Characterization of *Xanthomonas* spp. strains by bacteriocins

Marcel Bonini[1], Antonio Carlos Maringoni[1], Julio Rodrigues Neto[2]

[1]UNESP – São Paulo State University, Faculty of Agronomy, Department of Crop Production, P.O. Box 237, 18603-970 Botucatu, State of São Paulo, Brazil. [2]Biological Institute, Laboratory of Plant Pathogenic Bacteria, P.O. Box 70, 13001-970 Campinas, State of São Paulo, Brazil
Corresponding author: Antonio Carlos Maringoni e-mail: maringoni@fca.unesp.br
Data de chegada: 03/05/2005. Aceito para publicação em: 03/03/2006.

ABSTRACT

Bonini, M.; Maringoni, A. C.; Rodrigues Neto, J. Characterization of *Xanthomonas* spp. strains by bacteriocins. ***Summa Phytopathologica***, v.33, n.1, p.24-29, 2007.

Twenty-five strains of *Xanthomonas axonopodis* pv. *citri* and 14 strains of *Xanthomonas* spp. were tested for bacteriocin production. *X. axonopodis* pv. *passiflorae* strains were sensitive to the bacteriocins produced by the 25 *X. axonopodis* pv. *citri* strains evaluated in this study while strains of *X. axonopodis* pv. *manihotis* and *X. campestris* pv. *campestris* showed variable sensitivity. Only five of the 25 *X. axonopodis* pv. *citri* strains were not inhibited by the bacteriocins produced by the two *X. axonopodis* pv. *passiflorae* strains. The bacteriocins produced by the *Xanthomonas axonopodis* pv. *citri* (FDC-806) and *X. axonopodis* pv. *passiflorae* (Mar-2850 A) strains were thermolabile, resistant to lysozyme and sensitive to DNAse. The bacteriocin produced by *X. axonopodis* pv. *passiflorae* was resistant to the action of proteinase K, trypsin and RNAse while the bacteriocin produced by *X. axonopodis* pv. *citri* was sensitive to these enzymes. The bacteriocins produced by *X. axonopodis* pv. *passiflorae* and *X. axonopodis* pv. *citri* were called passifloricin and citricin, respectively.

Additional keywords: bacteria, citrus canker

RESUMO

Bonini, M.; Maringoni, A. C.; Rodrigues Neto, J. Caracterização de isolados de *Xanthomonas* spp. por bacteriocinas. ***Summa Phytopathologica***, v.33, n.1, p.24-29, 2007.

Vinte e cinco isolados de *Xanthomonas axonopodis* pv. *citri* e 14 isolados de *Xanthomonas* spp. foram comparados a fim de verificar a capacidade de produção de bacteriocina e a sua sensibilidade. Isolados de *X. axonopodis* pv. *passiflorae* foram sensíveis às bacteriocinas produzidas por 25 isolados de *X. axonopodis* pv. *citri* avaliados e os isolados de *X. axonopodis* pv. *manihotis* e *X. campestris* pv. *campestris* apresentaram sensibilidade variável. Dos 25 isolados de *X. axonopodis* pv. *citri* apenas cinco não foram inibidos pelas bacteriocinas produzidas por dois isolados de *X. axonopodis* pv. *passiflorae*. As bacteriocinas produzidas pelos isolados de *X. axonopodis* pv. *citri* (FDC-806) e de *X. axonopodis* pv. *passiflorae* (Mar-2850 A) foram termolábeis e resistentes à lisozima e sensíveis a DNAse. A bacteriocina produzida pelo isolado de *X. axonopodis* pv. *passiflorae* foi resistente à ação de proteinase K, tripsina e RNAse enquanto que a produzida pelo isolado de *X. axonopodis* pv. *citri* foi sensível a essas enzimas. As bacteriocinas produzidas por *X. axonopodis* pv. *passiflorae* e por *X. axonopodis* pv. *citri* foram denominadas passifloricina e citricina, respectivamente.

