Bacteriocinogeny in *Proteus rettgeri*

Bacteriocins are proteinaceous bactericidal substances produced by bacteria and generally of limited host range[1–3]. Bacteriocins have been detected in a variety of species of *Enterobacteriaceae*, including *Proteeae*[3–6]; however, bacteriocinogeny had not been shown previously in isolates of *P. rettgeri*[5,6]. Here we wish to report demonstration of bacteriocin production by *P. rettgeri* following induction with mitomycin C.

The bacteria studied included 7 isolates of wild-type *P. rettgeri* from various clinical sources (designated I–VII), 15 isolates of an unusual strain of *P. rettgeri*[7], all from urinary tract specimens (designated 1–15); 3 strains each of *P. vulgaris*, *P. mirabilis*, *P. morganii*, and 7 isolates of *Escherichia coli*. The organisms were identified according to previously published criteria[7,8], and were maintained on brain heart infusion agar (Difco) slants at 4 °C. Mitomycin C powder (lot 99B–0090, Sigma Chemical Co., St. Louis, Mo.) was dissolved in sterile distilled water to yield 10 μg/ml; the solution was passed through 0.2 μm membrane filters (Nalge Sybron Corp., Rochester, N.Y.) and dispensed in 2-ml aliquots into sterile screw-capped vials which were frozen and kept stored at –15°C in the dark.

All *P. rettgeri* isolates were induced with 1 μg/ml mitomycin C in tryptic soy broth (TSB; Difco) at 33 °C[9,10], controls consisted of mitomycin C in TSB. Indicator strains had been pregrown in TSB overnight; their turbidity was adjusted to 1.5×10^7 organisms/ml. Large Mueller-Hinton agar (Difco) plates (150 × 15 mm) were divided into appropriate numbers of sectors, including control sectors. The plates were streaked with the inocula in 3 planes and were allowed to dry for 10 min. One drop (0.05 ml) of each aerated bacteriocin preparation, including the control, was delivered to respective sectors. The drop inocula were allowed to 'dry' in, following which the plates were incubated at 33 °C overnight. Sectors were scored as positive if there were completely clear zones or if the areas of inhibition revealed fewer than 50 resistant variants against a clear background. For 'titration' purposes, the supernatant fluids were serially diluted twofold in TSB; 0.1 ml of each dilution were delivered to corresponding sectors of indicator strain-streaked Mueller-Hinton agar plates. 'Titers' were defined as the highest dilution of bacteriocin revealing activity. A 1% (w/v) solution of trypsin 1:250 (Difco certified) in phosphate buffered saline, pH 7.4 (Grand Island Biological Co., Grand Island, N.Y.) served to test bacteriocins for trypsin-sensitivity[11].

Two of the 7 wild type *P. rettgeri* isolates, i.e., isolates III and VI, consistently elaborated bacteriocins following induction with mitomycin C (Table); none of the isolates (1–15) comprising the unusual strain of *P. rettgeri* were bacteriocinogenic. The bacteriocins wre not produced spontaneously, and induction through UV-irradiation[4–6,11] gave consistent results. The 2 bacteriocins were species-specific in that they were active only against *P. rettgeri* (isolates VII, 9, 14, and 15; in addition, bacteriocin III was weakly active against isolate 4); the *P. vulgaris*, *P. mirabilis*, *P. morganii*, and *E. coli* isolates tested proved resistant.

Of the broths (Difco) employed, nutrient[5] and MacConkey broths were found unsuitable for bacteriocin induction, whereas brain heart infusion broth, Mueller-Hinton broth, and TSB could be used interchangeably. The 2 detected bacteriocins were not active against their own respective producer strains; trypsin treatment did not reduce their activity. The presence of bacteriophage was ruled out through failure to transfer activity in series[5,11]. Elaboration of these bacteriocins ensued at 2 h following induction; peak activity (1:128 to 1:256) was reached at 6 h and was maintained for 24 h. The bacteriocins were partially heat-labile; exposure to 56 °C for 1 h reduced activity roughly 4-fold, while boiling for 1 h completely abolished activity.

We were interested primarily to obtain an additional marker for our unusual strain of *P. rettgeri*[7], namely susceptibility to *P. rettgeri* bacteriocins. Fortuitous circumstance had it that 2 of the 7 wild-type *P. rettgeri* isolates examined proved bacteriocinogenic. However, only 4 of the 15 isolates of our unusual *P. rettgeri* were susceptible to these bacteriocins; conceivably, sensitivity to these 2 bacteriocins is a genetically unstable property of this particular strain of *P. rettgeri*[12].

Zusammenfassung. In 2 von 7 Stämmen von *Proteus rettgeri* gelang die Induktion von Bakteriozinen unter der Einwirkung von Mitomycin C. Die gewonnenen Bakteriozine waren chloroformbeständig, trypsinresistent und hitzelabil.

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Host range of *P. rettgeri* bacteriocins

P. rettgeri indicator strains	Activity of *P. rettgeri* bacteriocins			
	III		VI	
VII	+[a]	1:128[b]	+	1:64
4	+	1:4	—	–
9	+	1:256	+	1:256
14	+	1:256	+	1:64
15	+	1:256	+	1:128

[a] + and — denote presence and absence of activity, respectively.
[b] 'Titers' of activity.

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