MULTIRESISTANT *Serratia* SP. ISOLATED FROM THE MARINE ENVIRONMENT

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ABSTRACT: James P. Robeson[1], Carlos Riquelme[1*] and Ana María Skarmeta[1]: Multiresistant *Serratia* sp. isolated from the marine environment.

The genus *Serratia* is widespread in the environment including the marine milieu. Here we report data on plasmid determined, transmissible multiple drug resistance in a strain of *Serratia* isolated from polluted coastal seawater.

Key words: *Serratia*, marine environment, gene transfer.

RESUMEN: James P. Robeson[1], Carlos Riquelme[1*] y Ana María Skarmeta[1]: *Serratia* sp. multirresistente aislada del ambiente marino

El género *Serratia* es de amplia distribución en el ambiente, incluyendo el medio marino. Se comunican datos referente a resistencia múltiple a drogas, determinada por un plásmido transmisible en una cepa de *Serratia* aislada de agua de mar costera contaminada.

Palabras claves: *Serratia*, ambiente marino, transferencia génica.

INTRODUCTION

Serratia is an important and ubiquitous enterobacterial genus found in a wide variety of ecological niches (Grimont & Grimont 1978). It is found in soil, in association with plants and animals, and also as an opportunistic pathogen in humans (Daschner 1980). *Serratia marcescens*, in particular, produces a characteristic red pigment (Williams & Qadri 1980), hydrophobic surface molecules that seem to aid colonization (Maksuyama *et al.* 1986) and a variety of extracellular hydrolytic enzymes (Hines *et al.* 1988). With regard to the presence of *Serratia* in the marine environment some strains are found in the water column and also in association with marine mollusks (Daschner 1980). However, information about plasmid carriage in *Serratia* of marine origin is at best very scarce. Within this context we sought the isolation and genetic characterization of antibiotic resistant *Serratia* in our coastal environment, notoriously polluted by sewage discharges and influxes due to erosion (Zahr 1983). In this work we describe a strain of *Serratia* sp., recovered from the marine environment using a drug-based selective procedure to enhance the recovery of bacterial strains carrying R-factors. The strain contains a conjugative plasmid that codes for resistance to commonly used antibiotics.

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MATERIALS AND METHODS

Surface seawater samples were collected in sterile flasks in the intertidal zone at the Bay of Valparaíso, Chile, and transported to the laboratory in an ice box. Fifty ml of each sample was filtered through 0.45 µm Millipore GS filters. These were placed on Müeller-Hinton agar (Difco) plates supplemented with 0.8% NaCl (Merck) and appropriate antibiotics (Sigma). Resistant colonies were recovered after 48 h of incubation at 20°C and purified in the same medium. Tests for strain characterization were as described by Smibert and Krieg (Smibert & Krieg 1981). Resistance to antibiotics was determined by spotting exponentially growing bacterial cultures on plates supplemented with individual antibiotics in concentrations suggested for plasmid mediated resistance (Curtiss 1981). Genetic crosses were performed essentially as described by Curtiss (Curtiss 1981). In these experiments a modified L broth was used that contained (in g/l): Tryptone (Difco), 10; Yeast Extract (Difco), 5; NaCl (Merck), 5. The same medium was used for agar (Difco, 1,5%), plates. Plasmid DNA was detected by alkaline lysis followed by agarose gel electrophoresis (Sambrook *et al.* 1989).

A list of bacterial strains and plasmids used in this study is in Table 1.

RESULTS AND DISCUSSION

Our main purpose was to determine the presence of antibiotic resistant *Serratia* in polluted seawater and in one out of five samples examined we found a red pigmented isolate that was recovered from a filter incubated on L. agar containing 400 µg/ml of kanamycin. We used a higher concentration of this antibiotic based on the observation that our samples of polluted seawater normally yield a high bacterial growth background when plated at lower concentrations (50-100 µg/ml) of the drug (data not shown).

The red pigmented isolate, *Serratia* sp. BR, was further characterized on the basis of biochemical and physiological tests. The strain was a Gram negative, fermentative, motile rod which was oxidase negative and catalase positive. It produced acid from xylose, sucrose, arabinose, maltose and mannitol, was indole, methyl red, arginine dihydrolase and phenylalanine deaminase negative and Voges-Proskauer, citrate, lysine decarboxylase, ornithine decarboxylase, gelatinase and DNAase positive. These results suggest that the bacterium could be assigned to the species *Serratia marcescens* (Grimont & Grimont 1984).

We note that the BR strain grew both in the abscence and presence of NaCl in concentrations up to 6% (w/v) and at 15,28 and 37°C but not at 7°C. In addition, when grown in Nutrient Broth (Difco) prepared in seawater and at room temperature (c.a. 20°C) it reached concentrations about 5 x 10^8 cells/ml after overnight incubation. These observations suggest an adaptation of the bacterium to the marine milieu with a concomitant potential to proliferate in it, if provided with adequate nutrient and temperature conditions.

Table 1. Bacterial strains and plasmids.