Palavras-chave adicionais: bactéria, cancro cítrico

Citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin *et al.* (18), is one of the main bacterial diseases affecting the world's citrus crops. The pathogen causing citrus canker is originated from Southeast Asia and belongs to a group of diseases that cannot be eradicated by curative treatments and its causal agent spreads easily (1). The economic importance of the disease is a consequence of the reduction in the photosynthetic area of the affected leaves, causing stunted growth of the plant, a reduction in fruit size, premature fall of the fruits from the tree, and commercial depreciation of the fruit due to superficial lesions. The importation of fruits originating from countries where citrus canker occurs is prohibited by many consumer markets because the eradication of this disease is difficult and expensive (3).

Bacteriocins are substances produced by bacteria which are able to kill or inhibit the growth of other related bacteria (20). Bacteriocins are of protein origin and the genes responsible for their synthesis are located on plasmids or on the chromosome. These substances were originally called "colicins" since they were initially isolated from *Escherichia coli*, but on were later detected in other bacterial species and then called bacteriocins (19).

Bacteriocins have important applications in phytopathology, including their use in epidemiological studies, for typification of phytobacterial strains and for the identification and classification of bacteria, as well as a potential option for the control of

phytopathogenic bacteria (20).

Various genera of phytopathogenic bacteria have been characterized regarding the production of bacteriocins including *Clavibacter* (8, 10), *Erwinia* (5), *Pseudomonas* (2), *Ralstonia* (7), *Agrobacterium* (9), *Curtobacterium* (8, 12), and *Xanthomonas* (2, 6, 11, 13, 14, 15, 17).

In the case of *X. axonopodis* pv. c*itri*, studies conducted outside Brazil have indicated the presence of strains that produce bacteriocins and are also sensitive to the bacteriocins produced (11, 13). In Brazil, the study of Bonini (4) analyzing 48 *X. axonopodis* pv. *citri* strains demonstrated the lack of bacteriocinogenic strains able to inhibit them.

The objective of the present study was to evaluate the production and sensitivity to bacteriocins produced by *Xanthomonas* spp. strains (25 *X. axonopodis* pv. *citri* strains and 14 *Xanthomonas* strains belonging to four different species and various pathovars) as well as to characterize the bacteriocins produced by one *X. axonopodis* pv. *citri* strain and one *X. axonopodis* pv. *passiflorae* strain.

MATERIAL AND METHODS

Evaluation of bacteriocin production by *Xanthomonas* spp. strains

Twenty-five *Xanthomonas axonopodis* pv. *citri* strains and 14 *Xanthomonas* spp. strains (Table 1) were evaluated regarding their capacity to produce bacteriocins and their capacity to inhibit the growth of bacterial strains.

Bacteriocin production was evaluated according to the methods of Kurozawa (10), Matsuo et al. (13) and Maringoni & Kurozawa (12). The strains were transferred to Petri dishes containing YPDA medium (0.6 g peptone, 3 g dextrose, 3 g yeast extract, 15 g agar, and 1000 mL distilled water) according to Matsuo et al. (13). After growth at 30ºC for 24 h, the colonies were transferred to other Petri dishes containing YPDA medium with a dispenser content nine felt discs measuring 4 mm in diameter (12), with each disc corresponding to one strain. After growth of the strains on the culture surface (30ºC, 24 h), the Petri dishes were placed in an inverted position under an exhaust hood, 1 mL chloroform was added to the lid of each dish and the dishes were incubated for 2 h for bacterial inactivation. For the determination of bacteriocin production, the dishes were overlaid with 5 mL semisolid melting (45ºC) YPDA medium containing 1 mL of a bacterial suspension previously cultured in liquid YPD medium. The Petri dishes were incubated for 24 h at 30ºC and the presence or absence of an inhibition halo around the bacteriocin-producing colonies was recorded (11).

