Turkish Journal of Biology

Volume 24 | Number 4 Article 11

1-1-2000

Bacteriocin Typing of Some Turkish Isolates ofPseudomonas syringae pv. phaseolicola

KIYMET GÜVEN

Turk J Biol
24 (2000) 795–801

Bacteriocin Typing of Some Turkish Isolates of *Pseudomonas syringae* pv. *phaseolicola*

Kıymet GÜVEN
Anadolu University, Faculty of Science, Department of Biology, 26470 Eskişehir-TURKEY

Received: 11.02.1999

Abstract: Eighty-six *Pseudomonas syringae* pv. *phaseolicola* isolates collected from different bean growing areas in Eskişehir were typed for the production of bacteriocin.All the isolates tested produced bacteriocin and 24 bacteriosin groups were determined. No correlation was found between the bacteriocin groups and geographical origin. Authentic isolates of the bacterium representing 3 different races were also tested for bacteriocin production and bacteriocin types did not correlate with the races.

Key Words: Pseudomonas syringae pv. phaseolicola, bacteriocin, bacteriocin typing.

Türkiye Orijinli Bazı *Pseudomonas syringae* pv. *phaseolicola* İzolatlarının Bakteriosin Tiplendirmesi

Özet: Eskişehir'de çeşitli fasulye üretim alanlarından toplanan 86 *Pseudomonas syringae* pv. *phaselolicola* izolatı, bakteriyosin üretme yeteneğine göre tiplendirilmiştir. Test edilen tüm izolatlar bakteriosin üretmiş ve 24 bakteriosin grubu tespit edilmiştir. Bateriosin grupları ve strainlerin coğrafik orijinleri arasında bir ilişki bulunmamıştır. Bakterinin 3 farklı ırkını temsil eden tanınmış izolatları da bakteriosin üretim için test edilmiş ve bakteriosin grupları ile ırklar arasında da bir ilişki gözlenmemiştir.

Anahtar Sözcükler: P*seudomonas syringae* pv. *phaseolicola*, bateriosin, bakteriosin tiplendirmesi.

Introduction

Bacteriocins are non-replicating, proteinaceous antibiotics with specificity generally restricted to closely related bacterial strains and species. Small amounts of bacteriocins are produced spontaneously in cultures of bacteriocinogenic strains, but the substances can also be induced by trating cells with ultraviolet light or Mitomycin C. Because becteriocins specifically kill the cells of sensitive bacteria, bacterial isolates from distinct sources can be differentiated or typed by their sensitivity to panels of bacteriocins.

Bacteriocins have found widespread application in epidemiological studies as specific markers for bacteria. Various typing schemes have been based upon either the production of, or sensitivity to a range of different bacteriocins (1-5).

Bacteriocins have important applications in plant pathoogy. They can be used in epidemiological studies to type strains of phytobacteria. Identification and classification of bacteria can be carried out with bacteriocins. Plant pathogens can be controlled by use of bacteriocins (6).

Bacteriocins are produced by the major groups of plant pathogenic bacteria (7-17). Particular bacteriocins are produced by nearly all strains of *P. s.* pv. *syringae, P. s.* pv. *glycinea and P. s.* pv phaseolicola making them enable to type as producers (18-21).

Pseudomonas syringae pv. *phaseolicola* (Burkholder) Young, Dye and Wilkie is the causal agent of halo blight disease of beans worldwide and the bacterium has three races which were differentiated by their pathogenicity on *Phaseolus vulgaris* cultivars (22).

The present study is a survey of bacteriocin production by 86 isolates of *Pseudomonas syringae* pv. *phaseolicola*, the causal agent of halo blight of beans in Eskişehir. The bacteriocin typing pattern of the isolates tested were compared with authentic strains of three races.

Materials and Methods

Test strains

Eighty-six *P. s. pv. phaseolicola* isolates, obtained from different bean growing areas in a previous study (22) were used as the bacteriocin producer strains (Table 1). Conventional biochemical tests and LOPAT tests were carried out to identify the test strains as described by Sands et al. (23) and Fahy and Hayward (24).

Indicator strains

Six strains of *P. s. pv. syringae*, namely PS281, PS6, PSC1-B, PS14, PS17 and GN2, a strain of *P. s.* pv. *glycinea*, PG1-T, and a strain of *P. s.* pv. *phaseolicola*, HB6, which had been originally described by Vidaver et al. (19), were kindly provided by the University of Nebraska Lincoln, Institute of Agriculture and Natural Resources (Table 2).

