. campestris</i> pv. <i>citri</i>)		
	Ku 7101	16 isolates including Ku 7501	2 isolates including Ku 7508
<i>X. campestris</i>			
pv. <i>glycines</i>			
X1-7-1	+	(+)	(+)
pv. <i>physalidicola</i>			
X1-8-1	-	-	-
pv. <i>psi</i>	-	-	-
pv. <i>oryzae</i>			
H5809	(+)	+	(+)
H5846	-	-	-
H5854	+	+	+
H5808	-	-	-
pv. <i>hyacinthi</i>			
X1-4-1	-	-	-
pv. <i>cucurbitae</i>			
X1-3-1	+	+	+
pv. <i>campestris</i>			
X1-1-1	+	+	+
'64	(+)	(+)	(+)
'72	-	-	-
'76	-	-	-
pv. <i>citri</i>			
Ku 7101	+	-	-

+; Clear inhibition zone (+); Turbid inhibition zone -; Negative response

Table 3. Production of a specific bacteriocin by various pathovars of *X. campestris* treated with chloroform

Isolate	Productivity ^{a)}	Isolate	Productivity ^{a)}
<i>X. campestris</i>		pv. <i>vesicatoria</i>	-
pv. <i>citri</i>		pv. <i>campestris</i>	
48 isolates	+	X1-1-1	-
pv. <i>oryzae</i>		'64	-
20 isolates	-	'72	-
pv. <i>physalidicola</i>		'76	-
X1-8-1	-	pv. <i>vitians</i>	
pv. <i>glycines</i>		N 7501	-
X1-7-1	-	N 7509	-
pv. <i>hyacinthi</i>		pv. <i>translucens</i>	
X1-4-1	-	X1-11-2	-
pv. <i>psi</i>	-	pv. <i>pruni</i>	
pv. <i>cucurbitae</i>		X1-10-1	-
X1-3-1	(+)		

+; Clear inhibition zone (+); Turbid inhibition zone -; Negative response

a) Indicator: *Xanthomonas campestris* pv. *campestris* X1-1-1

From these results, it may be possible to suggest that there is an obvious difference between *X. campestris* pv. *citri* and other pathovars in bacteriocin productivity.

In general, bacteriocins are active against some kinds of heterologous isolates belonging to the species similar to or close to the producers¹³⁾. In the case of *Serratia marcescens*¹⁴⁾, however, it was reported that the bacteriocin was only active against some species other than producer. The bacteriocin commonly produced by all isolates of *X. campestris* pv. *citri* also did not show any inhibition zone on the same pathovar as in the case of *S. marcescens*, and the productivity of this bacteriocin is considered to be a property specific for *X. campestris* pv. *citri*.

Dye^{15,16,17)} reported that he could not find any significant difference among many species of xanthomonads in terms of bacteriological properties. In the 8th edition of Bergey's Manual of Determinative Bacteriology,¹⁸⁾ many xanthomonads listed in the 7th edition were put together into *X. campestris* group to divide all xanthomonads into 5 species of *X. ampelina*, *X. campestris*, *X. albilineans*, *X. fragariae* and *X. axonopodis*. An obvious difference between *X. campestris* pv. *citri* and other pathovars in bacteriocin productivity may suggest that some fundamental differences exist in properties related to pathogenicity.

Although it will be necessary to examine with greater number of xanthomonads, the detection of bacteriocin productivity by using *X. campestris* pv. *campestris* X1-1-1 could be a simple method for identification of *X. campestris* pv. *citri*.

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