Characterization of the bacteriocins produced by the bacteriocinogenic strains

Production of crude bacteriocin in liquid medium

The technique used for the production of crude bacteriocin in liquid medium was based on the method of Oliveira (14). The bacteriocinogenic strains *X. axonopodis* pv. *citri* (FDC-806) and *X. axonopodis* pv. *passiflorae* (Mar-2850 A) were transferred to 20 mL liquid YPD medium and incubated at 30ºC for 24 h under shaking. After incubation, the bacterial suspensions were centrifuged at 12,000 *g* for 2 min and the supernatants (crude bacteriocin) were transferred to sterile test tubes with screw-on caps. A 20-µL aliquot of the supernatant was deposited on a sterile filter paper disc (13 mm) which was placed in the center of a Petri dish on semisolid YPDA medium containing 1 mL of a suspension of the sensitive, previously cultured strain. The strains were tested against one another. The dishes were evaluated after 24 of incubation at 30ºC and the results were reported as the presence or absence of an inhibition halo.

Thermal sensitivity

Crude bacteriocin (500 µL) from each bacterial strain (FDC-806 and Mar-2850 A) was incubated at the following temperatures according to Oliveira (14): 37ºC (60 min), 42ºC (60 min), 65ºC (30 min), 80ºC (15 min), and 100ºC (15 min). The inhibitory activity of the bacteriocins was evaluated as described above.

Bacteriophage activity

Chloroform (10%, v/v) was added to 2 mL of crude bacteriocin obtained from the producer strains (FDC-806 and Mar-2850 A). The mixture was homogenized in an automatic shaker and left to decant for 30 min to separate the chloroform from the crude extract. About 100 µL crude extract was then added to 5 mL semisolid melting YPD medium containing 500 µL of the indicator strain (FDC-806 and Mar-2850 A) cultured in YPD medium for 24 h at 30ºC and overlaid on a Petri dish containing YPDA medium. The presence of lysis plaques on the culture surface where the bacteria had grown was evaluated after 24 h of incubation.

Enzymatic sensitivity

Aliquots (500 µL) of crude bacteriocin from strains FDC-806 and Mar-2850 A were separately treated with 15 µL/mL 2% proteinase K (Qbiogene®), 120 µL/mL 0.25% trypsin (Gibco®), 60 µL/mL 1% lysozyme (Sigma®), 25 µL/mL RNAse (2 U/µL, Invitrogen®), and 100 µL/mL DNAse (1 U/µL, Invitrogen®) for 2 h at 37ºC, and then analyzed against the sensitive strain (FDC-806 or Mar-2850 A) as described above.

RESULTS AND DISCUSSION

All 25 *X. axonopodis* pv. *citri* strains produced bacteriocin as indicated by the inhibition of the growth of *X. axonopodis* pv. *passiflorae* strains (Mar-2850 A and Mar-2850 B) and of one *X. campestris* pv. *campestris* strain (Br-2808). *X. axonopodis* pv. *manihotis* strain Man-7597 presented variable sensitivity to bacteriocins produced by strains of *X. axonopodis* pv. *citri* (FDC-213, FDC-106, FDC-585, FDC-625, FDC-601, FDC-007, 14002, and 12856), demonstrating differences in the action spectrum of these bacteriocins (Table 2 and Figure 1).

Twenty *X. axonopodis* pv. *citri* strains were sensitive to bacteriocins produced by strains Mar-2850 A and Mar-2850 B (*X. axonopodis* pv. *passiflorae*), except for *X. axonopodis* pv. *citri* strains FDC-585, FDC-625, FDC-601, FDC-011, and 14002 (Table 3 and Figure 2). The production of bacteriocins by the genus *Xanthomonas* that inhibit the growth of another pathovar is a common phenomenon (2, 6, 13, 15), as demonstrated by the sensitivity of most *X. axonopodis* pv. *citri* strains to bacteriocins produced by strains of *X. axonopodis* pv. *passiflorae*. However, strains of *X. axonopodis* pv. *vitians*, *X. axonopodis* pv. *phaseoli*, *X. vesicatoria* and *X. cucurbitae* exerted no bacteriocinogenic action on *X. axonopodis* pv. *citri* strains and were insensitive to bacteriocins produced by *X. axonopodis* pv. *citri* strains (Tables 2 and 3).