The Production of Bacteriocin by Induction

Production of bacteriocin by Mitomycin C and detection of the bacteriocins were carried out by procedure as described by Gross and Vidaver (21). Ninety-eight isolates of *P. s.* pv: *phaseolicola* shown in Table 1 were grown on Nutrient-broth yeast extract (NBY) agar plates (25) by incubating at 25°C for two days. The cultures were then inoculated into 10 ml of NBY broth and grown on a rotary shaker (250 rpm) to obtain 1 to $5X10^8$ cfu/ml. After addition of Mitomycin C to the bacterial cultures to obtain a final concentration of 1 μg/ml, the cultures were further incubated on a rotary shaker in the dark for 4 hours and then stored overnight at 2-5°C. The cells and debris of *P. s.* pv *phaseolicola* were pelleted by centrifugation at 12 100 g for 15 min. The supernatant fluids of the bacterial cultures were pipetted into a new bottle. Chloroform (10 % v/v) was added onto the supernatant fluids and then stored in screw-cap bottles at 2-5°C.

The Detection of Bacteriocin

Log phase cultures of the indicators were adjusted to a cell concentration of 10^8 cfu/ml. Then, 0.1 ml of the culture was suspended in 2.5 ml of NBY soft agar which was melted and cooled to 46°C. The mixture was poured over an NBY plate and once it solidified, 10 μl of test preparations from the producer strains were spotted onto the surface of an NBY plate. Plates

were incubated for 16 to 24 hours at 25°C to permit clear observation of zones of inhibition. All the experiments were carried out in duplicate. Bacteriocin groups were determined according to the presence of a clear (C) or a turbid (T) zone. If there was no inhibition zone results were recorded as negative (-).

Results and Discussion

All the isolates shown in Table 1 produced bacteriocin by Mitomycin C induction. The bacteriocin activity spectrum of the *P. s.* pv. *phaseolicola* isolates on eight indicator strains are listed in Table 2. Although the isolates tested were *P. s.* pv. *phaseolicola* and they do not have different plant species origin, the activity spectra of the bacteriocins were different.

Group A (Table 2) contained the majority of the isolates (57 isolates), and no correlation was obtained between the bacteriocin typing group and geographical origin. Group B (Table 2) representing mostly the authentic strains contained 8 of the isolates and only 3 of the isolates were from our collection. Group C (Table 2) represented 4 of the isolates obtained from different areas. Group H (Table 2) consisted of 7 of the isolates, 3 of which were authentic cultures. Group E and group T (table 2) contained 2 of the isolates which were collected in our study. Other groups were represented by only 1 isolate and groups Q and Y consisted of authentic isolates (Table 2).

Different races of the bacterium (race 1, race 2 and race 3) did not give specific typing patterns, therefore, no correlation between the race of the bacterium and the bacteriocin typing pattern was observed.

Typical typing experiments were performed to check the reproducibility of results by inducing bacteriocin production on at least two separate occasions by using strains of *P. syringae* pathovars known to produce characteristic bacteriocins. Bacteriocin preparations used in our study were tested immediately after production since some bacteriocins will lose activity or develop unusual typing patterns (21).

There are some reports (20, 26) on *P. s.* pv. s*yringae* isolates tested, 86 to 100% of which produced bacteriocins inhibitiory to the above indicators. In our study, all the isolates tested produced bacteriocins to the indicators. Less succesful results could be obtained if the conditions were changed or combinations of strains were used.

When we compare the bacteriocin groups of our study with Vidaver and Buckner's (20), only group A overlaps with their groups. Therefore, we obtained new sensitivity patterns by the isolates used in our study.

The isolation and characterisation of a particulate bacteriocin, syringacin W-1, from *Pseudomonas syringae* were described by Smidt and Vidaver (27). In the present study it was not necessary to purify the bacteriocins for typing of the strains.

In conclusion, the present study showed that the isolates of *P. s.* pv. *phaseolicola* from Turkey could be typed for the production of bacteriocin since all the isolates produced bacteriocin. Bacteriocins produced in this study can be isolated and purified for the control of plant pathogens in future studies.