Bacteria	Strain designation	Description	Plasmid and relevant markers	Source or reference
Serratia sp.	BR	wild type	pUCV1 55 Mda Gm,Km,Cm,Ap,Sm; Tra^+	This work
Escherichia coli	X1553	F-minA1 purE41 SupE42 - pdxC3-his-53 gyrA28 metC65 ilv-277 cycB2 cycA1 hsdR2	None	R. Curtiss III
	VAL 3	F- tonA53 dapD8 minA1 purE41 ginU42 (gal-uvrB) -minB2 hsd53 gyrA25 metC65 oms-1 T3 (bioH-asd)29 ilv-277 cycB2 cyc A1 hsdR2 Contains pUCV1	Same as for *Serratia* sp.BR	Ref. 16
Salmonella typhi	6011	wild type	None	M. Rodriguez
Vibrio fischeri	MJ-1	Lux^+	None	M. Silverman
Vibrio fluvialis	ATCC 33812	wild type	None	A. Baya
Vibrio damsela	ATCC 35083	wild type	None	A. Baya
		Plasmid size marker	TP116 143,6Mda Hg, Tra^+	J.C. Fry
		Plasmid size marker	LT2 60 Mda cryptic	R. Curtiss III
		Plasmid size marker	RP4 36,7 Mda Ap,Tc,Km;Tra^+	R. Curtiss III

The *Serratia* sp. isolate was also resistant to several antibiotics used at concentrations indicated for plasmid mediated antibiotic resistance (Curtiss 1981). These were the following: gentamicin (Gm, 25 µg/ml), chloramphenicol (Cm, 30 µg/ml), ampicillin (Ap, 50 µg/ml), kanamycin (Km,50 µg/ml and streptomycin (Sm, 25 µg/ml). As most enterobacteria the *Serratia* sp. isolate was sensitive to nalidixic acid (Nal, 50 µg/ml).

The antibiotic resistance markers could be transferred in liquid matings to *E. coli* XI553 at frequencies about 1×10^{-4} transconjugants per donor cell. The *E. coli* XI553 transconjugants became resistant "en bloc" to Gm, Cm, Ap, Km and Sm used at the same concentrations stated above. In addition, they consistently showed 100% coinheritance of unselected markers when selected on L agar + Nal plates supplemented with any of the above antibiotics used singly. Antibiotic multiresistance was associated to the presence of a single plasmid band shown in Figure 1. This plasmid, designated

pUCV1, had an approximate molecular mass of 55 MDa as determined by plotting the relative mobility against log molecular weight (Crosa & Falkow 1981) using the plasmid size markers in Table 1. These observations agree with the abundant information on plasmid mediated infectious multiple drug resistance in *S. marcescens* (Bukhari *et al.* 1977). Furthermore, the antibiotic resistance pattern determined, closely resembles that conferred by Inc S plasmids from *S. marcescens* (Bukhari *et al.* 1977).

Intergeneric transfer of pUCV1 antibiotic resistance markers was readily detected at various temperatures starting at 20°C. These results are summarized in Table 2. We did not, however, detect transfer at 15 or 10°C. This is in agreement with published reports that show a temperature dependent response of plasmid R1drd19 transfer among *E. coli* strains (Singleton & Anson 1981).

Taken together, the transfer data in Table 2 suggest that plasmid gene flow from *Serratia* to other bacteria in the environment could be possible in warm waters since this situation has been described for other Enterobacteriaceae (Mach & Grimes 1982, Altherr & Kasweck 1982).

In conclusion, we think that strains of *Serratia* as the one described might pose a health risk in polluted waters and, in addition, might represent a source of genetic variability for other bacteria in the aquatic environment.

Figure 1. Agarose (0.7%) gel electrophoresis of pUCV1.

1 : Molecular weight marker TP116
2 : Molecular weight marker LT2
3 : pUCV1
4 : Molecular weight marker RP4.

Table 2. Intergeneric transfer of pUCV1 antibiotic resistance markers.

Donor	Recipient	Mating (a) Conditions	Tranfer (b)
Serratia sp. BR	*E. coli* x1553	Liquid mating 1 hr: 20°C	Yes (7×10^{-5})
		As above but at 28°C	Yes ($3,5 \times 10^{-4}$)
		As above but at 37°C	Yes ($3,8 \times 10^{-4}$)
Serratia BR	*Salmonella typhi* 6011	Liquid mating 2 hr; 37°C	Yes ($1,3 \times 10^{-6}$)
E. coli VAL 3	*Vibrio fluvialis*	Spin mating 2 hr; 28°C	Yes (3×10^{-7})
	Vibrio damsela	As above	Yes (3×10^{-7})
	Other *Vibrio* strains (c)	As above	No

(a) Liquid matings were in L broth and spin matings performed by mixing 0,5 ml each of donor and recipient fresh liquid cultures, followed by centrifugation for 30 seconds in an Eppendorf 5414 centrifuge.

(b) Numbers in parentheses represent frequencies of transfer expressed as number of transconjugants per donor cell.

(c) *Vibrio fischeri*, *V. ordalii*, *V. aestuarinae*, *V. vulnificus*, and *V. tubiashi*. All strains were obtained from Dr. A. Baya, University of Maryland.

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