The results of the thermal treatment of crude bacteriocin extracts obtained from strains FDC-806 and Mar-2850 A at different temperatures and incubation times tested demonstrated that the

Table 1 – *Xanthomonas* spp. strains evaluated for bacteriocin production.

Strain	Species	Origin
FDC-806	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-213	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-022	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-616	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-121	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-609	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-075	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-106	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-767	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-714	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-118	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-585	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-625	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-601	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-561	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-553	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-007	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-011	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
12976	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
12864	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
14002	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-039	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-545	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
FDC-501	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
12856	*Xanthomonas axonopodis* pv. *citri*	FUNDECITRUS
Al-2937	*Xanthomonas axonopodis* pv. *vitians*	DPV-Phytosanitary Defense
Co-2909	*Xanthomonas campestris* pv. *campestris*	DPV-Phytosanitary Defense
Br-2908	*Xanthomonas campestris* pv. *campestris*	DPV-Phytosanitary Defense
I-4	*Xanthomonas axonopodis* pv. *phaseoli*	DPV-Phytosanitary Defense
F-41	*Xanthomonas axonopodis* pv. *phaseoli*	DPV-Phytosanitary Defense
Feij-2498	*Xanthomonas axonopodis* pv. *phaseoli*	DPV-Phytosanitary Defense
Feij-7631	*Xanthomonas axonopodis* pv. *phaseoli*	DPV-Phytosanitary Defense
Man-2763	*Xanthomonas axonopodis* pv. *manihotis*	DPV-Phytosanitary Defense
Mar-2850 A	*Xanthomonas axonopodis* pv. *passiflorae*	DPV-Phytosanitary Defense
Mar-2850 B	*Xanthomonas axonopodis* pv. *passiflorae*	DPV-Phytosanitary Defense
Pi-2046	*Xanthomonas vesicatoria*	DPV-Phytosanitary Defense
P-29	*Xanthomonas vesicatoria*	DPV-Phytosanitary Defense
7582	*Xanthomonas cucurbitae*	DPV-Phytosanitary Defense

bacteriocin produced by strain FDC-806 was inactivated at a temperature of 42°C or higher. In contrast, the bacteriocin produced by strain Mar-2850 A was only inactivated when treated at a temperature of 65ºC or higher for 15 min (Table 4). The bacteriocins produced by the bacterial strains were considered to be thermolabile, similar to those reported for *Erwinia* spp. (5) and *X. axonopodis* pv. *glycines* (6).

No lysis plaques were observed on the growth of the bacterial indicator strains, demonstrating the absence of bacteriophage particles in the crude extracts of the bacteriocins analyzed. According to Frey *et al.* (7), Oliveira & Rosato (15) and Tudor- Nelson *et al.* (17), analysis of bacteriophage activity is a standard procedure in the characterization of bacteriocins.

At the concentrations and treatment times used for the different enzymes, the bacteriocin produced by strain FDC-806 was resistant to lysozyme only whereas the bacteriocin produced by strain Mar-2850 A was resistant to proteinase K, trypsin, lysozyme and RNAse (Table 5).

Table 2 – Action of bacteriocins produced by 25 *Xanthomonas axonopodis* pv. *citri* (Xac) strains on *Xanthomonas* spp. strains.