Table 1. Bacterial strains used in the assay and groups of bacteriocin produciton test.

Strains	Geographical Source (Country, city or town)	Bacteriocin producer group (see Table 2)
Authentic isolates		
NCPPB 52* (race 1)	Canada	B
T 1302* (race 3)	Rwanda	B
T1817* (race 2)		B
T 1599* (race 2)		Y
T 1355* (race 1)		A
T 1516* (race 1)		B
T 1374* (race 2)		B
T 1360* (race 1)		C
Q 1945*(race 2)		H
T 1318* (race 3)		H
T1301* (race 3)	Tanzania	H
TPPB 4101**(race 1)	Turkey	Q
Eskişehir isolates		
	City center	
P3, P4, P45, P41, P42, P43, P44, P61, P35, P36, P37, P52, P53, P54, P55, P56, P57		A
P47		B
P64		C
P58		E
P46, P71, P84, P83		H
P62,		J
P63		K
P70		N
P73		O
P85		V
P74		P
P75		R
P81, P86		T
P82		U
P87		W
P88		X
	Sivrihisar	
P2, P5, P13, P9, P10, P11, P12 P6, P7, P14, P15, P16, P17, P19		

Table 1.

P20, P21, P22, P23, P18, P24, P80		A
P78		B
P25		D
P79		S
	Seyitgazi	
P26, P27, P28, P29, P30, P31		
P39, P48, P49, P50, P51, P76		
P32, P67, P68, P59, P60		A
P77		B
P33, P65		C
P34		E
P38		F
P40		G
P66		L
P69		M

* Obtained from Dr. John Turner, University of East Anglia, Norwich UK.

** Obtained from Dr. Kemal Benlioğlu, Adnan Menderes University, Aydın-Turkey.

Table 2. Bacteriocin groups of *Pseudomonas syringae* pv. *phaseolicola* isolates.

Ind. str.	Bacteriocin group																						
	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1	T	C	T	T	T	T	C	C	T	T	T	T	T	T	C	–	T	C	T	T	C	T	T	T
2	T	T	T	T	–	–	–	T	T	–	–	–	T	T	T	T	T	T	T	T	T	T	T	T
3	T	T	T	T	T	T	T	T	T	T	T	T	C	C	C	T	C	C	T	T	T	T	T	T
4	T	T	T	–	T	–	T	T	C	T	T	–	T	T	T	T	T	T	T	T	T	T	T	–
5	T	T	C	–	T	T	C	C	T	–	C	T	–	C	C	–	T	C	T	–	T	–	–	–
6	T	T	T	T	T	T	T	T	T	–	T	T	T	T	T	–	T	C	T	T	T	T	C	T
7	T	T	T	T	T	T	T	T	T	T	T	–	T	T	T	T	T	C	T	T	C	T	T	–
8	T	T	T	T	T	T	T	T	T	–	–	T	T	T	T	T	C	T	C	T	T	C	T	T

Indicator strain (Ind. str.): 1: Ps281, 2:Ps6, 3: PsC–1B, 4: Ps14, 5: PG1–T, 6: Ps17, 7: HB6, 8:GN–2.

C: clear zone, T: turbid zone, –: no zone.