Producer strain	Sensitive strain (*Xanthomonas* spp.)													
(Xac)	Al-2937	Al-2940	Man-2763	I-4	F-41	Feij-2498	Feij-7632	Pi-2046	P-29	Co-2909	Br-2908	Mar –2850 A	Mar-2850 B	7582
FDC-806	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-213	–	–	+	–	–	–	–	–	–	–	+	+	+	–
FDC-022	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-616	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-121	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-609	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-075	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-106	–	–	+	–	–	–	–	–	–	–	+	+	+	–
FDC-767	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-714	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-039	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-545	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-501	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-118	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-585	–	–	+	–	–	–	–	–	–	–	+	+	+	–
FDC-625	–	–	+	–	–	–	–	–	–	–	+	+	+	–
FDC-601	–	–	+	–	–	–	–	–	–	–	+	+	+	–
FDC-561	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-553	–	–	–	–	–	–	–	–	–	–	+	+	+	–
FDC-007	–	–	+	–	–	–	–	–	–	–	+	+	+	–
FDC-011	–	–	–	–	–	–	–	–	–	–	+	+	+	–
12864	–	–	–	–	–	–	–	–	–	–	+	+	+	–
14002	–	–	+	–	–	–	–	–	–	–	+	+	+	–
12856	–	–	+	–	–	–	–	–	–	–	+	+	+	–
12876	–	–	–	–	–	–	–	–	–	–	+	+	+	–

(+) presence of an inhibition halo; (-) absence of an inhibition halo.

Table 3 – Action of bacteriocins produced by 14 *Xanthomonas* spp. strains on 25 *Xanthomonas axonopodis* pv. *citri* (Xac) strains.

Producer strain	Sensitive strain (Xac)																								
(*Xanthomonas*	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC	FDC			
spp.)	806	213	022	616	121	609	075	106	767	714	039	545	501	118	585	625	601	561	553	007	011	12976	12864	14002	12856
Al-2937	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Al-2940	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Man-2763	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
I-4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F-41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Feij-2498	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Feij-7631	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pi-2046	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P-29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Co-2909	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Br-2908	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Mar-2850 A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	–	+	+	–	+
Mar-2850 B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+	+	+	–	+	+	–	+
7582	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

(+) presence of an inhibition halo; (-) absence of an inhibition halo.

Table 4 – Effect of thermal treatment at different temperatures and incubation times on bacteriocins produced by *Xanthomonas axonopodis* pv. *citri* (FDC-806) and *Xanthomonas axonopodis* pv. *passiflorae* (Mar-2850 A).

	Temperature/incubation time			
Strain	Control	37°C/60 min	42°C/60 min	65°C, 80°C and 100°C/15 min
FDC-806	+	+	-	-
Mar-2850 A	+	+	+	-

(+) presence of an inhibition halo; (-) absence of an inhibition halo.

Table 5 – Activity of the bacteriocin samples after treatment with proteinase K, trypsin, lysozyme, RNAse and DNAse for 2 h at 37°C.

Strain	Proteinase K	Trypsin	Lysozyme	RNAse	DNAse
FDC-806	-	-	+	-	-
Mar-2850 A	+	+	+	+	-

(+) resistant ; (-) sensitive.

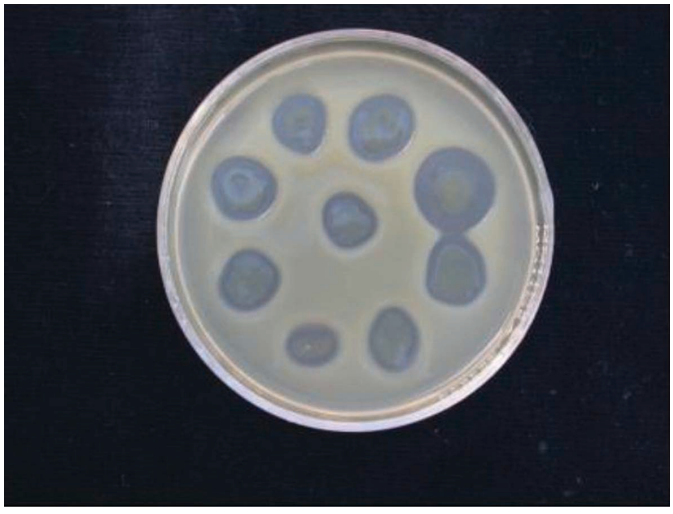

Figure 1. Sensitivity of *Xanthomonas axonopodis* pv. *passiflorae* strain Mar-2850 A to bacteriocins produced by nine *Xanthomonas axonopodis* pv. *citri* strains.