References

1. Shannon, R ., Colicine production as a method for typing E. coli O55:B5.J. Med. Lab. Technol., 14:199 214, 1957.
2. Anthony, B. D., Giuliano, D. M. and Oh, W., Nursery outbreak of Staphylococcal scalded skin syndrome. Am. J. Dis. Child., 124: 41-44, 1972.
3. Tagg, J. R., Dawani, A. S. and Wannamaker, L. W., Bacteriocins of Gram positive bacteria. Bacteriol. Rev., 40: 722-756, 1976.
4. Podschun, R., Bacteriocin typing of *Klebsiella* spp. isolated from different sources. Zentralblatt fur Hygiene and Umweldmedizin, 198:258, 1996.
5. Traub, W. H., Typing of nosocomial strains of *Serratia marcescens*: Comparison of restriction enzyme cleaved genomic DNA fragment (PFGE) analysis with bacteriocin typing, biochemical profiles and serotyping. Zentralbalatt fur Bacteriologic, 284:94, 1996.
6. Vidaver, A. K., Bacteriocins: The Lure and the Reality. Plant Disease, 67:471-474, 1983.
7. Okabe, N. and Goto, M., Bacteriophages o plant pathogens. Ann. Rev. Phytopathology, 1:397-418, 1963.
8. Kerr, A. and Htay, K., Biological control of crown gall through bacteriocin production. Physiol. Plant Pathol. 4: 37-44, 1974.
9. Echandi, E., Bacteriocin production by *Corynebacterium michiganense*. Phytopathology, 66:430-432, 1976.
10. Schwinghamer, E. A. and Brockwell, J., Competitive advantage of bacteriocin and phage producing strains of *Rhizobium trifolii* in mixed culture. Soil Biol. Biochem. 10: 383-387, 1978.
11. Cuppels, D., Hanson, R. S. and Kelman, A., 1978. Isolation and characterization of a bacteriocin produced by *Pseudomonas solanacearum*. J. Gen. Microbiol 109:295-303.
12. Echandi, E. and Moyer, J. W., Production, properties and morphology of bacteriocins from *Erwinia chrysanthemi*. Phytopathol. 69:1204-1207, 1979.
13. Gross, D. C. and Vidaver, A. K., Bacteriocin-like substrances produced by *Rhizobium japonicum* and other slow growing rhizobia. appl. Environ. Microbiol. 36: 936-943, 1978.
14. Gross, D. C. and Vidaver, A. K., Bacteriocins of phytopathogenic *Crynebacterium species*. Can. J. Microbiol. 25: 367-374, 1979.
15. Crowley, C. F. and DeBoer, S. H., Sensivity of some *Erwinia corotovora* serogroups to macromolecular bacteriocins. Can. J. Microbiol. 109: 295-303, 1980.
16. Shoeib, A. A., Bacteriocin productivity by *Erwinia amylovora* strains. Journal of Agricultural Research, 40: 21, 1995.
17. Shoeib, a. A., Bacteriocin production by *Erwinia amylovora* strains. Acta Horticulturae, 411:229, 1996.
18. Garrett, C. M. E., Panagopoulos, C. G. and Crosse, J. E. Comparison of plant pathogenic pseudomonads from fruit trees. J. Appl. Bacteriol. 29:342-356, 1966.

19. Vidaver, A. K., Mathys, M. L., Thomas M. E. and Schuster, M. L., Bacteriocins of phytopathogens *Pseudomonas syringae, P. glycinea* and *P. phaseolicola*. Can. J. Microbiol. 18: 705-713, 1972.

20. Vidaver, A. K. and Buckner, S., Typing of fluorescent phytopathogenic pseudomonads by bacteriocin production. Can. J. Microbiol. 24: 14-18, 1978.

21. Gross, D. C. and Vidaver, A. K. Bacteriocins. In:Methods in Phytobacteriology Klement, Z., K. Rudolph and D. C. Sands (eds.). Akademiai Kiado, Budapest, 245 p. 1990.

22. Ertuğrul, D. and Güven, K., Serological Properties of *Pseudomonas syringae pv. phaseolicola isolates collected* from Eskişehir, Turkish Journal of Biology, 22: 189-195, 1998.

23. Sands, D. C., Schroth, M. N. and Hildebrand. D. C., Pseudomonads. In: Schaad, N. W. (ed.), Laboratory guide for identification of plant pathogenic bacteria., St. Paul, Minnesota, American Phytopathological Society, 36 p. 1980.

24. Fahy, P. C. and Hayward, A. C., Media and methods for isolation and diagnostic tests, In:Fathy, P. C. and G. J. Persley (eds)., Plant bacterial diseases, *A diagnostic guide*, Academic Press, 337 p, 1983.

25. Vidaver, A. K., Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. Ann. Rev. Phytopathol. 14: 451-466, 1976.

26. Gross, D. C., Cody, Y. S. Proebsting, E. L., Radamaker, G. K. and Spotts, R. A., Ecotypes and Spotts, R. A., Ecotypes and Pathogenicity of Ice-Nucleation Active *Pseudomonas syringae* isolated From Dedicious Fruit Tree Orchards. Phytopathol., 74:241-248, 1984.

27. Smidt, M. L. and Vidaver, A. K., Isolation and characterization of syringacin W-1, a bacteriocin produced by *Pseudomonas syringae pv. syringae*. Can. J. Microbiol. 32:231-236, 1986.