A protein origin of the bacteriocin produced by strain FDC-806 can be suggested since Fett *et al.* (6), Oliveira & Rosato (15) and Tudor-Nelson *et al.* (17) have reported results similar to those reported in the present study. However, the hypothesis that the bacteriocin produced by strain Mar-2850 A is of protein origin cannot be ruled out, with this bacteriocin requiring a longer time of incubation for the occurrence of enzymatic reaction or of the formation of complexes with the different molecules present in the crude extract, a fact impairing the action of proteinase K as reported by Oliveira (14).

The sensitivity to DNAse can be explained by the presence of DNA fragments in the crude bacteriocin extract (14). Fett et al. (6) observed differences in the sensitivity to enzymatic treatment with trypsin and DNAse, indicating that *X. axonopodis* pv. *glycines* produces more than one type of bacteriocin. RNAse and DNAse did not affect the activity of bacteriocins produced by most coryneform

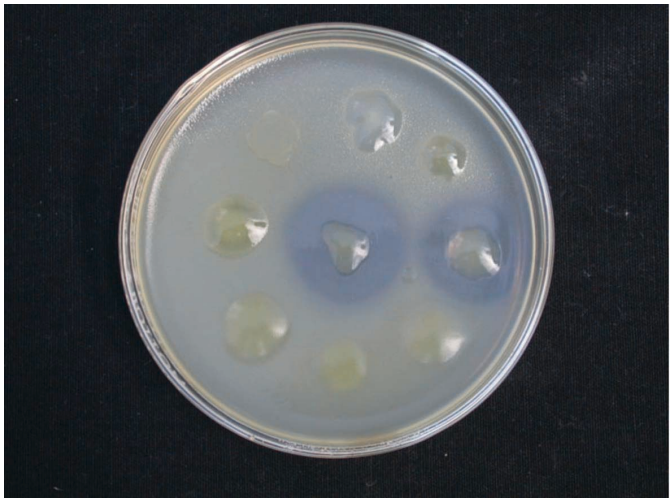

Figure 2. Sensitivity of *Xanthomonas axonopodis* pv. *citri* strain FDC-806 to bacteriocins produced by *Xanthomonas axonopodis* pv. *passiflorae* strains Mar-2850 A and Mar-2850 B.

bacteria (8), whereas the bacteriocin produced by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* was resistant to trypsin.

The bacteriocins produced by the strains tested here were resistant to the action of lysozyme, demonstrating that these bacteriocins do not require an association with lipid or carbohydrate molecules for their activity (16, 21). We suggest the denomination of passifloricin and citricin for the bacteriocins produced by *X. axonopodis* pv. *passiflorae* and *X. axonopodis* pv. *citri*, respectively. A further step would be to isolate, sequence and insert the genes responsible for the production of these bacteriocins into passion fruit and citrus plants, respectively, to obtain resistant plants to bacterial spot and citrus canker.

ACKNOWLEDGMENTS

The authors are grateful to FAPESP and FUNDECITROS for financial support.

REFERENCES

1.Amaral, S. F. Providências para erradicação do cancro cítrico. **O Biológico**, São Paulo, v.23, p.112-123, 1957.

2.Biagi, C.M.R.; Azevedo, J.L. Detecção de bacteriocinas produzidas por bactérias fitopatogênicas dos gêneros *Erwinia*, *Pseudomonas* e *Xanthomonas*. **Scientia Agricola**, Piracicaba, v.49, p.1-8, 1992.

3.Bitancourt, A. A. O cancro cítrico. **O Biológico**, São Paulo, v.23, p.101-111, 1957.

4.Bonini, M. **Produção e sensibilidade de isolados de *Xanthomonas axonopodis* pv. *citri* a bacteriocinas**. 2005. 42p. Master's thesis in Plant Protection – Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, Botucatu.

5.Echandi, E.; Moyer, J.W. Production, properties, and morphology of bacteriocins from *Erwinia chrysanthemi*. **Phytopathology**, St. Paul, v.69, p. 1204-1207, 1979.

6.Fett, W.F.; Dunn, M.F.; Maher, G.T.; Maleef, B.E. Bacteriocin and temperate phage of *Xanthomonas campestris* pv. *glycines*. **Current. Microbiology**, New York, v.16, p.137-144, 1987.

7.Frey, P.; Smith, J.J.; Albar, L.; Prior, P.; Saddler,G.S.; Trigalet-Demery, D.; Trigalet, A. Bacteriocin typing of *Burkholderia (Pseudomonas) solanacearum* race 1 of the French West Indies and correlation with genomic variation of pathogen. **Applied and Environmental Microbiology**, Washington, v.62, p.473-479, 1996.

8.Gross, D.C.; Vidaver, A.K. Bacteriocins of phytopathogenic *Corynebacterium* species. **Canadian Journal of Microbiology**, Toronto,

v.25, p.367-374, 1978.

9. Kerr, A.; Htay, K. Biological control of crown gall through bacteriocin production. **Physiology and Plant Pathology**, Bangor, v.4, p.37-44, 1974.

10. Kurozawa, C. **Caracterização de *Corynebacterium michiganense* (Smith) Jensen através de sorologia e sensibilidade a bacteriófagos, a drogas e a bacteriocina**. 1980. 61p. Thesis – Faculdade de Ciências Agronômicas, Universidade Estadual Paulista, Botucatu.

11. Mohammad, M.; Mizaee, M.R.; Rahimian, H. Physiological and biochemical characteristics of Iranian strains of *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus bacterial canker disease. **Journal of Phytopathology**, Berlin, v.149, p.65-75, 2001.

12. Maringoni, A. C.; Kurozawa, C. Tipificação de isolados de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* por bacteriocinas. **Pesquisa Agropecuária Brasileira**, Brasilia,v.37, n.9, p.1339-1345, 2002.

13. Matsuo, N.; Matsuyama, N.; Wakimoto, S. Production of a specific bacteriocin by *Xanthomonas campestris* pv. *citri*. **Annals of Phytopathological Society of Japan**, Tokyo, v. 47, p.571-574, 1981.

14. Oliveira, V.M. **Produção de bacteriocina por *Xanthomonas campestris* pv. *glycines*:** detecção, caracterização e potencial para proteção de plantas. 1992. 224p. Master's thesis in Genetics – Instituto de Biologia, Universidade de Campinas, Campinas.

15. Oliveira, V.M.; Rosato, Y.B. *In vitro* effect of a bacteriocinogenic strain over sensitive and non-sensitive strain of *Xanthomonas campestris* pv. *vesicatoria*. **Summa Phytopathologica**, Jaboticabal, v.22, p 162-168, 1996.

16. Park, S.H.; Itoh, K.; Fujisawa, T. Characteristics and identification of enterocins produced by *Enterococcus faecium* JCM 5804. **Journal of Applied Microbiology**, Washington, v. 95, p.294–300, 2003.

17. Tudor-Nelson, S.M.; Minsavage, G.V..; Stall, R.E.; Jones, J.B. Bacteriocin-like substances from tomato race 3 strains of *Xanthomonas campestris* pv. *vesicatoria*. **Phytopathology**, St. Paul, v.93, n.11, p.1415-1421, 2003.

18. Vauterin, L.; **Hoste**, B.; Kersters, K.; Swings, J. Reclassification of *Xanthomonas*. **International Journal of Systematic Bacteriology**, Bordeaux, v.45, p.472-489, 1995.

19. Vidaver, A . K. Bacteriocins: the lure and the reality. **Plant Disease**, St Paul, v.67, p.471-475, 1983.

20. Vidaver, A.K. Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. **Annual Review of Phytopathology**, Palo Alto, v.14, p.451-465, 1976.

21. Yildirim, Z.; Johnson, M.G. Detection and characterization of a bacteriocin produced by *Lactococcus lactis* subsp. *cremoris* R isolated from radish. **Letters in Applied Microbiology**, London, v.26, n.4, p.297-304, 1998.